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UNITED STATES DISTRICT COURT
DISTRICT OF NEW JERSEY

CELGENE CORPORATION, **Plaintiff,** **v.** **DR. REDDY'S LABORATORIES, INC. and DR. REDDY'S LABORATORIES, LTD.,** **Defendants.**	**Civil Action No. 18-11269 (MAS)(DEA)** **(Filed Electronically)**

SECOND AMENDED COMPLAINT FOR PATENT INFRINGEMENT[1]

Plaintiff Celgene Corporation ("Celgene" or "Plaintiff"), by its undersigned attorneys, brings this action against Defendants Dr. Reddy's Laboratories, Inc. ("DRL Inc.") and Dr.

[1] Plaintiff Celgene Corporation files this Second Amended Complaint with the consent of Defendants Dr. Reddy's Laboratories, Inc. and Dr. Reddy's Laboratories, Ltd. pursuant to Fed. R. Civ. P. 15(a)(2).

Reddy's Laboratories, Ltd. ("DRL Ltd.") (collectively, "DRL" or "Defendants"), and hereby alleges as follows:

NATURE OF THE ACTION

1. This is a civil action for patent infringement arising under the patent laws of the United States, 35 U.S.C. § 1, *et seq.*, and in particular under 35 U.S.C. § 271. This action relates to Abbreviated New Drug Application ("ANDA") No. 211756 (hereinafter, "DRL's ANDA"), filed by and for the benefit of DRL with the United States Food and Drug Administration ("FDA"). Through DRL's ANDA, DRL seeks approval to market generic versions of Celgene's OTEZLA® (apremilast) 10 mg, 20 mg, and 30 mg tablets (hereinafter, "DRL's Infringing ANDA Products"), prior to the expiration of Celgene's United States Patent Nos. 6,962,940 ("the '940 Patent"), 7,427,638 ("the '638 Patent"), 7,659,302 ("the '302 Patent"), 7,893,101 ("the '101 Patent"), 8,455,536 ("the '536 Patent"), 9,018,243 ("the '243 Patent"), 9,724,330 ("the '330 Patent"), and 10,092,541 ("the '541 Patent") (collectively, "the Patents-in-Suit").

THE PARTIES

2. Celgene is a biopharmaceutical company committed to improving the lives of patients worldwide. Celgene focuses on, and invests heavily in, the discovery and development of products for the treatment of severe and life-threatening conditions. Celgene is a world leader in the treatment of many such diseases. Celgene is a corporation organized and existing under the laws of the State of Delaware, having a principal place of business at 86 Morris Avenue, Summit, New Jersey 07901.

3. On information and belief, Defendant DRL Inc. is a corporation organized and existing under the laws of the State of New Jersey, having a principal place of business at 107 College Road East, Princeton, New Jersey 08540.

4. On information and belief, Defendant DRL Ltd. is a company organized and existing under the laws of India, having a principal place of business at 8-2-337, Road No. 3., Banjara Hills, Hyderabad, Telangana 500034, India.

5. On information and belief, DRL Inc. is a wholly owned subsidiary of DRL Ltd.

6. On information and belief, DRL Inc. acts at the direction, and for the benefit, of DRL Ltd., and is controlled and/or dominated by DRL Ltd.

7. On further information and belief, DRL Ltd. and DRL Inc. collaborate with respect to the development, regulatory approval, marketing, sale, and/or distribution of pharmaceutical products. On further information and belief, Defendants are agents of each other and/or operate in concert as integrated parts of the same business group, and enter into agreements with each other that are nearer than arm's length.

8. On information and belief, DRL Ltd. and DRL Inc. acted collaboratively in the preparation and submission of ANDA No. 211756.

9. On information and belief, DRL Inc. acts as the U.S. agent for DRL Ltd. for purposes of regulatory submissions to FDA in seeking approval for generic drugs.

10. On information and belief, DRL Inc. acts as the U.S. agent of DRL Ltd. for ANDA No. 211756.

11. On information and belief, DRL Ltd. relied on material assistance from DRL Inc. to market, distribute, offer to sell, or sell generic drugs in the U.S. market, including in the State of New Jersey. On information and belief, Defendants intend to act collaboratively to commercially manufacture, market, distribute, offer to sell, or sell DRL's Infringing ANDA Products in the event FDA approves ANDA No. 211756.

JURISDICTION AND VENUE

12. This is a civil action for patent infringement arising under the patent laws of the United States, including 35 U.S.C. § 271, for infringement of the Patents-in-Suit.

13. This Court has jurisdiction over the subject matter of this action pursuant to 28 U.S.C. §§ 1331, 1338, 2201, and 2202.

14. This Court has personal jurisdiction over each of the Defendants because, *inter alia*, on information and belief, Defendants have continuous and systematic contacts with the State of New Jersey, regularly conduct business in the State of New Jersey, either directly or through one or more wholly owned subsidiaries, agents, and/or alter egos, have purposefully availed themselves of the privilege of doing business in the State of New Jersey, and intend to sell DRL's Infringing ANDA Products in the State of New Jersey upon approval of ANDA No. 211756.

15. This Court also has personal jurisdiction over Defendant DRL Inc. at least because DRL Inc. is incorporated in New Jersey and has its principal place of business in New Jersey.

16. On information and belief, DRL is in the business of, *inter alia*, developing, manufacturing, obtaining regulatory approval, marketing, selling, and distributing generic copies of branded pharmaceutical products throughout the United States, including within the State of New Jersey, through its own actions and through the actions of its agents and subsidiaries, from which DRL derives a substantial portion of its revenue.

17. On information and belief, DRL, through its own actions and through the actions of its agents and subsidiaries, has engaged in the research and development, and the preparation and filing, of ANDA No. 211756, continues to engage in seeking FDA approval of this ANDA, intends to engage in the commercial manufacture, marketing, offer for sale, sale, or importation

of DRL's Infringing ANDA Products throughout the United States, including within the State of New Jersey, and stands to benefit from the approval of ANDA No. 211756.

18. On information and belief, DRL, through its own actions and through the actions of its agents and subsidiaries, prepared and submitted ANDA No. 211756 with a Paragraph IV Certification pursuant to 21 U.S.C. § 355(j)(2)(A)(vii)(IV).

19. On information and belief, following FDA approval of ANDA No. 211756, DRL intends to market, offer to sell, sell, or distribute DRL's Infringing ANDA Products throughout the United States and within the State of New Jersey, that will, as explained below, infringe upon Celgene's rights in the Patents-in-Suit protecting its OTEZLA® products. On information and belief, following FDA approval of ANDA No. 211756, DRL knows and intends that DRL's Infringing ANDA Products will be marketed, used, distributed, offered for sale, or sold in the United States and within the State of New Jersey.

20. On information and belief, DRL Inc. is the agent in the United States for DRL Ltd. On information and belief, DRL Inc. acts at the direction of, under the control of, and/or for the benefit of DRL Ltd. and is controlled by DRL Ltd.

21. On information and belief, DRL Inc. is registered to do business in the State of New Jersey under Entity Identification Number 0100518911 and is registered with the New Jersey Department of Health as a drug manufacturer and wholesaler under Registration Number 5002312.

22. DRL has consented to personal jurisdiction in this Court in numerous recent actions arising out of its ANDA filings and has filed counterclaims in such cases, including in this action, which arises out of DRL's filing of ANDA No. 211756. *See, e.g.*, *Celgene Corp. v. Dr. Reddy's Labs., Inc., et al.*, No. 18-cv-11269, D.I. 17 (D.N.J. Sept. 25, 2018); *Celgene Corp.*

v. Dr. Reddy's Labs., Ltd., et al., No. 18-cv-06378, D.I. 8 (D.N.J. Apr. 30, 2018); *Sumitomo Dainippon Pharma Co., et al. v. Aurobindo Pharma Ltd., et al.*, No. 18-cv-02620, D.I. 79 (D.N.J. Apr. 11, 2018); *Celgene Corp. v. Dr. Reddy's Labs., Ltd., et al.*, No. 17-cv-05314, D.I. 10 (D.N.J. Oct. 3, 2017); *Mitsubishi Tanabe Pharma Corp., et al. v. Prinston Pharm., Inc., et al.*, No. 17-cv-05135, D.I. 26 (D.N.J. Dec. 19, 2017). DRL has purposefully availed itself of the rights and benefits of this Court by asserting counterclaims in this Court.

23. This Court also has personal jurisdiction over DRL at least because, *inter alia*, (a) DRL has filed an ANDA seeking approval to engage in the commercial manufacture, use, offer for sale, sale, or importation of DRL's Infringing ANDA Products in the United States, including in the State of New Jersey; (b) DRL, through its own actions and through the actions of its agents and subsidiaries, will market, distribute, offer to sell, or sell DRL's Infringing ANDA Products in the United States, including in the State of New Jersey and to residents of this Judicial District, upon approval of ANDA No. 211756, and will derive substantial revenue from the use or consumption of DRL's Infringing ANDA Products in the State of New Jersey; and (c) DRL has purposefully availed itself of the privilege of doing business in the State of New Jersey by placing goods into the stream of commerce for distribution throughout the United States and within the State of New Jersey, and/or by selling, directly or through its agents, pharmaceutical products in the State of New Jersey. On information and belief, if ANDA No. 211756 is approved, DRL's Infringing ANDA Products charged with infringing the Patents-in-Suit would, *inter alia*, be marketed, distributed, offered for sale, or sold in the State of New Jersey, prescribed by physicians practicing in New Jersey, dispensed by pharmacies located within New Jersey, and used by patients in New Jersey, all of which would have a substantial effect on New Jersey.

24. This Court also has personal jurisdiction over DRL because DRL has committed, or aided, abetted, contributed to, and/or participated in the commission of, acts of patent infringement that will lead to foreseeable harm and injury to Celgene, a corporation headquartered in the State of New Jersey that manufactures OTEZLA® drug products for sale and use throughout the United States, including in this Judicial District. On information and belief, DRL filed ANDA No. 211756 with a Paragraph IV Certification, which was purposefully directed to the State of New Jersey, where Celgene is located. As a result, the consequences of DRL's actions were, and will be, suffered in the State of New Jersey. DRL knew or should have known that the consequences of its actions were, and will be, suffered in the State of New Jersey. At the time DRL sent notice of the Paragraph IV Certification, it was reasonably foreseeable that DRL would be sued within 45 days in this Judicial District, where Celgene is located. On information and belief, DRL's actions will injure Celgene by displacing at least some, if not all, of Celgene's sales of OTEZLA® drug products in this Judicial District, as well as resulting in price erosion and loss of goodwill with the purchasers and distributors of OTEZLA® drug products in this Judicial District.

25. In the alternative, this Court has personal jurisdiction over DRL Ltd. because the requirements of Federal Rule of Civil Procedure 4(k)(2) are met as (a) Celgene's claims arise under federal law; (b) DRL Ltd. is a foreign defendant not subject to general personal jurisdiction in the courts of any state; and (c) DRL Ltd. has sufficient contacts with the United States as a whole, including, but not limited to, preparing and submitting ANDAs to the FDA and/or manufacturing, importing, offering to sell, or selling pharmaceutical products that are distributed throughout the United States, such that this Court's exercise of jurisdiction over DRL Ltd. satisfies due process.

26. On information and belief, DRL has also engaged in substantial, systematic, and continuous contacts with New Jersey that satisfy due process and confer personal jurisdiction over DRL in New Jersey.

27. At least because, on information and belief, DRL Ltd. is a foreign company, venue is proper in this Judicial District pursuant to 28 U.S.C. §§ 1391(c)(3) and 1400(b).

28. At least because, on information and belief, DRL Inc. is incorporated in New Jersey, venue is proper in this Judicial District pursuant to 28 U.S.C. § 1400(b).

CELGENE'S PATENTS AND APPROVED OTEZLA® DRUG PRODUCTS

29. Celgene makes and sells OTEZLA® (apremilast) 10 mg, 20 mg, and 30 mg tablets (collectively, "OTEZLA®") for oral use to treat adult patients with active psoriatic arthritis (Indication 1.1), as well as patients with moderate to severe plaque psoriasis who are candidates for phototherapy or systemic therapy (Indication 1.2). The active ingredient in OTEZLA® is apremilast. A true and correct copy of the prescribing information for Celgene's OTEZLA® is attached as Exhibit A.

30. Celgene holds New Drug Application ("NDA") No. 205437, under which FDA approved the marketing of OTEZLA® on March 21, 2014.

31. OTEZLA® is the first approved pharmaceutical product to contain apremilast. In recognition of this, the FDA granted OTEZLA® five years of regulatory exclusivity for a new chemical entity, which expires on March 21, 2019, pursuant to 21 C.F.R. § 314.108.

32. OTEZLA® and one or more of its approved uses are covered by claims of the Patents-in-Suit.

33. The Patents-in-Suit are listed in *Approved Drug Products With Therapeutic Equivalence Evaluations* (an FDA publication commonly known as the "*Orange Book*") in connection with NDA No. 205437.

34. Celgene, as the assignee, owns the entire right, title, and interest in each of the Patents-in-Suit. Celgene has the right to enforce each of these Patents.

35. The '940 Patent is entitled, "(+)-2-[1-(3-Ethoxy-4-Methoxyphenyl)-2-Methylsulfonylethyl]-4-Acetylaminoisoindoline-1,3-Dione, Methods of Using and Compositions Thereof." The '940 Patent was duly and legally issued on November 8, 2005. The *Orange Book* presently shows that the '940 Patent's term ends on March 19, 2023. A true and correct copy of the '940 Patent is attached as Exhibit B.

36. The '638 Patent is entitled, "(+)-2-[1-(3-Ethoxy-4-Methoxyphenyl)-2-Methylsulfonylethyl]-4-Acetylaminoisoindoline-1,3-Dione, and Methods of Synthesis and Compositions Thereof." The '638 Patent was duly and legally issued on September 23, 2008. The *Orange Book* presently shows that the '638 Patent's term ends on February 16, 2028. A true and correct copy of the '638 Patent is attached as Exhibit C.

37. The '302 Patent is entitled, "Methods of Using (+)-2-[1-(3-Ethoxy-4-Methoxyphenyl)-2-Methylsulfonylethyl]-4-Acetylaminoisoindoline-1,3-Dione." The '302 Patent was duly and legally issued on February 9, 2010. The *Orange Book* presently shows that the '302 Patent's term ends on March 19, 2023. A true and correct copy of the '302 Patent is attached as Exhibit D.

38. The '101 Patent is entitled, "Solid Forms Comprising (+)-2-[1-(3-Ethoxy-4-Methoxyphenyl)-2-Methylsulfonylethyl]-4-Acetylaminoisoindoline-1,3-Dione, Compositions Thereof, and Uses Thereof." The '101 Patent was duly and legally issued on February 22, 2011.

The *Orange Book* presently shows that the '101 Patent's term ends on December 9, 2023. A true and correct copy of the '101 Patent is attached as Exhibit E.

39. The '536 Patent is entitled, "Methods of Using (+)-2-[1-(3-Ethoxy-4-Methoxyphenyl)-2-Methylsulfonylethyl]-4-Acetylaminoisoindoline-1,3-Dione." The '536 Patent was duly and legally issued on June 4, 2013. The *Orange Book* presently shows that the '536 Patent's term ends on March 19, 2023. A true and correct copy of the '536 Patent is attached as Exhibit F.

40. The '243 Patent is entitled, "Solid Forms Comprising (+)-2-[1-(3-Ethoxy-4-Methoxyphenyl)-2-Methylsulfonylethyl]-4-Acetylaminoisoindoline-1,3-Dione." The '243 Patent was duly and legally issued on April 28, 2015. The *Orange Book* presently shows that the '243 Patent's term ends on March 19, 2023. A true and correct copy of the '243 Patent is attached as Exhibit G.

41. The '330 Patent is entitled, "Methods of Using (+)-2-[1-(3-Ethoxy-4-Methoxyphenyl)-2-Methylsulfonylethyl]-4-Acetylaminoisoindoline-1,3-Dione." The '330 Patent was duly and legally issued on August 8, 2017. The Orange Book presently shows that the '330 Patent's term ends on March 19, 2023. A true and correct copy of the '330 Patent is attached as Exhibit H.

42. The '541 Patent is entitled, "Methods for the Treatment of Diseases Ameliorated by PDE4 Inhibition Using Dosage Titration of Apremilast." The '541 Patent was duly and legally issued on October 9, 2018. The *Orange Book* presently shows that the '541 Patent's term ends on May 29, 2034. A true and correct copy of the '541 Patent is attached as Exhibit I.

DRL'S ANDA AND NOTICE OF PARAGRAPH IV CERTIFICATION

43. On information and belief, DRL has submitted or caused to be submitted ANDA No. 211756 to FDA under 21 U.S.C. § 355(j), to obtain approval to engage in the commercial

manufacture, use, offer for sale, sale, or importation into the United States of the apremilast tablets described therein, as a purported generic version of OTEZLA®, prior to the expiration of the Patents-in-Suit.

44. On information and belief, DRL's Infringing ANDA Products are tablets that comprise 10 mg, 20 mg, or 30 mg of apremilast as the active pharmaceutical ingredient.

45. On information and belief, FDA has not yet approved ANDA No. 211756.

46. Celgene received a Notice of Paragraph IV Certification from DRL dated May 25, 2018 ("Notice Letter"). The Notice Letter represented that DRL had submitted to FDA ANDA No. 211756 with a purported Paragraph IV Certification pursuant to 21 U.S.C. § 355(j)(2)(A)(vii)(IV) to obtain approval to engage in the commercial manufacture, use, offer for sale, sale, or importation into the United States of the products described in ANDA No. 211756 before the expiration of the patents listed in the *Orange Book* for OTEZLA®. Hence, DRL's purpose in submitting ANDA No. 211756 is to obtain FDA approval to engage in the commercial manufacture, use, offer for sale, sale, or importation into the United States of DRL's Infringing ANDA Products before the expiration of the Patents-in-Suit.

47. The Notice Letter states that the Paragraph IV Certification in ANDA No. 211756 alleges that the Patents-in-Suit, other than the '330 Patent, which was not yet listed in the *Orange Book* by the date on the Notice Letter, and the '541 Patent, which issued after the date on the Notice Letter, are invalid, unenforceable, or would not be infringed by the commercial manufacture, use, offer for sale, sale, or importation into the United States of DRL's Infringing ANDA Products.

48. The Notice Letter contained a purported detailed statement of the factual and legal basis for DRL's opinion that the Patents-in-Suit, other than the '330 Patent, which was not yet

listed in the *Orange Book* by the date on the Notice Letter, and the '541 Patent, which issued after the date on the Notice Letter, are purportedly invalid, unenforceable, or not infringed by the manufacture, use, offer for sale, sale, or importation into the United States of DRL's Infringing ANDA Products ("Paragraph IV Statement").

49. On information and belief, DRL, through its own actions and through the actions of its agents and subsidiaries, has assisted with and participated in the preparation and submission of ANDA No. 211756, has provided material support to the preparation and submission of ANDA No. 211756, and intends to support the further prosecution of ANDA No. 211756. On information and belief, DRL Inc. and DRL Ltd. are acting in concert with one another with respect to the preparation, submission, and further prosecution of ANDA No. 211756.

50. On information and belief, if FDA approves ANDA No. 211756, DRL will manufacture, offer to sell, or sell DRL's Infringing ANDA Products within the United States, including within the State of New Jersey, or will import DRL's Infringing ANDA Products into the United States, including New Jersey.

51. On information and belief, if FDA approves ANDA No. 211756, DRL will actively induce or contribute to the manufacture, use, offer to sell, sale, or importation of DRL's Infringing ANDA Products in the United States.

52. Celgene initially brought this action pursuant to 21 U.S.C. § 355(j)(5)(B)(iii) within forty-five days of receipt of the Notice Letter. *See* 21 U.S.C. § 355(c)(3)(C).

53. After Celgene initially brought this action, the '330 Patent was listed in the *Orange Book* in connection with NDA No. 205437. One or more of the FDA-approved uses of OTEZLA® are covered by one or more claims of the '330 Patent. On August 16, 2018, Celgene

brought the First Amended Complaint to assert infringement of the '330 Patent in addition to the other Patents-in-Suit.

54. The '541 Patent issued on October 9, 2018, after Celgene brought the First Amended Complaint. One or more of the FDA-approved uses of OTEZLA® are covered by one or more claims of the '541 Patent. On information and belief, DRL intends to and will send Celgene a Paragraph IV Certification for the '541 Patent. Celgene brings this Second Amended Complaint to assert infringement of the '541 Patent in addition to the other Patents-in-Suit.

COUNT 1
INFRINGEMENT OF THE '940 PATENT

55. Celgene states, realleges, and incorporates by reference the foregoing paragraphs as if fully set forth herein.

56. On information and belief, DRL has submitted or caused the submission of ANDA No. 211756 to FDA and continues to seek FDA approval of ANDA No. 211756.

57. DRL has infringed the '940 Patent under 35 U.S.C. § 271(e)(2)(A) by submitting ANDA No. 211756 with a Paragraph IV Certification and seeking FDA approval of ANDA No. 211756 prior to the expiration of the '940 Patent.

58. The '940 Patent includes claims that recite methods of administering (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

59. On information and belief, DRL's Infringing ANDA Products contain (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

60. DRL's commercial manufacture, use, offer for sale, or sale within the United States, or importation into the United States of DRL's Infringing ANDA Products would directly infringe, or would actively induce or contribute to infringement of the '940 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a), (b), and (c). Accordingly, unless

enjoined by this Court, upon FDA approval of ANDA No. 211756, DRL will make, use, offer for sale, or sell DRL's Infringing ANDA Products within the United States, or will import DRL's Infringing ANDA Products into the United States, and will thereby infringe, contribute to the infringement of, or induce the infringement of one or more claims of the '940 Patent. *See id.*

61. On information and belief, upon FDA approval of ANDA No. 211756, DRL, through its own actions and through the actions of its agents and subsidiaries, will market and distribute DRL's Infringing ANDA Products to resellers, pharmacies, hospitals and other clinics, healthcare professionals, and end users of DRL's Infringing ANDA Products. On information and belief, DRL will also knowingly and intentionally accompany DRL's Infringing ANDA Products with a product label and product insert that will include instructions for using or administering DRL's Infringing ANDA Products. On information and belief, the product label and product insert accompanying DRL's Infringing ANDA Products will include instructions that are substantially similar to the instructions found in the prescribing information for OTEZLA®, attached as Exhibit A, and which, if followed, will infringe the '940 Patent. Accordingly, DRL will induce physicians and other healthcare professionals, resellers, pharmacies, and end users of DRL's Infringing ANDA Products to directly infringe one or more claims of the '940 Patent. In addition, on information and belief, DRL will encourage acts of direct infringement with knowledge of the '940 Patent and knowledge that it is encouraging infringement.

62. DRL had actual and constructive notice of the '940 Patent prior to filing DRL's ANDA and was aware that the filing of DRL's ANDA with the request for FDA approval prior to the expiration of the '940 Patent would constitute an act of infringement of the '940 Patent. DRL had no reasonable basis for asserting that the commercial manufacture, use, offer for sale,

sale, or importation of DRL's Infringing ANDA Products would not contribute to, or induce, the infringement of the '940 Patent.

63. DRL's Paragraph IV Statement in the Notice Letter lacks any sufficient contention that DRL's Infringing ANDA Products will not infringe, contribute to the infringement of, or induce the infringement of the '940 Patent.

64. In the Notice Letter, DRL does not allege non-infringement of one or more claims of the '940 Patent.

65. On information and belief, DRL filed ANDA No. 211756 without adequate justification for asserting the '940 Patent to be invalid, unenforceable, and/or not infringed by the commercial manufacture, use, offer for sale, sale, or importation into the United States of DRL's Infringing ANDA Products. DRL's conduct in certifying invalidity, unenforceability, and/or non-infringement with respect to the '940 Patent renders this case "exceptional" under 35 U.S.C. § 285.

66. Celgene will be irreparably harmed if DRL is not enjoined from infringing, and from actively inducing and contributing to the infringement of the '940 Patent. Celgene does not have an adequate remedy at law, and considering the balance of hardships between Celgene and DRL, a remedy in equity is warranted. Further, the public interest would not be disserved by the entry of a permanent injunction.

COUNT 2
DECLARATORY JUDGMENT OF INFRINGEMENT OF THE '940 PATENT

67. Celgene states, realleges, and incorporates by reference the foregoing paragraphs as if fully set forth herein.

68. Celgene's claims also arise under the Declaratory Judgment Act, 28 U.S.C. §§ 2201 and 2202.

69. The ’940 Patent includes claims that recite methods of administering (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

70. On information and belief, DRL’s Infringing ANDA Products contain (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

71. On information and belief, if DRL’s ANDA is approved, DRL’s Infringing ANDA Products will be made, offered for sale, sold, or otherwise distributed in the United States, including in the State of New Jersey, or will be imported into the United States, including the State of New Jersey, by or through DRL and its affiliates. DRL will therefore directly infringe one or more claims of the ’940 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a).

72. On information and belief, DRL knows that healthcare professionals or patients will use DRL’s Infringing ANDA Products in accordance with the labeling sought by DRL’s ANDA. On information and belief, the product label and product insert accompanying DRL’s Infringing ANDA Products will include instructions that are substantially similar to the instructions found in the prescribing information for OTEZLA®, attached as Exhibit A, and which, if followed, will infringe the ’940 Patent. DRL will therefore contribute to, or induce, the infringement of one or more claims of the ’940 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a), (b), and (c).

73. On information and belief, DRL’s infringing activity, including the commercial manufacture, use, offer for sale, sale, or importation of DRL’s Infringing ANDA Products complained of herein, will begin immediately after the FDA approves DRL’s ANDA. Any such conduct before the ’940 Patent expires will directly infringe, contribute to the infringement of, or

induce the infringement of one or more claims of the ’940 Patent under one or more of 35 U.S.C. § 271(a), (b), and (c).

74. As a result of the foregoing facts, there is a real, substantial, and continuing justiciable controversy between Celgene and DRL concerning liability for the infringement of the ’940 Patent for which this Court may grant declaratory relief consistent with Article III of the United States Constitution.

75. Celgene will be substantially and irreparably harmed by DRL’s infringing activities unless those activities are enjoined by this Court. Celgene has no adequate remedy at law.

76. This case is exceptional, and Celgene is entitled to an award of attorneys’ fees under 35 U.S.C. § 285.

COUNT 3
INFRINGEMENT OF THE ’638 PATENT

77. Celgene states, realleges, and incorporates by reference the foregoing paragraphs as if fully set forth herein.

78. On information and belief, DRL has submitted or caused the submission of ANDA No. 211756 to FDA and continues to seek FDA approval of ANDA No. 211756.

79. DRL has infringed the ’638 Patent under 35 U.S.C. § 271(e)(2)(A) by submitting ANDA No. 211756 with a Paragraph IV Certification and seeking FDA approval of ANDA No. 211756 prior to the expiration of the ’638 Patent.

80. The ’638 Patent includes claims that recite (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione; or a pharmaceutical composition or a single unit dosage form comprising (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

81. On information and belief, DRL's Infringing ANDA Products contain (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

82. On information and belief, DRL's Infringing ANDA Products are pharmaceutical compositions or single unit dosage forms containing (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

83. DRL's commercial manufacture, use, offer for sale, or sale within the United States, or importation into the United States of DRL's Infringing ANDA Products would directly infringe, or would actively induce or contribute to infringement of the '638 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a), (b), and (c). Accordingly, unless enjoined by this Court, upon FDA approval of ANDA No. 211756, DRL will make, use, offer for sale, or sell DRL's Infringing ANDA Products within the United States, or will import DRL's Infringing ANDA Products into the United States, and will thereby infringe, contribute to the infringement of, or induce the infringement of one or more claims of the '638 Patent. *See id.*

84. On information and belief, upon FDA approval of ANDA No. 211756, DRL, through its own actions and through the actions of its agents and subsidiaries, will market and distribute DRL's Infringing ANDA Products to resellers, pharmacies, hospitals and other clinics, healthcare professionals, and end users of DRL's Infringing ANDA Products. On information and belief, DRL will also knowingly and intentionally accompany DRL's Infringing ANDA Products with a product label and product insert that will include instructions for using or administering DRL's Infringing ANDA Products. On information and belief, the product label and product insert accompanying DRL's Infringing ANDA Products will include instructions that are substantially similar to the instructions found in the prescribing information for OTEZLA®, attached as Exhibit A, and which, if followed, will infringe the '638 Patent.

Accordingly, DRL will induce physicians and other healthcare professionals, resellers, pharmacies, and end users of DRL's Infringing ANDA Products to directly infringe one or more claims of the '638 Patent. In addition, on information and belief, DRL will encourage acts of direct infringement with knowledge of the '638 Patent and knowledge that it is encouraging infringement.

85. DRL had actual and constructive notice of the '638 Patent prior to filing DRL's ANDA and was aware that the filing of DRL's ANDA with the request for FDA approval prior to the expiration of the '638 Patent would constitute an act of infringement of the '638 Patent. DRL had no reasonable basis for asserting that the commercial manufacture, use, offer for sale, sale, or importation of DRL's Infringing ANDA Products would not contribute to, or induce, the infringement of the '638 Patent.

86. DRL's Paragraph IV Statement in the Notice Letter lacks any sufficient contention that DRL's Infringing ANDA Products will not infringe, contribute to the infringement of, or induce the infringement of the '638 Patent.

87. In the Notice Letter, DRL does not allege non-infringement of one or more claims of the '638 Patent.

88. On information and belief, DRL filed ANDA No. 211756 without adequate justification for asserting the '638 Patent to be invalid, unenforceable, and/or not infringed by the commercial manufacture, use, offer for sale, sale, or importation into the United States of DRL's Infringing ANDA Products. DRL's conduct in certifying invalidity, unenforceability, and/or non-infringement with respect to the '638 Patent renders this case "exceptional" under 35 U.S.C. § 285.

89. Celgene will be irreparably harmed if DRL is not enjoined from infringing, and from actively inducing and contributing to the infringement of the ’638 Patent. Celgene does not have an adequate remedy at law, and considering the balance of hardships between Celgene and DRL, a remedy in equity is warranted. Further, the public interest would not be disserved by the entry of a permanent injunction.

COUNT 4
DECLARATORY JUDGMENT OF INFRINGEMENT OF THE ’638 PATENT

90. Celgene states, realleges, and incorporates by reference the foregoing paragraphs as if fully set forth herein.

91. Celgene’s claims also arise under the Declaratory Judgment Act, 28 U.S.C. §§ 2201 and 2202.

92. The ’638 Patent includes claims that recite (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione; or a pharmaceutical composition or a single unit dosage form comprising (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

93. On information and belief, DRL’s Infringing ANDA Products contain (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

94. On information and belief, DRL’s Infringing ANDA Products are pharmaceutical compositions or single unit dosage forms containing (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

95. On information and belief, if DRL’s ANDA is approved, DRL’s Infringing ANDA Products will be made, offered for sale, sold, or otherwise distributed in the United States, including in the State of New Jersey, or will be imported into the United States, including the State of New Jersey, by or through DRL and its affiliates. DRL will therefore directly

infringe one or more claims of the '638 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a).

96. On information and belief, DRL knows that healthcare professionals or patients will use DRL's Infringing ANDA Products in accordance with the labeling sought by DRL's ANDA. On information and belief, the product label and product insert accompanying DRL's Infringing ANDA Products will include instructions that are substantially similar to the instructions found in the prescribing information for OTEZLA®, attached as Exhibit A, and which, if followed, will infringe the '638 Patent. DRL will therefore contribute to, or induce, the infringement of one or more claims of the '638 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a), (b), and (c).

97. On information and belief, DRL's infringing activity, including the commercial manufacture, use, offer for sale, sale, or importation of DRL's Infringing ANDA Products complained of herein, will begin immediately after the FDA approves DRL's ANDA. Any such conduct before the '638 Patent expires will directly infringe, contribute to the infringement of, or induce the infringement of one or more claims of the '638 Patent under one or more of 35 U.S.C. § 271(a), (b), and (c).

98. As a result of the foregoing facts, there is a real, substantial, and continuing justiciable controversy between Celgene and DRL concerning liability for the infringement of the '638 Patent for which this Court may grant declaratory relief consistent with Article III of the United States Constitution.

99. Celgene will be substantially and irreparably harmed by DRL's infringing activities unless those activities are enjoined by this Court. Celgene has no adequate remedy at law.

100. This case is exceptional, and Celgene is entitled to an award of attorneys' fees under 35 U.S.C. § 285.

COUNT 5
INFRINGEMENT OF THE '302 PATENT

101. Celgene states, realleges, and incorporates by reference the foregoing paragraphs as if fully set forth herein.

102. On information and belief, DRL has submitted or caused the submission of ANDA No. 211756 to FDA and continues to seek FDA approval of ANDA No. 211756.

103. DRL has infringed the '302 Patent under 35 U.S.C. § 271(e)(2)(A) by submitting ANDA No. 211756 with a Paragraph IV Certification and seeking FDA approval of ANDA No. 211756 prior to the expiration of the '302 Patent.

104. The '302 Patent includes claims that recite methods of administering (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

105. On information and belief, DRL's Infringing ANDA Products contain (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

106. DRL's commercial manufacture, use, offer for sale, or sale within the United States, or importation into the United States of DRL's Infringing ANDA Products would directly infringe, or would actively induce or contribute to infringement of the '302 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a), (b), and (c). Accordingly, unless enjoined by this Court, upon FDA approval of ANDA No. 211756, DRL will make, use, offer for sale, or sell DRL's Infringing ANDA Products within the United States, or will import DRL's Infringing ANDA Products into the United States, and will thereby infringe, contribute to the infringement of, or induce the infringement of one or more claims of the '302 Patent. *See id.*

107. On information and belief, upon FDA approval of ANDA No. 211756, DRL, through its own actions and through the actions of its agents and subsidiaries, will market and distribute DRL's Infringing ANDA Products to resellers, pharmacies, hospitals and other clinics, healthcare professionals, and end users of DRL's Infringing ANDA Products. On information and belief, DRL will also knowingly and intentionally accompany DRL's Infringing ANDA Products with a product label and product insert that will include instructions for using or administering DRL's Infringing ANDA Products. On information and belief, the product label and product insert accompanying DRL's Infringing ANDA Products will include instructions that are substantially similar to the instructions found in the prescribing information for OTEZLA®, attached as Exhibit A, and which, if followed, will infringe the '302 Patent. Accordingly, DRL will induce physicians and other healthcare professionals, resellers, pharmacies, and end users of DRL's Infringing ANDA Products to directly infringe one or more claims of the '302 Patent. In addition, on information and belief, DRL will encourage acts of direct infringement with knowledge of the '302 Patent and knowledge that it is encouraging infringement.

108. DRL had actual and constructive notice of the '302 Patent prior to filing DRL's ANDA and was aware that the filing of DRL's ANDA with the request for FDA approval prior to the expiration of the '302 Patent would constitute an act of infringement of the '302 Patent. DRL had no reasonable basis for asserting that the commercial manufacture, use, offer for sale, sale, or importation of DRL's Infringing ANDA Products would not contribute to, or induce, the infringement of the '302 Patent.

109. DRL's Paragraph IV Statement in the Notice Letter lacks any sufficient contention that DRL's Infringing ANDA Products will not infringe, contribute to the infringement of, or induce the infringement of the '302 Patent.

110. In the Notice Letter, DRL does not allege non-infringement of one or more claims of the '302 Patent.

111. On information and belief, DRL filed ANDA No. 211756 without adequate justification for asserting the '302 Patent to be invalid, unenforceable, and/or not infringed by the commercial manufacture, use, offer for sale, sale, or importation into the United States of DRL's Infringing ANDA Products. DRL's conduct in certifying invalidity, unenforceability, and/or non-infringement with respect to the '302 Patent renders this case "exceptional" under 35 U.S.C. § 285.

112. Celgene will be irreparably harmed if DRL is not enjoined from infringing, and from actively inducing and contributing to the infringement of the '302 Patent. Celgene does not have an adequate remedy at law, and considering the balance of hardships between Celgene and DRL, a remedy in equity is warranted. Further, the public interest would not be disserved by the entry of a permanent injunction.

COUNT 6
DECLARATORY JUDGMENT OF INFRINGEMENT OF THE '302 PATENT

113. Celgene states, realleges, and incorporates by reference the foregoing paragraphs as if fully set forth herein.

114. Celgene's claims also arise under the Declaratory Judgment Act, 28 U.S.C. §§ 2201 and 2202.

115. The '302 Patent includes claims that recite methods of administering (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

116. On information and belief, DRL's Infringing ANDA Products contain (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

117. On information and belief, if DRL's ANDA is approved, DRL's Infringing ANDA Products will be made, offered for sale, sold, or otherwise distributed in the United States, including in the State of New Jersey, or will be imported into the United States, including the State of New Jersey, by or through DRL and its affiliates. DRL will therefore directly infringe one or more claims of the '302 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a).

118. On information and belief, DRL knows that healthcare professionals or patients will use DRL's Infringing ANDA Products in accordance with the labeling sought by DRL's ANDA. On information and belief, the product label and product insert accompanying DRL's Infringing ANDA Products will include instructions that are substantially similar to the instructions found in the prescribing information for OTEZLA®, attached as Exhibit A, and which, if followed, will infringe the '302 Patent. DRL will therefore contribute to, or induce, the infringement of one or more claims of the '302 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a), (b), and (c).

119. On information and belief, DRL's infringing activity, including the commercial manufacture, use, offer for sale, sale, or importation of DRL's Infringing ANDA Products complained of herein, will begin immediately after the FDA approves DRL's ANDA. Any such conduct before the '302 Patent expires will directly infringe, contribute to the infringement of, or induce the infringement of one or more claims of the '302 Patent under one or more of 35 U.S.C. § 271(a), (b), and (c).

120. As a result of the foregoing facts, there is a real, substantial, and continuing justiciable controversy between Celgene and DRL concerning liability for the infringement of the ’302 Patent for which this Court may grant declaratory relief consistent with Article III of the United States Constitution.

121. Celgene will be substantially and irreparably harmed by DRL’s infringing activities unless those activities are enjoined by this Court. Celgene has no adequate remedy at law.

122. This case is exceptional, and Celgene is entitled to an award of attorneys’ fees under 35 U.S.C. § 285.

COUNT 7
INFRINGEMENT OF THE ’101 PATENT

123. Celgene states, realleges, and incorporates by reference the foregoing paragraphs as if fully set forth herein.

124. On information and belief, DRL has submitted or caused the submission of ANDA No. 211756 to FDA and continues to seek FDA approval of ANDA No. 211756.

125. DRL has infringed the ’101 Patent under 35 U.S.C. § 271(e)(2)(A) by submitting ANDA No. 211756 with a Paragraph IV Certification and seeking FDA approval of ANDA No. 211756 prior to the expiration of the ’101 Patent.

126. The ’101 Patent includes claims that recite a form of (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, wherein the form has an X-ray powder diffraction pattern comprising certain specified peaks.

127. On information and belief, DRL’s Infringing ANDA Products contain a form of (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, wherein the form has an X-ray powder diffraction pattern comprising the specified peaks.

128. DRL's commercial manufacture, use, offer for sale, or sale within the United States, or importation into the United States of DRL's Infringing ANDA Products would directly infringe, or would actively induce or contribute to infringement of the '101 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a), (b), and (c). Accordingly, unless enjoined by this Court, upon FDA approval of ANDA No. 211756, DRL will make, use, offer for sale, or sell DRL's Infringing ANDA Products within the United States, or will import DRL's Infringing ANDA Products into the United States, and will thereby infringe, contribute to the infringement of, or induce the infringement of one or more claims of the '101 Patent. *See id.*

129. On information and belief, upon FDA approval of ANDA No. 211756, DRL, through its own actions and through the actions of its agents and subsidiaries, will market and distribute DRL's Infringing ANDA Products to resellers, pharmacies, hospitals and other clinics, healthcare professionals, and end users of DRL's Infringing ANDA Products. On information and belief, DRL will also knowingly and intentionally accompany DRL's Infringing ANDA Products with a product label and product insert that will include instructions for using or administering DRL's Infringing ANDA Products. On information and belief, the product label and product insert accompanying DRL's Infringing ANDA Products will include instructions that are substantially similar to the instructions found in the prescribing information for OTEZLA®, attached as Exhibit A, and which, if followed, will infringe the '101 Patent. Accordingly, DRL will induce physicians and other healthcare professionals, resellers, pharmacies, and end users of DRL's Infringing ANDA Products to directly infringe one or more claims of the '101 Patent. In addition, on information and belief, DRL will encourage acts of direct infringement with knowledge of the '101 Patent and knowledge that it is encouraging infringement.

130. DRL had actual and constructive notice of the ’101 Patent prior to filing DRL’s ANDA and was aware that the filing of DRL’s ANDA with the request for FDA approval prior to the expiration of the ’101 Patent would constitute an act of infringement of the ’101 Patent. DRL had no reasonable basis for asserting that the commercial manufacture, use, offer for sale, sale, or importation of DRL’s Infringing ANDA Products would not contribute to, or induce, the infringement of the ’101 Patent.

131. DRL’s Paragraph IV Statement in the Notice Letter lacks any sufficient contention that DRL’s Infringing ANDA Products will not infringe, contribute to the infringement of, or induce the infringement of the ’101 Patent.

132. In the Notice Letter, DRL does not allege non-infringement of each of the claims of the ’101 Patent.

133. On information and belief, DRL filed ANDA No. 211756 without adequate justification for asserting the ’101 Patent to be invalid, unenforceable, and/or not infringed by the commercial manufacture, use, offer for sale, sale, or importation into the United States of DRL’s Infringing ANDA Products. DRL’s conduct in certifying invalidity, unenforceability, and/or non-infringement with respect to the ’101 Patent renders this case “exceptional” under 35 U.S.C. § 285.

134. Celgene will be irreparably harmed if DRL is not enjoined from infringing, and from actively inducing and contributing to the infringement of the ’101 Patent. Celgene does not have an adequate remedy at law, and considering the balance of hardships between Celgene and DRL, a remedy in equity is warranted. Further, the public interest would not be disserved by the entry of a permanent injunction.

COUNT 8
DECLARATORY JUDGMENT OF INFRINGEMENT OF THE '101 PATENT

135. Celgene states, realleges, and incorporates by reference the foregoing paragraphs as if fully set forth herein.

136. Celgene's claims also arise under the Declaratory Judgment Act, 28 U.S.C. §§ 2201 and 2202.

137. The '101 Patent includes claims that recite a form of (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, wherein the form has an X-ray powder diffraction pattern comprising certain specified peaks.

138. On information and belief, DRL's Infringing ANDA Products contain a form of (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, wherein the form has an X-ray powder diffraction pattern comprising the specified peaks.

139. On information and belief, if DRL's ANDA is approved, DRL's Infringing ANDA Products will be made, offered for sale, sold, or otherwise distributed in the United States, including in the State of New Jersey, or will be imported into the United States, including the State of New Jersey, by or through DRL and its affiliates. DRL will therefore directly infringe one or more claims of the '101 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a).

140. On information and belief, DRL knows that healthcare professionals or patients will use DRL's Infringing ANDA Products in accordance with the labeling sought by DRL's ANDA. On information and belief, the product label and product insert accompanying DRL's Infringing ANDA Products will include instructions that are substantially similar to the instructions found in the prescribing information for OTEZLA®, attached as Exhibit A, and which, if followed, will infringe the '101 Patent. DRL will therefore contribute to, or induce, the

infringement of one or more claims of the ’101 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a), (b), and (c).

141. On information and belief, DRL’s infringing activity, including the commercial manufacture, use, offer for sale, sale, or importation of DRL’s Infringing ANDA Products complained of herein, will begin immediately after the FDA approves DRL’s ANDA. Any such conduct before the ’101 Patent expires will directly infringe, contribute to the infringement of, or induce the infringement of one or more claims of the ’101 Patent under one or more of 35 U.S.C. § 271(a), (b), and (c).

142. As a result of the foregoing facts, there is a real, substantial, and continuing justiciable controversy between Celgene and DRL concerning liability for the infringement of the ’101 Patent for which this Court may grant declaratory relief consistent with Article III of the United States Constitution.

143. Celgene will be substantially and irreparably harmed by DRL’s infringing activities unless those activities are enjoined by this Court. Celgene has no adequate remedy at law.

144. This case is exceptional, and Celgene is entitled to an award of attorneys’ fees under 35 U.S.C. § 285.

COUNT 9
INFRINGEMENT OF THE ’536 PATENT

145. Celgene states, realleges, and incorporates by reference the foregoing paragraphs as if fully set forth herein.

146. On information and belief, DRL has submitted or caused the submission of ANDA No. 211756 to FDA and continues to seek FDA approval of ANDA No. 211756.

147. DRL has infringed the ’536 Patent under 35 U.S.C. § 271(e)(2)(A) by submitting ANDA No. 211756 with a Paragraph IV Certification and seeking FDA approval of ANDA No. 211756 prior to the expiration of the ’536 Patent.

148. The ’536 Patent includes claims that recite methods of administering (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

149. On information and belief, DRL’s Infringing ANDA Products contain (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

150. DRL’s commercial manufacture, use, offer for sale, or sale within the United States, or importation into the United States of DRL’s Infringing ANDA Products would directly infringe, or would actively induce or contribute to infringement of the ’536 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a), (b), and (c). Accordingly, unless enjoined by this Court, upon FDA approval of ANDA No. 211756, DRL will make, use, offer for sale, or sell DRL’s Infringing ANDA Products within the United States, or will import DRL’s Infringing ANDA Products into the United States, and will thereby infringe, contribute to the infringement of, or induce the infringement of one or more claims of the ’536 Patent. *See id.*

151. On information and belief, upon FDA approval of ANDA No. 211756, DRL, through its own actions and through the actions of its agents and subsidiaries, will market and distribute DRL’s Infringing ANDA Products to resellers, pharmacies, hospitals and other clinics, healthcare professionals, and end users of DRL’s Infringing ANDA Products. On information and belief, DRL will also knowingly and intentionally accompany DRL’s Infringing ANDA Products with a product label and product insert that will include instructions for using or administering DRL’s Infringing ANDA Products. On information and belief, the product label and product insert accompanying DRL’s Infringing ANDA Products will include instructions

that are substantially similar to the instructions found in the prescribing information for OTEZLA®, attached as Exhibit A, and which, if followed, will infringe the '536 Patent. Accordingly, DRL will induce physicians and other healthcare professionals, resellers, pharmacies, and end users of DRL's Infringing ANDA Products to directly infringe one or more claims of the '536 Patent. In addition, on information and belief, DRL will encourage acts of direct infringement with knowledge of the '536 Patent and knowledge that it is encouraging infringement.

152. DRL had actual and constructive notice of the '536 Patent prior to filing DRL's ANDA and was aware that the filing of DRL's ANDA with the request for FDA approval prior to the expiration of the '536 Patent would constitute an act of infringement of the '536 Patent. DRL had no reasonable basis for asserting that the commercial manufacture, use, offer for sale, sale, or importation of DRL's Infringing ANDA Products would not contribute to, or induce, the infringement of the '536 Patent.

153. DRL's Paragraph IV Statement in the Notice Letter lacks any sufficient contention that DRL's Infringing ANDA Products will not infringe, contribute to the infringement of, or induce the infringement of the '536 Patent.

154. In the Notice Letter, DRL does not allege non-infringement of one or more claims of the '536 Patent.

155. On information and belief, DRL filed ANDA No. 211756 without adequate justification for asserting the '536 Patent to be invalid, unenforceable, and/or not infringed by the commercial manufacture, use, offer for sale, sale, or importation into the United States of DRL's Infringing ANDA Products. DRL's conduct in certifying invalidity, unenforceability, and/or

non-infringement with respect to the ’536 Patent renders this case “exceptional” under 35 U.S.C. § 285.

156. Celgene will be irreparably harmed if DRL is not enjoined from infringing, and from actively inducing and contributing to the infringement of the ’536 Patent. Celgene does not have an adequate remedy at law, and considering the balance of hardships between Celgene and DRL, a remedy in equity is warranted. Further, the public interest would not be disserved by the entry of a permanent injunction.

COUNT 10
DECLARATORY JUDGMENT OF INFRINGEMENT OF THE ’536 PATENT

157. Celgene states, realleges, and incorporates by reference the foregoing paragraphs as if fully set forth herein.

158. Celgene’s claims also arise under the Declaratory Judgment Act, 28 U.S.C. §§ 2201 and 2202.

159. The ’536 Patent includes claims that recite methods of administering (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

160. On information and belief, DRL’s Infringing ANDA Products contain (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

161. On information and belief, if DRL’s ANDA is approved, DRL’s Infringing ANDA Products will be made, offered for sale, sold, or otherwise distributed in the United States, including in the State of New Jersey, or will be imported into the United States, including the State of New Jersey, by or through DRL and its affiliates. DRL will therefore directly infringe one or more claims of the ’536 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a).

162. On information and belief, DRL knows that healthcare professionals or patients will use DRL's Infringing ANDA Products in accordance with the labeling sought by DRL's ANDA. On information and belief, the product label and product insert accompanying DRL's Infringing ANDA Products will include instructions that are substantially similar to the instructions found in the prescribing information for OTEZLA®, attached as Exhibit A, and which, if followed, will infringe the '536 Patent. DRL will therefore contribute to, or induce, the infringement of one or more claims of the '536 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a), (b), and (c).

163. On information and belief, DRL's infringing activity, including the commercial manufacture, use, offer for sale, sale, or importation of DRL's Infringing ANDA Products complained of herein, will begin immediately after the FDA approves DRL's ANDA. Any such conduct before the '536 Patent expires will directly infringe, contribute to the infringement of, or induce the infringement of one or more claims of the '536 Patent under one or more of 35 U.S.C. § 271(a), (b), and (c).

164. As a result of the foregoing facts, there is a real, substantial, and continuing justiciable controversy between Celgene and DRL concerning liability for the infringement of the '536 Patent for which this Court may grant declaratory relief consistent with Article III of the United States Constitution.

165. Celgene will be substantially and irreparably harmed by DRL's infringing activities unless those activities are enjoined by this Court. Celgene has no adequate remedy at law.

166. This case is exceptional, and Celgene is entitled to an award of attorneys' fees under 35 U.S.C. § 285.

COUNT 11
INFRINGEMENT OF THE '243 PATENT

167. Celgene states, realleges, and incorporates by reference the foregoing paragraphs as if fully set forth herein.

168. On information and belief, DRL has submitted or caused the submission of ANDA No. 211756 to FDA and continues to seek FDA approval of ANDA No. 211756.

169. DRL has infringed the '243 Patent under 35 U.S.C. § 271(e)(2)(A) by submitting ANDA No. 211756 with a Paragraph IV Certification and seeking FDA approval of ANDA No. 211756 prior to the expiration of the '243 Patent.

170. The '243 Patent includes claims that recite methods of administering a form of (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, wherein the form has an X-ray powder diffraction pattern comprising certain specified peaks.

171. On information and belief, DRL's Infringing ANDA Products contain a form of (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, wherein the form has an X-ray powder diffraction pattern comprising the specified peaks.

172. DRL's commercial manufacture, use, offer for sale, or sale within the United States, or importation into the United States of DRL's Infringing ANDA Products would directly infringe, or would actively induce or contribute to infringement of the '243 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a), (b), and (c). Accordingly, unless enjoined by this Court, upon FDA approval of ANDA No. 211756, DRL will make, use, offer for sale, or sell DRL's Infringing ANDA Products within the United States, or will import DRL's Infringing ANDA Products into the United States, and will thereby infringe, contribute to the infringement of, or induce the infringement of one or more claims of the '243 Patent. *See id.*

173. On information and belief, upon FDA approval of ANDA No. 211756, DRL, through its own actions and through the actions of its agents and subsidiaries, will market and distribute DRL's Infringing ANDA Products to resellers, pharmacies, hospitals and other clinics, healthcare professionals, and end users of DRL's Infringing ANDA Products. On information and belief, DRL will also knowingly and intentionally accompany DRL's Infringing ANDA Products with a product label and product insert that will include instructions for using or administering DRL's Infringing ANDA Products. On information and belief, the product label and product insert accompanying DRL's Infringing ANDA Products will include instructions that are substantially similar to the instructions found in the prescribing information for OTEZLA®, attached as Exhibit A, and which, if followed, will infringe the '243 Patent. Accordingly, DRL will induce physicians and other healthcare professionals, resellers, pharmacies, and end users of DRL's Infringing ANDA Products to directly infringe one or more claims of the '243 Patent. In addition, on information and belief, DRL will encourage acts of direct infringement with knowledge of the '243 Patent and knowledge that it is encouraging infringement.

174. DRL had actual and constructive notice of the '243 Patent prior to filing DRL's ANDA and was aware that the filing of DRL's ANDA with the request for FDA approval prior to the expiration of the '243 Patent would constitute an act of infringement of the '243 Patent. DRL had no reasonable basis for asserting that the commercial manufacture, use, offer for sale, sale, or importation of DRL's Infringing ANDA Products would not contribute to, or induce, the infringement of the '243 Patent.

175. DRL's Paragraph IV Statement in the Notice Letter lacks any sufficient contention that DRL's Infringing ANDA Products will not infringe, contribute to the infringement of, or induce the infringement of the '243 Patent.

176. In the Notice Letter, DRL does not allege non-infringement of one or more claims of the '243 Patent.

177. On information and belief, DRL filed ANDA No. 211756 without adequate justification for asserting the '243 Patent to be invalid, unenforceable, and/or not infringed by the commercial manufacture, use, offer for sale, sale, or importation into the United States of DRL's Infringing ANDA Products. DRL's conduct in certifying invalidity, unenforceability, and/or non-infringement with respect to the '243 Patent renders this case "exceptional" under 35 U.S.C. § 285.

178. Celgene will be irreparably harmed if DRL is not enjoined from infringing, and from actively inducing and contributing to the infringement of the '243 Patent. Celgene does not have an adequate remedy at law, and considering the balance of hardships between Celgene and DRL, a remedy in equity is warranted. Further, the public interest would not be disserved by the entry of a permanent injunction.

COUNT 12
DECLARATORY JUDGMENT OF INFRINGEMENT OF THE '243 PATENT

179. Celgene states, realleges, and incorporates by reference the foregoing paragraphs as if fully set forth herein.

180. Celgene's claims also arise under the Declaratory Judgment Act, 28 U.S.C. §§ 2201 and 2202.

181. The '243 Patent includes claims that recite methods of administering a form of (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-

dione, wherein the form has an X-ray powder diffraction pattern comprising certain specified peaks.

182. On information and belief, DRL's Infringing ANDA Products contain a form of (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, wherein the form has an X-ray powder diffraction pattern comprising the specified peaks.

183. On information and belief, if DRL's ANDA is approved, DRL's Infringing ANDA Products will be made, offered for sale, sold, or otherwise distributed in the United States, including in the State of New Jersey, or will be imported into the United States, including the State of New Jersey, by or through DRL and its affiliates. DRL will therefore directly infringe one or more claims of the '243 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a).

184. On information and belief, DRL knows that healthcare professionals or patients will use DRL's Infringing ANDA Products in accordance with the labeling sought by DRL's ANDA. On information and belief, the product label and product insert accompanying DRL's Infringing ANDA Products will include instructions that are substantially similar to the instructions found in the prescribing information for OTEZLA®, attached as Exhibit A, and which, if followed, will infringe the '243 Patent. DRL will therefore contribute to, or induce, the infringement of one or more claims of the '243 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a), (b), and (c).

185. On information and belief, DRL's infringing activity, including the commercial manufacture, use, offer for sale, sale, or importation of DRL's Infringing ANDA Products complained of herein, will begin immediately after the FDA approves DRL's ANDA. Any such conduct before the '243 Patent expires will directly infringe, contribute to the infringement of, or

induce the infringement of one or more claims of the ’243 Patent under one or more of 35 U.S.C. § 271(a), (b), and (c).

186. As a result of the foregoing facts, there is a real, substantial, and continuing justiciable controversy between Celgene and DRL concerning liability for the infringement of the ’243 Patent for which this Court may grant declaratory relief consistent with Article III of the United States Constitution.

187. Celgene will be substantially and irreparably harmed by DRL’s infringing activities unless those activities are enjoined by this Court. Celgene has no adequate remedy at law.

188. This case is exceptional, and Celgene is entitled to an award of attorneys’ fees under 35 U.S.C. § 285.

COUNT 13
INFRINGEMENT OF THE ’330 PATENT

189. Celgene states, realleges, and incorporates by reference the foregoing paragraphs as if fully set forth herein.

190. On information and belief, DRL has submitted or caused the submission of ANDA No. 211756 to FDA and continues to seek FDA approval of ANDA No. 211756.

191. DRL has infringed the ’330 Patent under 35 U.S.C. § 271(e)(2)(A) by submitting ANDA No. 211756 and seeking FDA approval of ANDA No. 211756 prior to the expiration of the ’330 Patent.

192. The ’330 Patent includes claims that recite methods of administering (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

193. On information and belief, DRL’s Infringing ANDA Products contain (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

194. DRL's commercial manufacture, use, offer for sale, or sale within the United States, or importation into the United States of DRL's Infringing ANDA Products would directly infringe, or would actively induce or contribute to infringement of the '330 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a), (b), and (c). Accordingly, unless enjoined by this Court, upon FDA approval of ANDA No. 211756, DRL will make, use, offer for sale, or sell DRL's Infringing ANDA Products within the United States, or will import DRL's Infringing ANDA Products into the United States, and will thereby infringe, contribute to the infringement of, or induce the infringement of one or more claims of the '330 Patent. *See id.*

195. On information and belief, upon FDA approval of ANDA No. 211756, DRL, through its own actions and through the actions of its agents and subsidiaries, will market and distribute DRL's Infringing ANDA Products to resellers, pharmacies, hospitals and other clinics, healthcare professionals, and end users of DRL's Infringing ANDA Products. On information and belief, DRL will also knowingly and intentionally accompany DRL's Infringing ANDA Products with a product label and product insert that will include instructions for using or administering DRL's Infringing ANDA Products. On information and belief, the product label and product insert accompanying DRL's Infringing ANDA Products will include instructions that are substantially similar to the instructions found in the prescribing information for OTEZLA®, attached as Exhibit A, and which, if followed, will infringe the '330 Patent. Accordingly, DRL will induce physicians and other healthcare professionals, resellers, pharmacies, and end users of DRL's Infringing ANDA Products to directly infringe one or more claims of the '330 Patent. In addition, on information and belief, DRL will encourage acts of direct infringement with knowledge of the '330 Patent and knowledge that it is encouraging infringement.

196. On information and belief, DRL had actual and constructive notice of the '330 Patent prior to filing DRL's ANDA and was aware that the filing of DRL's ANDA with the request for FDA approval prior to the expiration of the '330 Patent would constitute an act of infringement of the '330 Patent.

197. This case is exceptional, and Celgene is entitled to an award of attorneys' fees under 35 U.S.C. § 285.

198. Celgene will be irreparably harmed if DRL is not enjoined from infringing, and from actively inducing and contributing to the infringement of the '330 Patent. Celgene does not have an adequate remedy at law, and considering the balance of hardships between Celgene and DRL, a remedy in equity is warranted. Further, the public interest would not be disserved by the entry of a permanent injunction.

COUNT 14
DECLARATORY JUDGMENT OF INFRINGEMENT OF THE '330 PATENT

199. Celgene states, realleges, and incorporates by reference the foregoing paragraphs as if fully set forth herein.

200. Celgene's claims also arise under the Declaratory Judgment Act, 28 U.S.C. §§ 2201 and 2202.

201. The '330 Patent includes claims that recite methods of administering (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

202. On information and belief, DRL's Infringing ANDA Products contain (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

203. On information and belief, if DRL's ANDA is approved, DRL's Infringing ANDA Products will be made, offered for sale, sold, or otherwise distributed in the United States, including in the State of New Jersey, or will be imported into the United States, including

the State of New Jersey, by or through DRL and its affiliates. DRL will therefore directly infringe one or more claims of the '330 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a).

204. On information and belief, DRL knows that healthcare professionals or patients will use DRL's Infringing ANDA Products in accordance with the labeling sought by DRL's ANDA. On information and belief, the product label and product insert accompanying DRL's Infringing ANDA Products will include instructions that are substantially similar to the instructions found in the prescribing information for OTEZLA®, attached as Exhibit A, and which, if followed, will infringe the '330 Patent. DRL will therefore contribute to, or induce, the infringement of one or more claims of the '330 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a), (b), and (c).

205. On information and belief, DRL's infringing activity, including the commercial manufacture, use, offer for sale, sale, or importation of DRL's Infringing ANDA Products complained of herein, will begin immediately after the FDA approves DRL's ANDA. Any such conduct before the '330 Patent expires will directly infringe, contribute to the infringement of, or induce the infringement of one or more claims of the '330 Patent under one or more of 35 U.S.C. § 271(a), (b), and (c).

206. As a result of the foregoing facts, there is a real, substantial, and continuing justiciable controversy between Celgene and DRL concerning liability for the infringement of the '330 Patent for which this Court may grant declaratory relief consistent with Article III of the United States Constitution.

207. Celgene will be substantially and irreparably harmed by DRL's infringing activities unless those activities are enjoined by this Court. Celgene has no adequate remedy at law.

208. This case is exceptional, and Celgene is entitled to an award of attorneys' fees under 35 U.S.C. § 285.

COUNT 15
INFRINGEMENT OF THE '541 PATENT

209. Celgene states, realleges, and incorporates by reference the foregoing paragraphs as if fully set forth herein.

210. On information and belief, DRL has submitted or caused the submission of ANDA No. 211756 to FDA and continues to seek FDA approval of ANDA No. 211756.

211. DRL has infringed the '541 Patent under 35 U.S.C. § 271(e)(2)(A) by submitting ANDA No. 211756 with a Paragraph IV Certification and seeking FDA approval of ANDA No. 211756 prior to the expiration of the '541 Patent.

212. The '541 Patent includes claims that recite methods of administering (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

213. On information and belief, DRL's Infringing ANDA Products contain (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

214. DRL's commercial manufacture, use, offer for sale, or sale within the United States, or importation into the United States of DRL's Infringing ANDA Products would directly infringe, or would actively induce or contribute to infringement of the '541 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a), (b), and (c). Accordingly, unless enjoined by this Court, upon FDA approval of ANDA No. 211756, DRL will make, use, offer for sale, or sell DRL's Infringing ANDA Products within the United States, or will import

DRL's Infringing ANDA Products into the United States, and will thereby infringe, contribute to the infringement of, or induce the infringement of one or more claims of the '541 Patent. *See id.*

215. On information and belief, upon FDA approval of ANDA No. 211756, DRL, through its own actions and through the actions of its agents and subsidiaries, will market and distribute DRL's Infringing ANDA Products to resellers, pharmacies, hospitals and other clinics, healthcare professionals, and end users of DRL's Infringing ANDA Products. On information and belief, DRL will also knowingly and intentionally accompany DRL's Infringing ANDA Products with a product label and product insert that will include instructions for using or administering DRL's Infringing ANDA Products. On information and belief, the product label and product insert accompanying DRL's Infringing ANDA Products will include instructions that are substantially similar to the instructions found in the prescribing information for OTEZLA®, attached as Exhibit A, and which, if followed, will infringe the '541 Patent. Accordingly, DRL will induce physicians and other healthcare professionals, resellers, pharmacies, and end users of DRL's Infringing ANDA Products to directly infringe one or more claims of the '541 Patent. In addition, on information and belief, DRL will encourage acts of direct infringement with knowledge of the '541 Patent and knowledge that it is encouraging infringement.

216. This case is exceptional, and Celgene is entitled to an award of attorneys' fees under 35 U.S.C. § 285.

217. Celgene will be irreparably harmed if DRL is not enjoined from infringing, and from actively inducing and contributing to the infringement of the '541 Patent. Celgene does not have an adequate remedy at law, and considering the balance of hardships between Celgene and

DRL, a remedy in equity is warranted. Further, the public interest would not be disserved by the entry of a permanent injunction.

COUNT 16
DECLARATORY JUDGMENT OF INFRINGEMENT OF THE '541 PATENT

218. Celgene states, realleges, and incorporates by reference the foregoing paragraphs as if fully set forth herein.

219. Celgene's claims also arise under the Declaratory Judgment Act, 28 U.S.C. §§ 2201 and 2202.

220. The '541 Patent includes claims that recite methods of administering (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

221. On information and belief, DRL's Infringing ANDA Products contain (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

222. On information and belief, if DRL's ANDA is approved, DRL's Infringing ANDA Products will be made, offered for sale, sold, or otherwise distributed in the United States, including in the State of New Jersey, or will be imported into the United States, including the State of New Jersey, by or through DRL and its affiliates. DRL will therefore directly infringe one or more claims of the '541 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a).

223. On information and belief, DRL knows that healthcare professionals or patients will use DRL's Infringing ANDA Products in accordance with the labeling sought by DRL's ANDA. On information and belief, the product label and product insert accompanying DRL's Infringing ANDA Products will include instructions that are substantially similar to the instructions found in the prescribing information for OTEZLA®, attached as Exhibit A, and which, if followed, will infringe the '541 Patent. DRL will therefore contribute to, or induce, the

infringement of one or more claims of the '541 Patent either literally or under the doctrine of equivalents. *See* 35 U.S.C. § 271(a), (b), and (c).

224. On information and belief, DRL's infringing activity, including the commercial manufacture, use, offer for sale, sale, or importation of DRL's Infringing ANDA Products complained of herein, will begin immediately after the FDA approves DRL's ANDA. Any such conduct before the '541 Patent expires will directly infringe, contribute to the infringement of, or induce the infringement of one or more claims of the '541 Patent under one or more of 35 U.S.C. § 271(a), (b), and (c).

225. As a result of the foregoing facts, there is a real, substantial, and continuing justiciable controversy between Celgene and DRL concerning liability for the infringement of the '541 Patent for which this Court may grant declaratory relief consistent with Article III of the United States Constitution.

226. Celgene will be substantially and irreparably harmed by DRL's infringing activities unless those activities are enjoined by this Court. Celgene has no adequate remedy at law.

227. This case is exceptional, and Celgene is entitled to an award of attorneys' fees under 35 U.S.C. § 285.

REQUEST FOR RELIEF

WHEREFORE, Celgene respectfully requests the following relief:

(a) The entry of a judgment, in favor of Celgene and against DRL, that DRL's submission of ANDA No. 211756 to the FDA seeking approval for the commercial manufacture, use, offer for sale, or sale in the United States, or importation into the United States, of DRL's Infringing ANDA Products before the expiration of the Patents-in-Suit was an act of infringement of one or more claims of the Patents-in-Suit under 35 U.S.C. § 271(e)(2)(A);

(b) The entry of a declaratory judgment, in favor of Celgene and against DRL, declaring that DRL's commercial manufacture, use, offer for sale, or sale in the United States, or importation into the United States, of DRL's Infringing ANDA Products, or inducing or contributing to such conduct, would constitute infringement of one or more claims of the Patents-in-Suit by DRL under one or more of 35 U.S.C. § 271(a), (b), and (c);

(c) The entry of a judgment declaring that the Patents-in-Suit remain valid and enforceable;

(d) The entry of preliminary and permanent injunctions enjoining DRL and its officers, directors, agents, servants, employees, parents, subsidiaries, affiliates, other related business entities, and all other persons and entities acting in concert, participation, or in privity with DRL, and their successors or assigns, from commercially manufacturing, using, offering to sell, or selling DRL's Infringing ANDA Products within the United States, or importing DRL's Infringing ANDA Products into the United States, or inducing or contributing to such conduct, until the last of the expiration dates of the Patents-in-Suit, including any extensions or regulatory exclusivities, or any later expiration of exclusivity to which Celgene is or becomes entitled;

(e) The entry of a permanent injunction, pursuant to 35 U.S.C. § 271(e)(4)(B), enjoining DRL and its officers, directors, agents, servants, employees, parents, subsidiaries, affiliates, other related business entities, and all other persons and entities acting in concert, participation, or in privity with DRL, and their successors or assigns, from commercially manufacturing, using, offering to sell, or selling DRL's Infringing ANDA Products within the United States, or importing DRL's Infringing ANDA Products into the United States, or inducing or contributing to such conduct, until the last of the expiration dates of the Patents-in-

Suit, including any extensions or regulatory exclusivities, or any later expiration of exclusivity to which Celgene is or becomes entitled;

(f) The entry of an order, pursuant to 35 U.S.C. § 271(e)(4)(A), that the effective date of any FDA approval of ANDA No. 211756 shall be a date that is not earlier than the last of the expiration dates of the Patents-in-Suit, including any extensions or regulatory exclusivities, or any later expiration of exclusivity to which Celgene is or becomes entitled;

(g) A declaration under 28 U.S.C. § 2201 that if DRL, its officers, directors, agents, servants, employees, representatives, attorneys, parents, subsidiaries, affiliates, other related business entities, or other persons or entities acting or attempting to act in concert, participation, or in privity with DRL, or acting on DRL's behalf, engage in the commercial manufacture, use, offer for sale, or sale in the United States, or importation into the United States, of DRL's Infringing ANDA Products, then it will constitute an act of direct or indirect infringement of the Patents-in-Suit;

(h) An award of damages or other relief, pursuant to 35 U.S.C. § 271(e)(4)(C), if DRL engages in the commercial manufacture, use, offer for sale, sale, or importation of DRL's Infringing ANDA Products, or any product that infringes the Patents-in-Suit, or induces or contributes to such conduct, prior to the expiration of such patents, including any extensions or regulatory exclusivities;

(i) The entry of judgment declaring that DRL's acts render this case an exceptional case and awarding Celgene its attorneys' fees pursuant to 35 U.S.C. §§ 271(e)(4) and 285;

(j) An award to Celgene of its costs and expenses in this action; and

(k) Such other and further relief this Court deems just and proper.

Dated: November 6, 2018

By: s/ Charles M. Lizza

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EXHIBIT A

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use OTEZLA safely and effectively. See full prescribing information for OTEZLA.

OTEZLA® (apremilast) tablets, for oral use
Initial U.S. approval: 2014

----------RECENT MAJOR CHANGES----------
Warnings and Precautions (5.1) 06/2017

----------INDICATIONS AND USAGE----------
OTEZLA, an inhibitor of phosphodiesterase 4 (PDE4), is indicated for the treatment of:
- Adult patients with active psoriatic arthritis (1.1)
- Patients with moderate to severe plaque psoriasis who are candidates for phototherapy or systemic therapy (1.2)

----------DOSAGE AND ADMINISTRATION----------
- To reduce risk of gastrointestinal symptoms, titrate to recommended dose of 30 mg twice daily according to the following schedule (2.1)
  - Day 1: 10 mg in morning
  - Day 2: 10 mg in morning and 10 mg in evening
  - Day 3: 10 mg in morning and 20 mg in evening
  - Day 4: 20 mg in morning and 20 mg in evening
  - Day 5: 20 mg in morning and 30 mg in evening
  - Day 6 and thereafter: 30 mg twice daily
- Dosage in Severe Renal Impairment:
  - Recommended dose is 30 mg once daily (2.2)
  - For initial dosage titration, titrate using only morning schedule listed in Table 1 and skip afternoon doses (2.2)

----------DOSAGE FORMS AND STRENGTHS----------
Tablets: 10 mg, 20 mg, 30 mg (3)

----------CONTRAINDICATIONS----------
Known hypersensitivity to apremilast or any excipients in formulation (4)

----------WARNINGS AND PRECAUTIONS----------
- Diarrhea, Nausea, and Vomiting: Consider OTEZLA dose reduction or suspension if patients develop severe diarrhea, nausea, or vomiting. (5.1)
- Depression: Advise patients, their caregivers, and families to be alert for the emergence or worsening of depression, suicidal thoughts or other mood changes and if such changes occur to contact their healthcare provider. Carefully weigh risks and benefits of treatment with OTEZLA in patients with a history of depression and/or suicidal thoughts or behavior. (5.2)
- Weight Decrease: Monitor weight regularly. If unexplained or clinically significant weight loss occurs, evaluate weight loss and consider discontinuation of OTEZLA (5.3)
- Drug Interactions: Use with strong cytochrome P450 enzyme inducers (e.g., rifampin, phenobarbital, carbamazepine, phenytoin) is **not** recommended because loss of efficacy may occur (5.4, 7.1)

----------ADVERSE REACTIONS----------
- Psoriatic Arthritis: The most common adverse reactions (≥5%) are diarrhea, nausea, and headache (6.1)
- Psoriasis: The most common adverse reactions (≥5%) are diarrhea, nausea, upper respiratory tract infection, and headache, including tension headache (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Celgene Corporation at 1-888-423-5436 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch

----------USE IN SPECIFIC POPULATIONS----------
Severe Renal Impairment: Increased systemic exposure of OTEZLA has been observed, reduction in dose to 30 mg once daily is recommended (2.2, 8.6)

See 17 for PATIENT COUNSELING INFORMATION

Revised: 06/2017

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the Full Prescribing Information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

1.1 Psoriatic Arthritis

OTEZLA is indicated for the treatment of adult patients with active psoriatic arthritis.

1.2 Psoriasis

OTEZLA is indicated for the treatment of patients with moderate to severe plaque psoriasis who are candidates for phototherapy or systemic therapy.

2 DOSAGE AND ADMINISTRATION

2.1 Dosage in Psoriatic Arthritis and Psoriasis

The recommended initial dosage titration of OTEZLA from Day 1 to Day 5 is shown in Table 1. Following the 5-day titration, the recommended maintenance dosage is 30 mg twice daily taken orally starting on Day 6. This titration is intended to reduce the gastrointestinal symptoms associated with initial therapy.

OTEZLA can be administered without regard to meals. Do not crush, split, or chew the tablets.

Table 1: Dosage Titration Schedule

Day 1	Day 2		Day 3		Day 4		Day 5		Day 6 & thereafter	
AM	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**
10 mg	10 mg	10 mg	10 mg	20 mg	20 mg	20 mg	20 mg	30 mg	30 mg	30 mg

2.2 Dosage Adjustment in Patients with Severe Renal Impairment

OTEZLA dosage should be reduced to 30 mg once daily in patients with severe renal impairment (creatinine clearance (CLcr) of less than 30 mL per minute estimated by the Cockcroft–Gault equation) [*see Use in Specific Populations (8.6) and Clinical Pharmacology (12.3)*]. For initial dosage titration in this group, it is recommended that OTEZLA be titrated using only the AM schedule listed in Table 1 and the PM doses be skipped.

3 DOSAGE FORMS AND STRENGTHS

OTEZLA is available as diamond shaped, film coated tablets in the following dosage strengths:

- 10-mg pink tablet engraved with “APR” on one side and “10” on the other side
- 20-mg brown tablet engraved with “APR” on one side and “20” on the other side
- 30-mg beige tablet engraved with “APR” on one side and “30” on the other side.

4 CONTRAINDICATIONS

OTEZLA is contraindicated in patients with a known hypersensitivity to apremilast or to any of the excipients in the formulation [*see Adverse Reactions (6.1)*].

5 WARNINGS AND PRECAUTIONS

5.1 Diarrhea, Nausea, and Vomiting

There have been postmarketing reports of severe diarrhea, nausea, and vomiting associated with the use of OTEZLA. Most events occurred within the first few weeks of treatment. In some cases patients were hospitalized. Patients 65 years of age or older and patients taking medications that can lead to volume depletion or hypotension may be at a higher risk of complications from severe diarrhea, nausea, or vomiting. Monitor patients who are more susceptible to complications of diarrhea or vomiting. Patients who reduced dosage or discontinued OTEZLA generally improved quickly. Consider OTEZLA dose reduction or suspension if patients develop severe diarrhea, nausea, or vomiting.

5.2 Depression

Treatment with OTEZLA is associated with an increase in adverse reactions of depression. Before using OTEZLA in patients with a history of depression and/or suicidal thoughts or behavior prescribers should carefully weigh the risks and benefits of treatment with OTEZLA in such patients. Patients, their caregivers, and families should be advised of the need to be alert for the emergence or worsening of depression, suicidal thoughts or other mood changes, and if such changes occur to contact their healthcare provider. Prescribers should carefully evaluate the risks and benefits of continuing treatment with OTEZLA if such events occur.

Psoriatic arthritis: During the 0 to 16 week placebo-controlled period of the 3 controlled clinical trials, 1.0% (10/998) of subjects treated with OTEZLA reported depression or depressed mood compared to 0.8% (4/495) treated with placebo. During the clinical trials, 0.3% (4/1441) of subjects treated with OTEZLA discontinued treatment due to depression or depressed mood compared with none in placebo treated subjects (0/495). Depression was reported as serious in 0.2% (3/1441) of subjects exposed to OTEZLA, compared to none in placebo-treated subjects (0/495). Instances of suicidal ideation and behavior have been observed in 0.2% (3/1441) of subjects while receiving OTEZLA, compared to none in placebo treated subjects (0/495). In the clinical trials, 2 subjects who received placebo committed suicide compared to none in OTEZLA-treated subjects.

Psoriasis: During the 0 to 16 week placebo-controlled period of the 3 controlled clinical trials, 1.3% (12/920) of subjects treated with OTEZLA reported depression compared to 0.4% (2/506) treated with placebo. During the clinical trials, 0.1% (1/1308) of subjects treated with OTEZLA discontinued treatment due to depression compared with none in placebo-treated subjects (0/506). Depression was reported as serious in 0.1% (1/1308) of subjects exposed to OTEZLA, compared to none in placebo-treated subjects (0/506). Instances of suicidal behavior have been observed in 0.1% (1/1308) of subjects while receiving OTEZLA, compared to 0.2% (1/506) in placebo-treated subjects. In the clinical trials, one subject treated with OTEZLA attempted suicide while one who received placebo committed suicide.

5.3 Weight Decrease

During the controlled period of the studies in psoriatic arthritis (PsA), weight decrease between 5%-10% of body weight was reported in 10% (49/497) of subjects treated with OTEZLA 30 mg twice daily compared to 3.3% (16/495) treated with placebo [*see Adverse Reactions (6.1)*].

During the controlled period of the trials in psoriasis, weight decrease between 5%-10% of body weight occurred in 12% (96/784) of subjects treated with OTEZLA compared to 5% (19/382) treated with placebo. Weight decrease of ≥10% of body weight occurred in 2% (16/784) of subjects treated with OTEZLA 30 mg twice daily compared to 1% (3/382) subjects treated with placebo.

Patients treated with OTEZLA should have their weight monitored regularly. If unexplained or clinically significant weight loss occurs, weight loss should be evaluated, and discontinuation of OTEZLA should be considered.

5.4 Drug Interactions

Co-administration of strong cytochrome P450 enzyme inducer, rifampin, resulted in a reduction of systemic exposure of apremilast, which may result in a loss of efficacy of OTEZLA. Therefore, the use of cytochrome P450 enzyme inducers (e.g., rifampin, phenobarbital, carbamazepine, phenytoin) with OTEZLA is not recommended [*see Drug Interactions (7.1)* and *Clinical Pharmacology (12.3)*].

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in clinical practice.

Psoriatic Arthritis Clinical Trials

OTEZLA was evaluated in 3 multicenter, randomized, double-blind, placebo-controlled trials [Studies PsA-1, PsA-2, and PsA-3] of similar design in adult patients with active psoriatic arthritis *[see Clinical Studies (14.1)]*. Across the 3 studies, there were 1493 patients randomized equally to placebo, OTEZLA 20 mg twice daily or OTEZLA 30 mg twice daily. Titration was used over the first 5 days *[see Dosage and Administration (2.1)*]. Placebo patients whose tender and swollen joint counts had not improved by at least 20% were re-randomized 1:1 in a blinded fashion to either OTEZLA 20 mg twice daily or 30 mg twice daily at week 16 while OTEZLA patients remained on their initial treatment. Patients ranged in age from 18 to 83 years, with an overall median age of 51 years.

The majority of the most common adverse reactions presented in Table 2 occurred within the first 2 weeks of treatment and tended to resolve over time with continued dosing. Diarrhea, headache, and nausea were the most commonly reported adverse reactions. The most common adverse reactions leading to discontinuation for patients taking OTEZLA were nausea (1.8%), diarrhea (1.8%), and headache

(1.2%). The proportion of patients with psoriatic arthritis who discontinued treatment due to any adverse reaction was 4.6% for patients taking OTEZLA 30 mg twice daily and 1.2% for placebo-treated patients.

Table 2: Adverse Reactions Reported in ≥2% of Patients on OTEZLA 30 mg Twice Daily and ≥1% Than That Observed in Patients on Placebo for up to Day 112 (Week 16)

	Placebo		OTEZLA 30 mg BID	
Preferred Term	**Day 1 to 5 (N=495) n (%)[c]**	**Day 6 to Day 112 (N=490) n (%)**	**Day 1 to 5 (N=497) n (%)**	**Day 6 to Day 112 (N=493) n (%)**
Diarrhea[a]	6 (1.2)	8 (1.6)	46 (9.3)	38 (7.7)
Nausea[a]	7 (1.4)	15 (3.1)	37 (7.4)	44 (8.9)
Headache[a]	9 (1.8)	11 (2.2)	24 (4.8)	29 (5.9)
Upper respiratory tract infection[b]	3 (0.6)	9 (1.8)	3 (0.6)	19 (3.9)
Vomiting[a]	2 (0.4)	2 (0.4)	4 (0.8)	16 (3.2)
Nasopharyngitis[b]	1 (0.2)	8 (1.6)	1 (0.2)	13 (2.6)
Abdominal pain upper[b]	0 (0.0)	1 (0.2)	3 (0.6)	10 (2.0)

[a] Of the reported gastrointestinal adverse reactions, 1 subject experienced a serious adverse reaction of nausea and vomiting in OTEZLA 30 mg twice daily; 1 subject treated with OTEZLA 20 mg twice daily experienced a serious adverse reaction of diarrhea; 1 patient treated with OTEZLA 30 mg twice daily experienced a serious adverse reaction of headache.
[b] Of the reported adverse drug reactions none were serious.
[c] n (%) indicates number of patients and percent.

Other adverse reactions reported in patients on OTEZLA in clinical studies including extension studies:
Immune system disorders: Hypersensitivity
Investigations: Weight decrease
Gastrointestinal Disorders: Frequent bowel movement, gastroesophageal reflux disease, dyspepsia
Metabolism and Nutrition Disorders: Decreased appetite*
Nervous System Disorders: Migraine
Respiratory, Thoracic, and Mediastinal Disorders: Cough
Skin and Subcutaneous Tissue Disorders: Rash
*1 patient treated with OTEZLA 30 mg twice daily experienced a serious adverse reaction.

Psoriasis Clinical Trials
The safety of OTEZLA® was assessed in 1426 subjects in 3 randomized, double-blind, placebo-controlled trials in adult subjects with moderate to severe plaque psoriasis who were candidates for phototherapy or systemic therapy. Subjects were randomized to receive OTEZLA 30 mg twice daily or placebo twice daily. Titration was used over the first 5 days *[see Dosage and Administration (2.1)]*. Subjects ranged in age from 18 to 83 years, with an overall median age of 46 years.

Diarrhea, nausea, and upper respiratory tract infection were the most commonly reported adverse reactions. The most common adverse reactions leading to discontinuation for subjects taking OTEZLA were nausea (1.6%), diarrhea (1.0%), and headache (0.8%). The proportion of subjects with psoriasis who discontinued treatment due to any adverse reaction was 6.1% for subjects treated with OTEZLA 30 mg twice daily and 4.1% for placebo-treated subjects.

Table 3: Adverse Reactions Reported in ≥1% of Subjects on OTEZLA and With Greater Frequency Than in Subjects on Placebo; up to Day 112 (Week 16)

Preferred Term	**Placebo (N=506) n (%)**	**OTEZLA 30 mg BID (N=920) n (%)**
Diarrhea	32 (6)	160 (17)
Nausea	35 (7)	155 (17)
Upper respiratory tract infection	31 (6)	84 (9)
Tension headache	21 (4)	75 (8)
Headache	19 (4)	55 (6)
Abdominal pain*	11 (2)	39 (4)
Vomiting	8 (2)	35 (4)

Fatigue	9 (2)	29 (3)
Dyspepsia	6 (1)	29 (3)
Decreased appetite	5 (1)	26 (3)
Insomnia	4 (1)	21 (2)
Back pain	4 (1)	20 (2)
Migraine	5 (1)	19 (2)
Frequent bowel movements	1 (0)	17 (2)
Depression	2 (0)	12 (1)
Bronchitis	2 (0)	12 (1)
Tooth abscess	0 (0)	10 (1)
Folliculitis	0 (0)	9 (1)
Sinus headache	0 (0)	9 (1)

*Two subjects treated with OTEZLA experienced serious adverse reaction of abdominal pain.

Severe worsening of psoriasis (rebound) occurred in 0.3% (4/1184) subjects following discontinuation of treatment with OTEZLA.

7 DRUG INTERACTIONS

7.1 Strong CYP450 Inducers

Apremilast exposure is decreased when OTEZLA is co-administered with strong CYP450 inducers (such as rifampin) and may result in loss of efficacy [*see Warnings and Precautions (*5.3*) and Clinical Pharmacology (12.3)*].

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C:

Pregnancy Exposure Registry
There is a pregnancy exposure registry that monitors pregnancy outcomes in women exposed to OTEZLA during pregnancy. Information about the registry can be obtained by calling 1-877-311-8972.

Risk Summary

Adequate and well-controlled studies with OTEZLA have not been conducted in pregnant women. In animal embryo-fetal development studies, the administration of apremilast to cynomolgus monkeys during organogenesis resulted in dose-related increases in abortion/embryo-fetal death at dose exposures 2.1-times the maximum recommended human therapeutic dose (MRHD) and no adverse effect at an exposure of 1.4-times the MRHD. In mice, there were no apremilast induced malformations up to exposures 4.0-times the MRHD. The incidences of malformations and pregnancy loss in human pregnancies have not been established for OTEZLA. However, all pregnancies, regardless of drug exposure, have a background rate of 2% to 4% for major malformations, and 15% to 20% for pregnancy loss. OTEZLA should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Clinical Considerations

Labor or delivery
The effects of OTEZLA on labor and delivery in pregnant women are unknown. In mice, dystocia was noted at doses corresponding to ≥4.0-times the MRHD (on an AUC basis at doses ≥80 mg/kg/day) of apremilast.

Animal Data
Monkey embryo-fetal development: In an embryo-fetal developmental study, cynomolgus monkeys were administered apremilast at doses of 20, 50, 200, or 1000 mg/kg/day during the period of organogenesis (gestation Days 20 through 50). There was a dose-related increase in spontaneous abortions, with most abortions occurring during weeks 3 to 4 of dosing in the first trimester, at doses approximately 2.1-times the MRHD and greater (on an AUC basis at doses ≥50 mg/kg/day). No abortifacient effects were observed at a dose approximately 1.4-times the MRHD (on an AUC basis at a dose of 20 mg/kg/day). Although, there was no evidence for a teratogenic effect at doses of 20 mg/kg/day and greater when examined at day 100, aborted fetuses were not examined.

Mouse embryo-fetal development: In an embryo-fetal development study, apremilast was administered at doses of 250, 500, or 750 mg/kg/day to dams during organogenesis (gestation Day 6 through 15). In a combined fertility and embryo-fetal development study, apremilast was administered at doses of 10, 20, 40 or 80 mg/kg/day starting 15 days before cohabitation and continuing through gestation Day 15. No teratogenic findings attributed to apremilast were observed in either study; however, there was an increase in postimplantation loss at doses corresponding to a systemic exposure of 2.3-times the MRHD and greater (≥20 mg/kg/day). At doses of ≥20 mg/kg/day skeletal variations included incomplete ossification sites of tarsals, skull, sternebra, and vertebrae. No effects were

observed at a dose approximately 1.3-times the MRHD (10 mg/kg/day).

Mouse pre- and postnatal development: In a pre- and postnatal study in mice, apremilast was administered to pregnant female mice at doses of 10, 80, or 300 mg/kg/day from Day 6 of gestation through Day 20 of lactation, with weaning on day 21. Dystocia, reduced viability, and reduced birth weights occurred at doses corresponding to ≥4.0-times the MRHD (on an AUC basis at doses ≥80 mg/kg/day). No adverse effects occurred at a dose 1.3-times the MRHD (10 mg/kg/day). There was no evidence for functional impairment of physical development, behavior, learning ability, immune competence, or fertility in the offspring at doses up to 7.5-times the MRHD (on an AUC basis at a dose of 300 mg/kg/day).

8.3 Nursing Mothers

It is not known whether OTEZLA or its metabolites are present in human milk; however apremilast was detected in milk of lactating mice. Because many drugs are present in human milk, caution should be exercised when OTEZLA is administered to a nursing woman.

8.4 Pediatric use

The safety and effectiveness of OTEZLA in pediatric patients less than18 years of age have not been established.

8.5 Geriatric use

Of the 1493 subjects who enrolled in Studies PsA-1, PsA-2, and PsA-3 a total of 146 psoriatic arthritis subjects were 65 years of age and older, including 19 subjects 75 years and older. No overall differences were observed in the safety profile of elderly subjects ≥65 years of age and younger adult subjects <65 years of age in the clinical studies.

Of the 1257 subjects who enrolled in two placebo-controlled psoriasis trials (PSOR 1 and PSOR 2), a total of 108 psoriasis subjects were 65 years of age and older, including 9 subjects who were 75 years of age and older. No overall differences were observed in the efficacy and safety in elderly subjects ≥65 years of age and younger adult subjects <65 years of age in the clinical trials.

8.6 Renal Impairment

Apremilast pharmacokinetics were characterized in subjects with mild, moderate, and severe renal impairment as defined by a creatinine clearance of 60-89, 30-59, and less than 30 mL per minute, respectively, by the Cockcroft–Gault equation. While no dose adjustment is needed in patients with mild or moderate renal impairment, the dose of OTEZLA should be reduced to 30 mg once daily in patients with severe renal impairment [*see Dosage and Administration (2.2) and Clinical Pharmacology (12.3)*].

8.7 Hepatic Impairment

Apremilast pharmacokinetics were characterized in subjects with moderate (Child Pugh B) and severe (Child Pugh C) hepatic impairment. No dose adjustment is necessary in these patients.

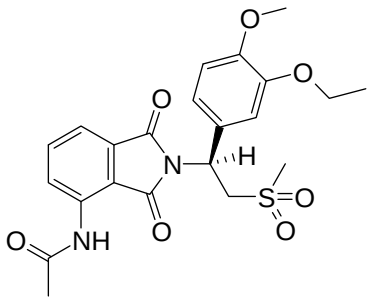
10 OVERDOSAGE

In case of overdose, patients should seek immediate medical help. Patients should be managed by symptomatic and supportive care should there be an overdose.

11 DESCRIPTION

The active ingredient in OTEZLA tablets is apremilast. Apremilast is a phosphodiesterase 4 (PDE4) inhibitor. Apremilast is known chemically as N-[2-[(1S)-1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl)ethyl]-2,3-dihydro-1,3-dioxo-1H-isoindol-4-yl]acetamide. Its empirical formula is $C_{22}H_{24}N_2O_7S$ and the molecular weight is 460.5.

The chemical structure is:



OTEZLA tablets are supplied in 10-, 20-, and 30-mg strengths for oral administration. Each tablet contains apremilast as the active ingredient and the following inactive ingredients: lactose monohydrate, microcrystalline cellulose, croscarmellose sodium, magnesium stearate, polyvinyl alcohol, titanium dioxide, polyethylene glycol, talc, iron oxide red, iron oxide yellow (20 and 30 mg only) and iron oxide black (30 mg only).

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of action

Apremilast is an oral small-molecule inhibitor of phosphodiesterase 4 (PDE4) specific for cyclic adenosine monophosphate (cAMP). PDE4 inhibition results in increased intracellular cAMP levels. The specific mechanism(s) by which apremilast exerts its therapeutic action in psoriatic arthritis patients and psoriasis patients is not well defined.

12.3 Pharmacokinetics

Absorption

Apremilast when taken orally is absorbed with an absolute bioavailability of ~73%, with peak plasma concentrations (C_{max}) occurring at a median time (t_{max}) of ~2.5 hours. Co-administration with food does not alter the extent of absorption of apremilast.

Distribution

Human plasma protein binding of apremilast is approximately 68%. Mean apparent volume of distribution (Vd) is 87 L.

Metabolism

Following oral administration in humans, apremilast is a major circulating component (45%) followed by inactive metabolite M12 (39%), a glucuronide conjugate of O-demethylated apremilast. It is extensively metabolized in humans with up to 23 metabolites identified in plasma, urine and feces. Apremilast is metabolized by both cytochrome (CYP) oxidative metabolism with subsequent glucuronidation and non-CYP mediated hydrolysis. In vitro, CYP metabolism of apremilast is primarily mediated by CYP3A4, with minor contributions from CYP1A2 and CYP2A6.

Elimination

The plasma clearance of apremilast is about 10 L/hr in healthy subjects, with a terminal elimination half-life of approximately 6-9 hours. Following oral administration of radio-labeled apremilast, about 58% and 39% of the radioactivity is recovered in urine and feces, respectively, with about 3% and 7% of the radioactive dose recovered as apremilast in urine and feces, respectively.

Specific Populations

Hepatic Impairment: The pharmacokinetics of apremilast is not affected by moderate or severe hepatic impairment.

Renal Impairment: The pharmacokinetics of apremilast is not affected by mild or moderate renal impairment. In 8 subjects with severe renal impairment administered a single dose of 30 mg apremilast, the AUC and C_{max} of apremilast increased by approximately 88% and 42%, respectively [*see Dosage and Administration (2.2) and Use in Specific Populations (8.6)*].

Age: A single oral dose of 30-mg apremilast was studied in young adults and elderly healthy subjects. The apremilast exposure in elderly subjects (65 to 85 years of age) was about 13% higher in AUC and about 6% higher in C_{max} than in young subjects (18 to 55 years of age). [*see Use in Specific Populations (8.5)*].

Gender: In pharmacokinetic studies in healthy volunteers, the extent of exposure in females was about 31% higher and C_{max} was about 8% higher than that in male subjects.

Race and Ethnicity: The pharmacokinetics of apremilast in Chinese and Japanese healthy male subjects is comparable to that in Caucasian healthy male subjects. In addition, apremilast exposure is similar among Hispanic Caucasians, non-Hispanic Caucasians, and African Americans.

Drug Interactions

In vitro data: Apremilast is not an inhibitor of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 and not an inducer of CYP1A2, CYP2B6, CYP2C9, CYP2C19, or CYP3A4. Apremilast is a substrate, but not an inhibitor of P-glycoprotein (P-gp) and is not a substrate or an inhibitor of organic anion transporter (OAT)1 and OAT3, organic cation transporter (OCT)2, organic anion transporting polypeptide (OATP)1B1 and OATP1B3, or breast cancer resistance protein (BCRP).

Drug interaction studies were performed with apremilast and CYP3A4 substrates (oral contraceptive containing ethinyl estradiol and norgestimate), CYP3A and P-gp inhibitor (ketoconazole), CYP450 inducer (rifampin) and frequently co-administered drug in this patient population (methotrexate).

No significant pharmacokinetic interactions were observed when 30-mg oral apremilast was administered with either oral contraceptive, ketoconazole, or methotrexate. Co-administration of the CYP450 inducer rifampin (600 mg once daily for 15 days) with a single oral dose of 30-mg apremilast resulted in reduction of apremilast AUC and C_{max} by 72% and 43%, respectively [*see Warnings and Precautions (5.3) and Drug Interactions (7.1)*].

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term studies were conducted in mice and rats with apremilast to evaluate its carcinogenic potential. No evidence of apremilast-induced tumors was observed in mice at oral doses up to 8.8-times the Maximum Recommended Human Dose (MRHD) on an AUC basis (1000 mg/kg/day) or in rats at oral doses up to approximately 0.08- and 1.1-times the MRHD, (20 mg/kg/day in males and 3 mg/kg/day in females, respectively).

Apremilast tested negative in the Ames assay, in vitro chromosome aberration assay of human peripheral blood lymphocytes, and the in vivo mouse micronucleus assay.

In a fertility study of male mice, apremilast at oral doses up to approximately 3-times the MRHD based on AUC (up to 50 mg/kg/day) produced no effects on male fertility. In a fertility study of female mice, apremilast was administered at oral doses of 10, 20, 40, or 80 mg/kg/day. At doses ≥1.8-times the MRHD (≥20 mg/kg/day), estrous cycles were prolonged, due to lengthening of diestrus which resulted in a longer interval until mating. Mice that became pregnant at doses of 20 mg/kg/day and greater also had increased incidences of early postimplantation losses. There was no effect of apremilast approximately 1.0-times the MRHD (10 mg/kg/day).

14 CLINICAL STUDIES

14.1 Psoriatic Arthritis

The safety and efficacy of OTEZLA was evaluated in 3 multi-center, randomized, double-blind, placebo-controlled trials (Studies PsA-1, PsA-2, and PsA-3) of similar design. A total of 1493 adult patients with active PsA (≥3 swollen joints and ≥3 tender joints) despite prior or current treatment with disease-modifying antirheumatic drug (DMARD) therapy were randomized. Patients enrolled in these studies had a diagnosis of PsA for at least 6 months. One qualifying psoriatic skin lesion of at least 2 cm in diameter was required in Study PsA-3. Previous treatment with a biologic, including TNF-blockers was allowed (up to 10% could be TNF-blocker therapeutic failures). Across the 3 studies, patients were randomly assigned to placebo (n=496), OTEZLA 20 mg (n=500), or OTEZLA 30 mg (n=497) given orally twice daily. Titration was used over the first 5 days *[see Dosage and Administration (2.1)]*. Patients were allowed to receive stable doses of concomitant methotrexate [MTX (≤25 mg/week)], sulfasalazine [SSZ (≤2 g/day)], leflunomide [LEF (≤20 mg/day)], low dose oral corticosteroids (equivalent to ≤10 mg of prednisone a day), and/or nonsteroidal anti-inflammatory drugs (NSAIDs) during the trial. Treatment assignments were stratified based on small-molecule DMARD use at baseline in Studies PsA-1, PsA-2 and PsA-3. There was an additional stratification of BSA >3% with psoriasis in study PsA-3. The patients who were therapeutic failures of >3 agents for PsA (small molecules or biologics), or >1 biologic TNF blocker were excluded.

The primary endpoint was the percentage of patients achieving American College of Rheumatology (ACR) 20 response at Week 16. Placebo-controlled efficacy data were collected and analyzed through Week 24. Patients whose tender and swollen joint counts had not improved by at least 20% were considered non-responders at Week 16. Placebo non-responders were re-randomized 1:1 in a blinded fashion to either OTEZLA 20 mg twice daily or 30 mg twice daily following the titration schema *[see Dosage and Administration (2.1)]*. OTEZLA patients remained on their initial treatment. At Week 24, all remaining placebo patients were re-randomized to either 20 mg twice daily or 30 mg twice daily.

Patients with subtypes of PsA were enrolled across the 3 studies, including symmetric polyarthritis (62.0%), asymmetric oligoarthritis (27.0%), distal interphalangeal (DIP) joint arthritis (6.0%), arthritis mutilans (3.0%), and predominant spondylitis (2.1%). The median duration of PsA disease was 5 years. Patients received concomitant therapy with at least one DMARD (65.0%), MTX (55.0%), SSZ (9.0%), LEF (7.0%), low dose oral corticosteroids (14.0%), and NSAIDs (71.0%). Prior treatment with small-molecule DMARDs only was reported in 76.0% of patients and prior treatment with biologic DMARDs was reported in 22.0% of patients, which includes 9.0% who had failed prior biologic DMARD treatment.

Clinical Response in Patients with Psoriatic Arthritis

The percent of patients achieving ACR 20, 50 and 70 responses in Studies PsA-1, PsA-2, and PsA-3 are presented in Table 4 below. OTEZLA ± DMARDs, compared with Placebo ± DMARDs resulted in a greater improvement in signs and symptoms of psoriatic arthritis as demonstrated by the proportion of patients with an ACR 20 response at Week 16.

Table 4: Proportion of Patients With ACR Responses in Studies PsA-1, PsA-2 and PsA-3

	PsA-1		PsA-2		PsA-3	
N[a]	**Placebo ± DMARDs N=168**	**OTEZLA 30 mg twice daily ± DMARDs N=168**	**Placebo ± DMARDs N=159**	**OTEZLA 30 mg twice daily ± DMARDs N=162**	**Placebo ± DMARDs N=169**	**OTEZLA 30 mg twice daily ± DMARDs N=167**
ACR 20 Week 16	19%	38% [b]	19%	32% [b]	18%	41% [b]
ACR 50 Week 16	6%	16%	5%	11%	8%	15%
ACR 70 Week 16	1%	4%	1%	1%	2%	4%

[a] N is number of randomized and treated patients.
[b] Statistically significantly different from placebo ($p<0.05$).

OTEZLA 30 mg twice daily resulted in improvement for each ACR component, compared to placebo at Week 16 in Study PsA-1 (Table 5). Consistent results were observed in Studies PsA-2 and PsA-3.

Table 5: ACR Components Mean Change from Baseline at Week 16 in Study PsA- 1

	Placebo (N*=168)	OTEZLA 30 mg twice daily (N*=168)
Number of tender joints[a]		
Sample Size	166	164
Baseline	23	23
Mean Change at Week 16	-2	-7
Number of swollen joints[b]		
Sample Size	166	164
Baseline	13	13
Mean Change at Week 16	-2	-5
Patient's assessment of pain[c]		
Sample Size	165	159
Baseline	61	58
Mean Change at Week 16	-6	-14
Patient's global assessment of disease activity[c]		
Sample Size	165	159
Baseline	59	56
Mean Change at Week 16	-3	-10
Physician's global assessment of disease activity[c]		
Sample Size	158	159
Baseline	55	56
Mean Change at Week 16	-8	-19

HAQ-DI[d] score		
Sample Size	165	159
Baseline	1.2	1.2
Mean Change at Week 16	-0.09	-0.2
CRP[e]		
Sample Size	166	167
Baseline	1.1	0.8
Mean Change at Week 16	0.1	-0.1

Mean changes from baseline are least square means from analyses of covariance.
[a] Scale 0-78.
[b] Scale 0-76.
[c] VAS=Visual Analog Scale; 0=best, 100=worst.
[d] HAQ-DI = Health Assessment Questionnaire-Disability Index; 0=best, 3=worst; measures the subject's ability to perform the following: dress/groom, arise, eat, walk, reach, grip, maintain hygiene, and maintain daily activity.
[e] CRP = C-reactive protein; Reference range 0-0.5 mg/dL
* N reflects randomized patients; actual number of patients evaluable for each endpoint may vary by timepoint.

Treatment with OTEZLA resulted in improvement in dactylitis and enthesitis in patients with pre-existing dactylitis or enthesitis.

Physical Function Response

OTEZLA 30 mg twice daily demonstrated a greater improvement compared to placebo in mean change from baseline for the Health Assessment Questionnaire Disability Index (HAQ-DI) score at Week 16 [-0.244 vs. -0.086, respectively; 95% CI for the difference was (-0.26, -0.06)] in Study PsA-1. The proportions of HAQ-DI responders (≥0.3 improvement from baseline) at Week 16 for the OTEZLA 30 mg twice daily group were 38%, compared to 27%, for the placebo group in Study PsA-1. Consistent results were observed in Studies PsA-2 and PsA-3.

14.2 Psoriasis

Two multicenter, randomized, double-blind, placebo-controlled trials (Studies PSOR-1 and PSOR-2) enrolled a total of 1257 subjects 18 years of age and older with moderate to severe plaque psoriasis [body surface area (BSA) involvement of ≥10%, static Physician Global Assessment (sPGA) of ≥3 (moderate or severe disease), Psoriasis Area and Severity Index (PASI) score ≥12, candidates for phototherapy or systemic therapy]. Subjects were allowed to use low-potency topical corticosteroids on the face, axilla and groin. Subjects with scalp psoriasis were allowed to use coal tar shampoo and/or salicylic acid scalp preparations on scalp lesions.

Study PSOR-1 enrolled 844 subjects and Study PSOR-2 enrolled 413 subjects. In both studies, subjects were randomized 2:1 to OTEZLA 30 mg BID or placebo for 16 weeks. Both studies assessed the proportion of subjects who achieved PASI-75 at Week 16 and the proportion of subjects who achieved a sPGA score of clear (0) or almost clear (1) at Week 16. Across both studies, subjects ranged in age from 18 to 83 years, with an overall median age of 46 years. The mean baseline BSA involvement was 25.19% (median 21.0%), the mean baseline PASI score was 19.07 (median 16.80), and the proportion of subjects with sPGA score of 3 (moderate) and 4 (severe) at baseline were 70.0% and 29.8%, respectively. Approximately 30% of all subjects had received prior phototherapy and 54% had received prior conventional systemic and/or biologic therapy for the treatment of psoriasis with 37% receiving prior conventional systemic therapy and 30% receiving prior biologic therapy. Approximately one-third of subjects had not received prior phototherapy, conventional systemic nor biologic therapy. A total of 18% of subjects had a history of psoriatic arthritis.

Clinical Response in Subjects with Plaque Psoriasis

The proportion of subjects who achieved PASI -75 responses, and sPGA score of clear (0) or almost clear (1), are presented in Table 6.

Table 6: Clinical Response at Week 16 in Studies PSOR-1 and PSOR-2

	Study PSOR-1		**Study PSOR-2**	
	Placebo	**OTEZLA 30 mg BID**	**Placebo**	**OTEZLA 30 mg BID**
N[a]	**N=282**	**N=562**	**N=137**	**N=274**
PASI[b] -75, n (%)	15 (5.3)	186 (33.1)	8 (5.8)	79 (28.8)
sPGA[c] of Clear or Almost Clear, n (%)	11 (3.9)	122 (21.7)	6 (4.4)	56 (20.4)

[a] N is number of randomized and treated patients.
[b] PASI=Psoriasis Area and Severity Index.
[c] sPGA=Static Physician Global Assessment.

The median time to loss of PASI-75 response among the subjects re-randomized to placebo at Week 32 during the Randomized Treatment Withdrawal Phase was 5.1 weeks.

16 HOW SUPPLIED/STORAGE AND HANDLING

OTEZLA is available as diamond-shaped, film-coated tablets in the following dosage strengths: 10-mg pink tablet engraved with "APR" on one side and "10" on the other side; 20-mg brown tablet engraved with "APR" on one side and "20" on the other side; 30-mg beige tablet engraved with "APR" on one side and "30" on the other side.

Tablets are supplied in the following strengths and package configurations:

Package configuration	Tablet strength	NDC number
Bottles of 60	30 mg	59572-631-06
Two-week starter pack	13-tablet blister titration pack containing: (4) 10-mg, (4) 20-mg, and (5) 30-mg tablets with an additional (14) 30-mg tablets	59572-630-27
28-count carton	Two 30-mg blister cards containing (14) 30-mg tablets	59572-631-28
28-day starter pack	13-tablet blister titration pack containing: (4) 10-mg, (4) 20-mg, and (5) 30-mg tablets with an additional (42) 30-mg tablets	59572-632-55

Storage and Handling
Store tablets below 30°C (86°F).

17 PATIENT COUNSELING INFORMATION

- **Diarrhea, Nausea, and Vomiting**
  Instruct patients to contact their healthcare provider if they experience severe diarrhea, nausea, or vomiting. Prescribers should advise patients of the potential complications of severe diarrhea, nausea, or vomiting. Consider OTEZLA dose reduction or suspension if patients develop severe diarrhea, nausea, or vomiting [*see Warnings and Precautions (5.1)*].
- **Depression**
  Before using OTEZLA in patients with a history of depression and/or suicidal thoughts or behavior, prescribers should carefully weigh the risks and benefits of treatment with OTEZLA in such patients. Patients, their caregivers, and families should be advised of the need to be alert for the emergence or worsening of depression, suicidal thoughts or other mood changes, and if such changes occur to contact their healthcare provider. Prescribers should carefully evaluate the risks and benefits of continuing treatment with OTEZLA if such events occur [*see Warnings and Precautions (5.2)*].
- **Weight Decrease**
  Patients treated with OTEZLA should have their weight monitored regularly. If unexplained or clinically significant weight loss occurs, weight loss should be evaluated, and discontinuation of OTEZLA should be considered [*see Warnings and Precautions (5.3)*].
- **Drug Interactions**
  The use of strong cytochrome P450 enzyme inducers (e.g., rifampin, phenobarbital, carbamazepine, phenytoin) with OTEZLA is not recommended [*see Warnings and Precautions (5.4), Drug Interactions (7.1),* and *Clinical Pharmacology (12.3)*].
- Instruct patients to take OTEZLA only as prescribed.
- Advise patients OTEZLA can be taken with or without food.
- Advise patients that the tablets should not be crushed, split, or chewed.
- Advise patients about the side effects associated with OTEZLA [*see Adverse Reactions (6.1)*].

Manufactured for: Celgene Corporation
Summit, NJ 07901

OTEZLA® is a registered trademark of Celgene Corporation.

Pat. http://www.celgene.com/therapies

APRPI.006 06/17

EXHIBIT B

US006962940B2

(12) **United States Patent**
Muller et al.

(10) **Patent No.: US 6,962,940 B2**
(45) **Date of Patent: Nov. 8, 2005**

(54) **(+)-2-[1-(3-ETHOXY-4-METHOXYPHENYL)-2-METHYLSULFONYLETHYL]-4-ACETYLAMINOISOINDOLINE-1,3-DIONE: METHODS OF USING AND COMPOSITIONS THEREOF**

(75) Inventors: **George W. Muller**, Bridgewater, NJ (US); **Peter H. Schafer**, Somerset, NJ (US); **Hon-Wah Man**, Princeton, NJ (US); **Chuansheng Ge**, Belle Mead, NJ (US)

(73) Assignee: **Celgene Corporation**, Summit, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **10/392,195**

(22) Filed: **Mar. 19, 2003**

(65) **Prior Publication Data**

US 2003/0187052 A1 Oct. 2, 2003

Related U.S. Application Data

(60) Provisional application No. 60/366,515, filed on Mar. 20, 2002, and provisional application No. 60/438,450, filed on Jan. 7, 2003.

(51) **Int. Cl.**[7] **A61K 31/4035**; C07D 209/44

(52) **U.S. Cl.** **514/417**; 548/478; 548/469

(58) **Field of Search** 548/478, 469; 514/417

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,031,450	A	4/1962	Fischer et al.
3,322,755	A	5/1967	Roch et al.
3,920,636	A	11/1975	Takahashi et al.
4,001,237	A	1/1977	Partyka et al.
4,001,238	A	1/1977	Partyka et al.
4,047,404	A	9/1977	Hayashi
4,060,615	A	11/1977	Matier et al.
4,101,548	A	7/1978	Crenshaw et al.
4,162,316	A	7/1979	Nishimura et al.
4,209,623	A	6/1980	Juby
4,880,810	A	11/1989	Lowe, III
4,885,301	A	12/1989	Coates
5,147,875	A	9/1992	Coates et al.
5,354,571	A	10/1994	Morikawa et al.
5,401,774	A	3/1995	Pamukcu et al.
5,439,895	A	8/1995	Lee et al.
5,488,055	A	1/1996	Kumar et al.
5,608,914	A	3/1997	Keesler
5,614,530	A	3/1997	Kumar et al.
5,614,627	A	3/1997	Takase et al.
5,658,940	A	8/1997	Muller et al.
5,698,579	A	12/1997	Muller
5,703,098	A	12/1997	Muller et al.
5,710,170	A	1/1998	Guay et al.
5,728,844	A	3/1998	Muller et al.
5,728,845	A	3/1998	Muller et al.

(Continued)

FOREIGN PATENT DOCUMENTS

EP	0 347 146	6/1989
EP	0 349 239 A2	6/1989
EP	0 351 058	6/1989
EP	0 352 960 A2	7/1989
EP	0 395 328	4/1990
EP	0 428 268 A2	10/1990
EP	0 463 756 A1	6/1991
EP	0 526 004 A1	7/1992
EP	0 607 439 A1	9/1992
EP	0 722 937 A1	1/1996
EP	0 722 943 A1	1/1996
EP	0 722 944 A1	1/1996
GB	2 063 249 A	9/1980
WO	WO 93/07149	4/1993
WO	WO 93/12095	6/1993
WO	WO 94/01728	1/1994
WO	WO 94/05661	3/1994
WO	WO 94/29277	12/1994
WO	WO 95/19978	7/1995
WO	WO 96/32379	10/1996
WO	WO 97/03070	1/1997
WO	WO 97/03675	2/1997
WO	WO 97/03985	2/1997
WO	WO 97/24334	7/1997
WO	WO 98/06722	2/1998
WO	WO 98/08848	3/1998
WO	WO 98/14448	4/1998
WO	WO 98/16521	4/1998
WO	WO 98/17668	4/1998
WO	WO 98/23597	6/1998
WO	WO 98/38168	9/1998
WO	WO 99/06041	2/1999

OTHER PUBLICATIONS

U.S. Appl. No. 60/454,155, G. Muller et al., filed Mar. 12, 2003.

U.S. Appl. No. 60/454,159, G. Muller et al., filed Mar. 12, 2003.

U.S. Appl. No. 60/438,448, G. Muller et al., filed Jan. 7, 2003.

U.S. Appl. No. 60/436,975, G. Muller et al., filed Dec. 30, 2002.

U.S. Appl. No. 60/366,515, G. Muller et al., filed Mar. 20, 2002.

(Continued)

Primary Examiner—Golam M. M. Shameem
(74) *Attorney, Agent, or Firm*—Jones Day

(57) **ABSTRACT**

Stereomerically pure (+)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, substantially free of its (−) isomer, and prodrugs, metabolites, polymorphs, salts, solvates, hydrates, and clathrates thereof are discussed. Also discussed are methods of using and pharmaceutical compositions comprising the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione are disclosed. The methods include methods of treating and/or preventing disorders ameliorated by the reduction of levels of TNF-α or the inhibition of PDE4.

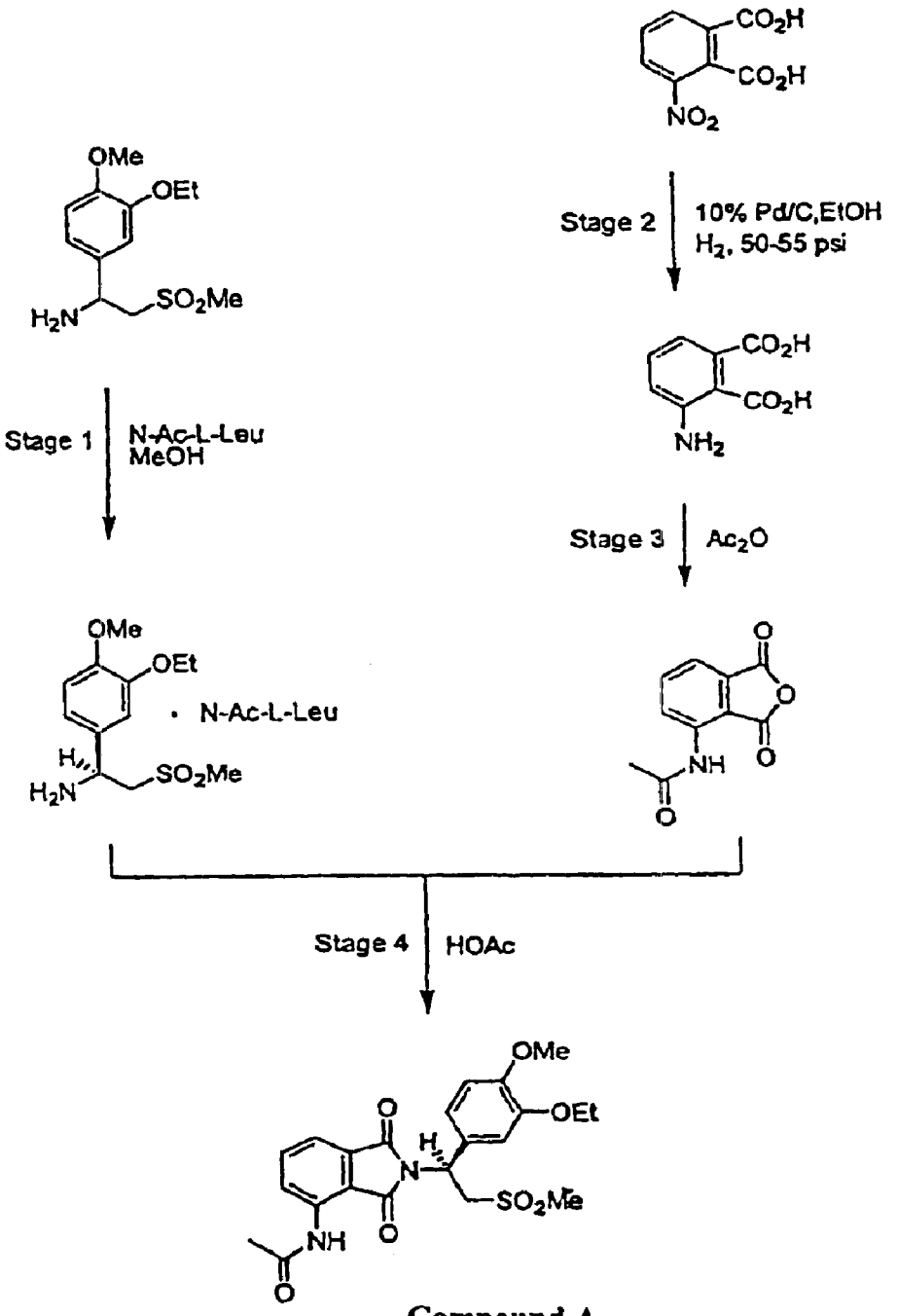
23 Claims, 2 Drawing Sheets

U.S. PATENT DOCUMENTS

5,736,570	A	4/1998	Muller et al.
5,798,373	A	8/1998	Warrellow
5,801,195	A	9/1998	Muller et al.
5,849,770	A	12/1998	Head et al.
5,877,200	A	3/1999	Muller
5,891,896	A	4/1999	Warrellow et al.
6,011,060	A	1/2000	Laurent et al.
6,020,339	A	2/2000	Perrier et al.
6,020,358	A	2/2000	Muller et al.
6,034,089	A	3/2000	Han et al.
6,046,221	A	4/2000	Muller et al.
6,069,156	A	5/2000	Oku et al.
6,162,830	A	12/2000	Connor et al.
6,166,041	A	12/2000	Cavalla et al.
6,177,471	B1	1/2001	Menander et al.
6,204,275	B1	3/2001	Friesen et al.
6,218,400	B1	4/2001	Daugan et al.
6,300,335	B1	10/2001	Campbell et al.
6,316,472	B1	11/2001	Frenette et al.
6,333,354	B1	12/2001	Schudt

OTHER PUBLICATIONS

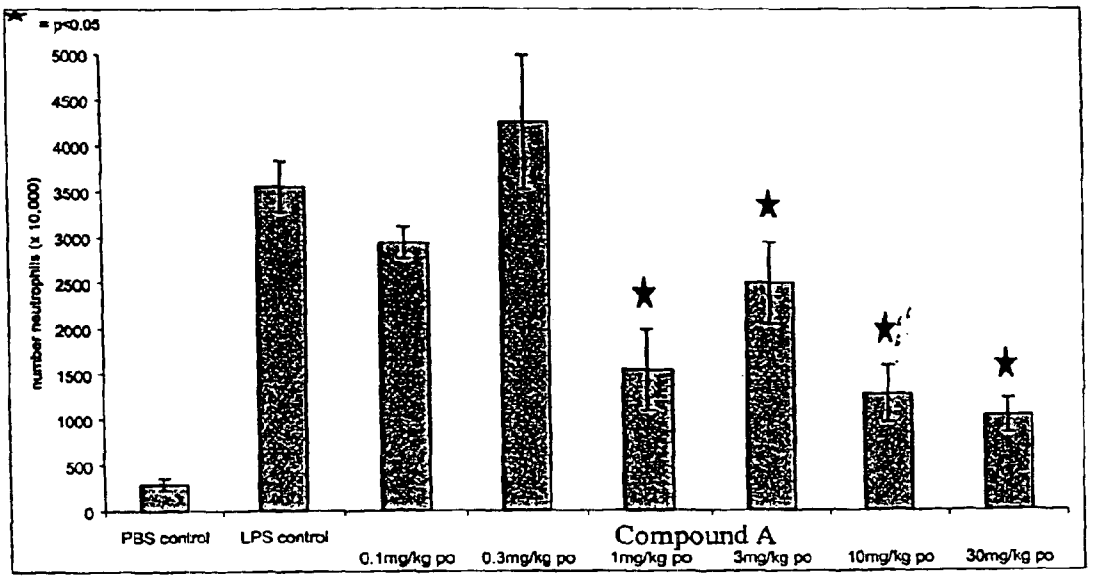
Au et al., 1998, *Brit. J. Pharm.* 123:1260–1266.
Baehr et al., 1979, *J. Biol. Chem.* 254:11669.
Baughman et al., 1990, *J. Lab. Clin. Med.* 115:36–42.
Beavo and Reifsnyder, *Trends in Pharm.,* 11, 150–55, 1990.
Bissonnette et al., 1989, *Inflammation* 13:329–339.
Bloom and Beavo 1996, *Proc. Natl. Acad. Sci. USA* 93:14188–14192.
Brackeen, M.F. et al., 1995, "Design and synthesis of conformationally constrained analogues of 4–(3–butoxy–4–methoxybenzyl) imidazolidin–2–one (Ro 20–1724) as potent inhibitors of cAMP–specific phosphodiesterase", *J. Med. Chem.* 38:4848–54.
Carstensen, Jens T., 1995, *Drug Stability: Principles & Practice*, 2nd ed., Marcel Dekker, New York, NY pp. 379–380.
Casini et al., 1964, Farmaco Ed. Sci. 19:563.
Clouse et al., 1989, *J. Immunol.* 142:431–438.
Derian et al., 1995, *J. Immunol.* 154:308–317.
Duh et al., 1989, *Proc. Nat. Acad. Sci.* 86:5974–5978.
Featherstone, R.L., et al., 2000, "Comparison of phosphodiesterase inhibitors of differing isoenzyme selectivity added to St. Thomas' hospital cardioplegic solution used for hypothermic preservation of rat lungs", Am. J. Respir. Crit. Care Med. 162:850–6.
Gillespie et al., 1989, *Mol. Pharm.* 36:773.
Hidaka and Asano 1976, *Biochem. Biophys. Acta* 429:485.
Hinshaw et al. 1990, *Circ. Shock* 30:2797–292.
Holler et al., 1990, *Blood* 75:1101–1016.
Johnson et al., 1989, *Endocrinology* 124:1424–1427.
List et al., 1990, *J. Clin. Oncol.* 8:1424.MDS.
Merck Manual (1999) 17th ed., 953.
Monté et al., 1990, *Blood* 79:2670.
Muller, George, et al., 1999, *Bioorganic& Medicinal Chemistry Letters* 9; pp. 1625–1630.
Muller et al., 1998, *Bioorg.& Med Chem Lett.* 8:2669–2674.
Muller, et al., 1996, *J. Med. Chem.* 39:3238.
Nicholson et al., 1991, *Trends Pharmaco. Sci.* 12:19.
Shealy et al., 1965, "D–and L–thalidomide." *Chem. Indus.* 12;24:1030–1.
Tierney, et al.,ed., 1998, Current Medical Diagnosis & Treatment, 37th ed., Appleton & Lange, pp. 499.
Verghese, et al., *Journal of Pharmacology and Experimental Therapeutics*, 272(3), 1313–1320, 1995.
Van Dullemen et al., 1995, *Gastroenterology*, 109:129–135.
Wilen, S.H., et al., 1977, *Tetrahedron* 33:2725.
Wilen, S.H., 1972, *Tables of Resolving Agents and Optical Resoutions* (E.L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, IN) p. 268.
Wolff, Manfred E., *ed., Burger's Medicinal Chemistry and Drug Discovery*, 5th ed. 1995 172–178, 949–982.
Akazome, M. et al., 1997, "Asymmetric recognition of 1–arylethylamines by (R)–phenylglycyl–(R)–phenylglycine and its mechanism," Tetrahedron: Asymmetry, Elsevier Scince Publishers, Amsterdam, NL, 8(14):2331–2336.
Luke, G.P. et al., 1999, "Synthesis of(S)–5–(1–aminoethyl)–2–(cyclohexylmethoxy) benzamide," Tetrahedron: Asymmetry, Elsevier Science Publishers, Amsterdam, NL, 10(22):4393–4403.



Compound A

FIG. 1

FIG 2.


* = p<0.05
number neutrophils (x 10,000)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
PBS control
LPS control
0.1mg/kg po
0.3mg/kg po
1mg/kg po
3mg/kg po
10mg/kg po
30mg/kg po
Compound A

(+)-2-[1-(3-ETHOXY-4-METHOXYPHENYL)-2-METHYLSULFONYLETHYL]-4-ACETYLAMINOISOINDOLINE-1,3-DIONE: METHODS OF USING AND COMPOSITIONS THEREOF

This application claims the benefit of U.S. Provisional Application No. 60/366,515 filed Mar. 20, 2002 and U.S. Provisional Application No. 60/438,450 filed Jan. 7, 2003 both of which are incorporated herein by reference in their entireties.

1. FIELD OF INVENTION

The invention relates to methods of using and compositions comprising the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

2. BACKGROUND OF THE INVENTION

Tumor necrosis factor alpha, (TNF-α) is a cytokine that is released primarily by mononuclear phagocytes in response to immunostimulators. TNF-α is capable of enhancing most cellular processes, such as differentiation, recruitment, proliferation, and proteolytic degradation. At low levels, TNF-α confers protection against infective agents, tumors, and tissue damage. But TNF-α also has a role in many diseases. When administered to mammals or humans, TNF-α causes or aggravates inflammation, fever, cardiovascular effects, hemorrhage, coagulation, and acute phase responses similar to those seen during acute infections and shock states. Enhanced or unregulated TNF-α production has been implicated in a number of diseases and medical conditions, for example, cancers, such as solid tumors and blood-born tumors; heart disease, such as congestive heart failure; and viral, genetic, inflammatory, allergic, and autoimmune diseases.

Adenosine 3',5'-cyclic monophosphate (cAMP) also plays a role in many diseases and conditions, such as but not limited to asthma and inflammation, and other conditions (Lowe and Cheng, *Drugs of the Future,* 17(9), 799–807, 1992). It has been shown that the elevation of cAMP in inflammatory leukocytes inhibits their activation and the subsequent release of inflammatory mediators, including TNF-α and NF-κB. Increased levels of cAMP also leads to the relaxation of airway smooth muscle.

It is believed that the primary cellular mechanism for the inactivation of cAMP is the breakdown of cAMP by a family of isoenzymes referred to as cyclic nucleotide phosphodiesterases (PDE) (Beavo and Reitsnyder, *Trends in Pharm.,* 11, 150–155, 1990). There are eleven known PDE families. It is recognized, for example, that the inhibition of PDE type IV is particularly effective in both the inhibition of inflammatory mediator release and the relaxation of airway smooth muscle (Verghese, et al., *Journal of Pharmacology and Experimental Therapeutics,* 272(3), 1313–1320, 1995). Thus, compounds that inhibit PDE4 (PDE IV) specifically, may inhibit inflammation and aid the relaxation of airway smooth muscle with a minimum of unwanted side effects, such as cardiovascular or anti-platelet effects. Currently used PDE4 inhibitors lack the selective action at acceptable therapeutic doses.

Cancer is a particularly devastating disease, and increases in blood TNF-α levels are implicated in the risk of and the spreading of cancer. Normally, in healthy subjects, cancer cells fail to survive in the circulatory system, one of the reasons being that the lining of blood vessels acts as a barrier

to tumor-cell extravasation. But increased levels of cytokines have been shown to substantially increase the adhesion of cancer cells to endothelium in vitro. One explanation is that cytokines, such as TNF-α, stimulate the biosynthesis and expression of a cell surface receptors called ELAM-1 (endothelial leukocyte adhesion molecule). ELAM-1 is a member of a family of calcium-dependent cell adhesion receptors, known as LEC-CAMs, which includes LECAM-1 and GMP-140. During an inflammatory response, ELAM-1 on endothelial cells functions as a "homing receptor" for leukocytes. Recently, ELAM-1 on endothelial cells was shown to mediate the increased adhesion of colon cancer cells to endothelium treated with cytokines (Rice et al., 1989, *Science* 246:1303–1306).

Inflammatory diseases such as arthritis, related arthritic conditions (e.g., osteoarthritis and rheumatoid arthritis), inflammatory bowel disease (e.g., Crohn's disease and ulcerative colitis), sepsis, psoriasis, atopic dermatitis, contact dermatitis, and chronic obstructive pulmonary disease, chronic inflammatory pulmonary diseases are also prevalent and problematic ailments. TNF-α plays a central role in the inflammatory response and the administration of their antagonists block chronic and acute responses in animal models of inflammatory disease.

Enhanced or unregulated TNF-α production has been implicated in viral, genetic, inflammatory, allergic, and autoimmune diseases. Examples of such diseases include but are not limited to: HIV; hepatitis; adult respiratory distress syndrome; bone-resorption diseases; chronic obstructive pulmonary diseases; chronic pulmonary inflammatory diseases; asthma, dermatitis; cystic fibrosis; septic shock; sepsis; endotoxic shock; hemodynamic shock; sepsis syndrome; post ischemic reperfusion injury; meningitis; psoriasis; fibrotic disease; cachexia; graft rejection; autoimmune disease; rheumatoid spondylitis; arthritic conditions, such as rheumatoid arthritis and osteoarthritis; osteoporosis; Crohn's disease; ulcerative colitis; inflammatory-bowel disease; multiple sclerosis; systemic lupus erythrematosus; ENL in leprosy; radiation damage; asthma; and hyperoxic alveolar injury. Tracey et al., 1987, *Nature* 330:662–664 and Hinshaw et al., 1990, *Circ. Shock* 30:279–292 (endotoxic shock); Dezube et al., 1990, *Lancet,* 335:662 (cachexia); Millar et al., 1989, *Lancet* 2:712–714 and Ferrai-Baliviera et al., 1989, *Arch. Surg.* 124:1400–1405 (adult respiratory distress syndrome); Bertolini et al., 1986, *Nature* 319:516–518, Johnson et al., 1989, *Endocrinology* 124:1424–1427, Holler et al., 1990, *Blood* 75:1011–1016, and Grau et al., 1989, *N. Engl. J. Med.* 320:1586–1591 (bone resorption diseases); Pignet et al., 1990, *Nature,* 344:245–247, Bissonnette et al., 1989, *Inflammation* 13:329–339 and Baughman et al., 1990, *J. Lab. Clin. Med.* 115:36–42 (chronic pulmonary inflammatory diseases); Elliot et al., 1995, *Int. J. Pharmac.* 17:141–145 (rheumatoid arthritis); von Dullemen et al., 1995, *Gastroenterology,* 109:129–135 (Crohn's disease); Duh et al., 1989, *Proc. Nat. Acad. Sci.* 86:5974–5978, Poll et al., 1990, *Proc. Nat. Acad. Sci.* 87:782–785, Monto et al., 1990, *Blood* 79:2670, Clouse et al., 1989, *J. Immunol.* 142, 431–438, Poll et al., 1992, *AIDS Res. Hum. Retrovirus,* 191–197, Poli et al. 1990, *Proc. Natl. Acad. Sci.* 87:782–784, Folks et al., 1989, PNAS 86:2365–2368 (HIV and opportunistic infections resulting from HIV).

Pharmaceutical compounds that can block the activity or inhibit the production of certain cytokines, including TNF-α, may be beneficial therapeutics. Many small-molecule inhibitors have demonstrated an ability to treat or prevent inflammatory diseases implicated by TNF-α (for a review,

see Lowe, 1998 *Exp. Opin. Ther. Patents* 8:1309–1332). One such class of molecules are the substituted phenethylsulfones described in U.S. Pat. No. 6,020,358.

3. SUMMARY OF THE INVENTION

This invention relates to methods of treating diseases and disorders utilizing an enantiomer of a substituted phenethylsulfone compound and pharmaceutically acceptable salts, hydrates, solvates, clathrates, prodrugs and polymorphs thereof and methods for reducing the level of cytokines and their precursors in mammals. The invention also relates to pharmaceutical compositions comprising an enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione and a pharmaceutically acceptable carrier. The invention further relates to an enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione substantially free of its other enantiomer.

This invention particularly relates to the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione. This compound is believed to have increased potency and other benefits as compared to its racemate—2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

The invention encompasses the use of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione for treating or preventing diseases or disorders ameliorated by the inhibition of TNF-α production in mammals. In certain embodiments, this treatment includes the reduction or avoidance of adverse effects. Such disorders include, but are not limited to, cancers, including, but not limited to cancer of the head, thyroid, neck, eye, skin, mouth, throat, esophagus, chest, bone, blood, bone marrow, lung, colon, sigmoid, rectum, stomach, prostate, breast, ovaries, kidney, liver, pancreas, brain, intestine, heart, adrenal, subcutaneous tissue, lymph nodes, heart, and combinations thereof. Specific cancers that can be treated by this method are multiple myeloma, malignant melanoma, malignant glioma, leukemia and solid tumors.

The invention also encompasses the use of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione in the treatment or prevention of heart disease, including, but not limited to congestive heart failure, cardiomyopathy, pulmonary edema, endotoxin-mediated septic shock, acute viral myocarditis, cardiac allograft rejection, and myocardial infarction.

The invention also encompasses the use of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione to treat diseases or disorders ameliorated by the inhibition of PDE4. For example, the compounds and compositions of the invention may be useful to treat or prevent viral, genetic, inflammatory, allergic, and autoimmune diseases. Examples of such diseases include, but are not limited to: HIV; hepatitis; adult respiratory distress syndrome; bone-resorption diseases; chronic obstructive pulmonary diseases; chronic pulmonary inflammatory diseases; dermatitis; inflammatory skin disease, atopic dermatitis, cystic fibrosis; septic shock; sepsis; endotoxic shock; hemodynamic shock; sepsis syndrome; post ischemic reperfusion injury; meningitis; psoriasis; fibrotic disease; cachexia; graft rejection including graft versus host disease; auto-immune disease; rheumatoid spondylitis; arthritic conditions, such as rheumatoid arthritis and osteoarthritis; osteoporosis; Crohn's disease; ulcerative colitis; inflammatory-bowel disease; multiple sclerosis; systemic lupus erythrematosus; erythema nodosum leprosum (ENL) in leprosy; radiation damage; asthma; and hyperoxic alveolar injury.

In yet another embodiment, the stereomerically pure (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is also useful in the treatment or prevention of microbial infections or the symptoms of microbial infections including, but not limited to, bacterial infections, fungal infections, malaria, mycobacterial infection, and opportunistic infections resulting from HIV.

The invention further encompasses pharmaceutical compositions and single unit dosage forms comprising an enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione and pharmaceutically acceptable polymorphs, prodrugs, salts, hydrates, clathrates, and solvates thereof.

In a separate embodiment, the invention encompasses the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

In a further embodiment, the invention encompasses a method of producing a stereomerically pure enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione which comprises contacting 1-(3-Ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethylamine with a chiral amino acid and contacting the product of the first step with N-(1,3-Dioxo-1,3-dihydro-isobenzofuran-4-yl)-acetamide. In a related embodiment the invention encompasses a chiral salt of 1-(3-Ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethylamine.

3.1. BRIEF DESCRIPTION OF THE FIGURES

FIG. **1**. illustrates the preparation of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

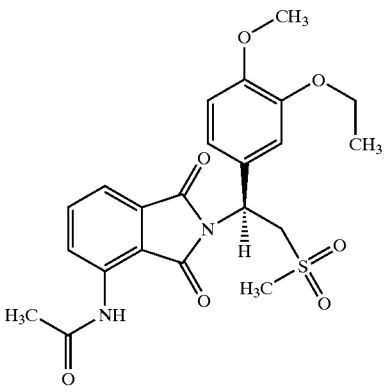
FIG. **2**. illustrates the effect of the enantiomer of the invention on LPS-induced neutrophilia in the lungs of conscious ferrets.

3.2. DEFINITIONS

As used herein, term "Compound A" refers to an enantiomerically pure form of 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione which comes off of an HPLC column at about 25.4 minutes when that column is a 150 mm×4.6 mm Ultron Chiral ES-OVS chiral HPLC column (Agilent Technology), the eluent is 15:85 ethanol: 20 mM KH_2PO_4 at pH 3.5, and the observation wavelength is 240 nm. The ^1H NMR spectrum of compound A is substantially as follows: δ($CDCl_3$): 1.47 (t, 3H), 2.26 (s, 3H), 2.87 (s, 3H), 3.68–3.75 (dd, 1H), 3.85 (s, 3H), 4.07–4.15 (q, 2H), 4.51–4.61 (dd, 1H), 5.84–5.90 (dd, 1H), 6.82–8.77 (m, 6H), 9.46 (s, 1H). The ^{13}C NMR spectrum of Compound A is substantially as follows δ(DMSO-d_6): 14.66, 24.92, 41.61, 48.53, 54.46, 55.91, 64.51, 111.44, 112.40, 115.10, 118.20, 120.28, 124.94, 129.22, 131.02, 136.09, 137.60, 148.62, 149.74, 167.46, 169.14, 169.48. Compound A dissolved in methanol also rotates plane polarized light in the (+) direction.

Without being limited by theory, Compound A is believed to be S-{2-[1-(3-ethoxy-4-methoxyphenyl)-2-

methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione}, which has the following structure:



As used herein, the term "patient" refers to a mammal, particularly a human.

As used herein, the term "pharmaceutically acceptable salts" refer to salts prepared from pharmaceutically acceptable non-toxic acids or bases including inorganic acids and bases and organic acids and bases. Suitable pharmaceutically acceptable base addition salts for the compound of the present invention include metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from lysine, N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. Suitable non-toxic acids include, but are not limited to, inorganic and organic acids such as acetic, alginic, anthranilic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethenesulfonic, formic, fumaric, furoic, galacturonic, gluconic, glucuronic, glutamic, glycolic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phenylacetic, phosphoric, propionic, salicylic, stearic, succinic, sulfanilic, sulfuric, tartaric acid, and p-toluenesulfonic acid. Specific non-toxic acids include hydrochloric, hydrobromic, phosphoric, sulfuric, and methanesulfonic acids. Examples of specific salts thus include hydrochloride and mesylate salts.

As used herein and unless otherwise indicated, the term "prodrug" means a derivative of a compound that can hydrolyze, oxidize, or otherwise react under biological conditions (in vitro or in vivo) to provide the compound. Examples of prodrugs include, but are not limited to, derivatives and metabolites of Compound A that include biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Prodrugs can typically be prepared using well-known methods, such as those described by 1 *Burger's Medicinal Chemistry and Drug Discovery,* 172–178, 949–982 (Manfred E. Wolff ed., 5th ed. 1995).

As used herein and unless otherwise indicated, the terms "biohydrolyzable amide," "biohydrolyzable ester," "biohydrolyzable carbamate," "biohydrolyzable carbonate," "biohydrolyzable ureide," "biohydrolyzable phosphate" mean an amide, ester, carbamate, carbonate, ureide, or phosphate, respectively, of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties in vivo, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted in vivo to the biologically active compound. Examples of biohydrolyzable esters include, but are not limited to, lower alkyl esters, alkoxyacyloxy esters, alkyl acylamino alkyl esters, and choline esters. Examples of biohydrolyzable amides include, but are not limited to, lower alkyl amides, α-amino acid amides, alkoxyacyl amides, and alkylaminoalkylcarbonyl amides. Examples of biohydrolyzable carbamates include, but are not limited to, lower alkylamines, substituted ethylenediamines, aminoacids, hydroxyalkylamines, heterocyclic and heteroaromatic amines, and polyether amines.

As used herein and unless otherwise indicated, the term "stereomerically pure" means a composition that comprises one stereoisomer of a compound and is substantially free of other stereoisomers of that compound. For example, a stereomerically pure composition of a compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically pure composition of a compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, more preferably greater than about 90% by weight of one stereoisomer of the compound and less than about 10% by weight of the other stereoisomers of the compound, even more preferably greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, and most preferably greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound.

As used herein and unless otherwise indicated, the term "enantiomerically pure" means a stereomerically pure composition of a compound having one chiral center.

As used herein, term "adverse effects" includes, but is not limited to gastrointestinal, renal and hepatic toxicities, leukopenia, increases in bleeding times due to, e.g., thrombocytopenia, and prolongation of gestation, nausea, vomiting, somnolence, asthenia, dizziness, teratogenicity, extra-pyramidal symptoms, akathisia, cardiotoxicity including cardiovascular disturbances, inflammation, male sexual dysfunction, and elevated serum liver enzyme levels. The term "gastrointestinal toxicities" includes but is not limited to gastric and intestinal ulcerations and erosions. The term "renal toxicities" includes but is not limited to such conditions as papillary necrosis and chronic interstitial nephritis.

As used herein and unless otherwise indicated, the phrases "reduce or avoid adverse effects" and "reducing or avoiding adverse effects" mean the reduction of the severity of one or more adverse effects as defined herein.

It should be noted that if there is a discrepancy between a depicted structure and a name given that structure, the depicted structure is to be accorded more weight. In addition, if the stereochemistry of a structure or a portion of a structure is not indicated with, for example, bold or dashed lines, the structure or portion of the structure is to be interpreted as encompassing all stereoisomers of it.

4. DETAILED DESCRIPTION OF THE INVENTION

This invention relates to stereomerically pure Compound A, which is an enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, substantially free of its

other enantiomer, as well as novel methods using, and compositions comprising stereomerically pure Compound A. For example, the present invention encompasses the in vitro and in vivo use of Compound A, and the incorporation of Compound A into pharmaceutical compositions and single unit dosage forms useful in the treatment and prevention of a variety of diseases and disorders. Diseases and disorders which are ameliorated by the reduction of levels of TNF-α or inhibition of PDE4 are well known in the art and are described herein. Specific methods of the invention reduce or avoid the adverse effects associated with compounds used as TNF-α inhibitor. Other specific methods of the invention reduce or avoid the adverse effects associated with use of racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

Specific methods of the invention include methods of treating or preventing diseases and disorders including, but not limited to, solid tumor cancers, blood-born cancers and inflammatory diseases.

Pharmaceutical and dosage forms of the invention, which comprise Compound A or a pharmaceutically acceptable polymorph, prodrug, salt, clathrate, solvate or hydrate thereof, can be used in the methods of the invention.

Without being limited by theory, it is believed that Compound A can inhibit TNF-α production. Consequently, a first embodiment of the invention relates to a method of inhibiting TNF-α production which comprises contacting a cell exhibiting abnormal TNF-α production with an effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. In a particular embodiment, the invention relates to a method of inhibiting TNF-α production which comprises contacting a mammalian cell exhibiting abnormal TNF-α production with an effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof.

The invention also relates to a method of treating or preventing disorders ameliorated by the reduction of levels of TNF-α in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof.

A further embodiment of the invention relates to a method of treating or preventing cancer, including but not limited to, solid tumor, blood-born tumor, leukemias, and in particular, multiple myeloma in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically effective amount of stereomerically pure compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof; in particular wherein the patient is a mammal.

In another embodiment, the invention relates to a method of inhibiting PDE4 which comprises contacting PDE4 with an effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof.

In another embodiment, the invention relates to a method of controlling cAMP levels in a cell which comprises contacting a cell with an effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. As used herein the term "controlling cAMP levels" includes preventing or reducing the rate of the breakdown of Adenosine 3',5'-cyclic monophosphate (cAMP) in a cell or increasing the amount of Adenosine 3',5'-cyclic monophosphate present in a cell, preferably a mammalian cell, more preferably a human cell. In a particular method, the rate of cAMP breakdown is reduced by about 10, 25, 50, 100, 200, or 500 percent as compared to the rate in comparable cells which have not been contacted with a compound of the invention.

A further embodiment of the invention relates to a method of treating or preventing diseases or disorders ameliorated by the inhibition of PDE4 in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. Disorders ameliorated by the inhibition of PDE4 include, but are not limited to, asthma, inflammation (e.g., inflammation due to reperfusion), chronic or acute obstructive pulmonary diseases, chronic or acute pulmonary inflammatory diseases, inflammatory bowel disease, Crohn's Disease, Bechet's Disease, or colitis.

A further embodiment of the invention relates to a method of treating or preventing depression, asthma, inflammation (e.g., contact dermatitis, atopic dermatitis, psoriais, rheumatoid arthritis, osteoarthritis, inflammatory skin disease, inflammation due to reperfusion), chronic or acute obstructive pulmonary diseases, chronic or pulmonary inflammatory diseases, inflammatory bowel disease, Crohn's Disease, Bechet's Disease or colitis in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof; in particular wherein the patient is a mammal.

A separate embodiment of the invention encompasses methods of treating or preventing Myclodysplastic syndrome (MDS) which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. MDS refers to a diverse group of hematopoietic stem cell disorders. MDS is characterized by a cellular marrow with impaired morphology and maturation (dysmyelopoiesis), peripheral blood cytopenias, and a variable risk of progression to acute leukemia, resulting from ineffective blood cell production. See The Merck Manual 953 (17th ed. 1999) and List et al., 1990, *J. Clin. Oncol.* 8:1424.MDS

A separate embodiment of the invention encompasses methods of treating or preventing Myeloproliferative disease (MPD) which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. Myeloproliferative disease (MPD) refers to a group of disorders characterized by clonal abnormalities of the hematopoietic stem cell. See e.g., Current Medical Diagnosis & Treatment, pp. 499 (37th ed., Tierney et al. ed, Appleton & Lange, 1998).

The invention also encompasses a method of treating, preventing or managing complex regional pain syndrome, which comprises administering to a patient in need of such treatment, prevention or management a therapeutically or

prophylactically effective amount of a stereomerically pure Compound A, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. In a specific embodiment, the administration is before, during or after surgery or physical therapy directed at reducing or avoiding a symptom of complex regional pain syndrome in the patient.

In particular methods of the invention, stereomerically pure Compound A, or a pharmaceutically acceptable polymorph, prodrug, salt, solvate, hydrate, or clathrate thereof, is adjunctively administered with at least one additional therapeutic agent. Examples of additional therapeutic agents include, but are not limited to, anti-cancer drugs, anti-inflammatories, antihistamines and decongestants.

4.1. Synthesis and Preparation

Racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is readily prepared using the methods in U.S. Pat. No. 6,020,358, which is incorporated herein by reference.

Compound A can be isolated from the racemic compound by techniques known in the art. Examples include, but are not limited to, the formation of chiral salts and the use of chiral or high performance liquid chromatography "HPLC" and the formation and crystallization of chiral salts. See, e.g., Jacques, J., et al., *Enantiomers, Racemates and Resolutions* (Wiley-Interscience, New York, 1981); Wilen, S. H., et al, *Tetrahedron* 33:2725 (1977); Eliel, E. L., *Stereochemistry of Carbon Compounds* (McGraw-Hill, NY, 1962); and Wilen, S. H., *Tables of Resolving Agents and Optical Resolutions* p. 268 (E. L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, Ind., 1972).

In a specific method, Compound A is synthesized from 3-acetamidophthalic anhydride and a chiral amino acid salt of (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine. Chiral amino acid salts of (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine include, but not limited to salts formed with the L isomers of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, ornithine, 4-aminobutyric acid, 2 amino isobutyric acid, 3 amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, and N-acetyl-leucine. A specific chiral amino acid salt is (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine N-acetyl-L-leucine salt, which is resolved from 2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine and N-acetyl-L-leucine in methanol.

4.2. Methods of Treatment

The invention encompasses methods of treating and preventing diseases or disorders ameliorated by the reduction of levels of TNF-α in a patient which comprise administering to a patient in need of such treatment or prevention a therapeutically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof.

Disorders ameliorated by the inhibition of TNF-α include, but are not limited to: heart disease, such as congestive heart failure, cardiomyopathy, pulmonary edema, endotoxin-mediated septic shock, acute viral myocarditis, cardiac allograft rejection, and myocardial infarction; solid tumors, including but not limited to, sarcoma, carcinomas, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, Kaposi's sarcoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma; and blood-born tumors including but not limited to, acute lymphoblastic leukemia "ALL", acute lymphoblastic B-cell leukemia, acute lymphoblastic T-cell leukemia, acute myeloblastic leukemia "AML", acute promyelocytic leukemia "APL", acute monoblastic leukemia, acute erythroleukemic leukemia, acute megakaryoblastic leukemia, acute myelomonocytic leukemia, acute nonlymphocyctic leukemia, acute undifferentiated leukemia, chronic myelocytic leukemia "CML", chronic lymphocytic leukemia "CLL", hairy cell leukemia, multiple myeloma and acute and chronic leukemias, for example, lymphoblastic, myelogenous, lymphocytic, and myelocytic leukemias.

Specific methods of the invention further comprise the administration of an additional therapeutic agent (i.e., a therapeutic agent other than Compound A). Examples of additional therapeutic agents include, but are not limited to, anti-cancer drugs such as, but are not limited to: alkylating agents, nitrogen mustards, ethylenimines, methylmelamines, alkyl sulfonates, nitrosoureas, triazenes, folic acid analofs, pyrimidine analogs, purine analogs, vinca alkaloids, epipodophyllotoxins, antibiotics, topoisomerase inhibitors and anti-cancer vaccines.

Specific additional therapeutic agents include, but are not limited to: acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carnustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cisplatin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; dactinomycin; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; docetaxel; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; flurocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; ilmofosine; interleukin II (including recombinant interleukin II,

or rIL2), interferon alfa-2a; interferon alfa-2b; interferon alfa-n1; interferon alfa-n3; interferon beta-I a; interferon gamma-I b; iproplatin; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedepa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; paclitaxel; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; rogletimide; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; triciribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin hydrochloride. Other anti-cancer drugs include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorlns; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl spiromustine; docetaxel; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bisacridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras famesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol;

saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofuran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

The invention further encompasses a method of treating or preventing diseases or disorders ameliorated by the inhibition of PDE4 in a patient which comprise administering to a patient in need of such treatment or prevention a therapeutically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. Disorders ameliorated by the inhibition of PDE4 include, but are not limited to, asthma, inflammation, chronic or acute obstructive pulmonary disease, chronic or acute pulmonary inflammatory disease, inflammatory bowel disease, Crohn's Disease, Bechet's Disease, colitis, ulcerative colitis and arthritis or inflammation due to reperfusion. In a preferred embodiment, the disease or disorder to be treated or prevented is chronic obstructive pulmonary disease.

Specific methods of the invention can comprise the administration of an additional therapeutic agent such as, but not limited to, anti-inflammatory drugs, antihistamines and decongestants. Examples of such additional therapeutic agents include, but are not limited to: antihistamines including, but not limited to, ethanolamines, ethylenediamines, piperazines, and phenothiazines; antinflammatory drugs; NSAIDS, including, but not limited to, aspirin, salicylates, acetominophen, indomethacin, sulindac, etodolac, fenamates, tolmetin, ketorolac, diclofenac, ibuprofen, naproxen, fenoprofen, ketoprofen, flurbiprofen, oxaprozin, piroxicam, meloxicam, pyrazolon derivatives; and steriods including, but not limited to, cortical steroids and adrenocortical steroids.

Specific methods of the invention avoid or reduce drug-drug interactions and other adverse effects associated with agents used in the treatment of such disorders, including racemic substituted phenylethylsulfones. Without being limited by any theory, stereomerically pure Compound A may further provide an overall improved therapeutic effectiveness, or therapeutic index, over racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione. For example, a smaller amount of the drug may in some circumstances be administered to attain the same level of effectiveness.

As stated above, the active compound of the invention (i.e., Compound A) may be used in the treatment or prevention of a wide range of diseases and conditions. The magnitude of a prophylactic or therapeutic dose of a particular active ingredient of the invention in the acute or chronic management of a disease or condition will vary, however, with the nature and severity of the disease or condition, and the route by which the active ingredient is administered. The dose, and perhaps the dose frequency, will also vary according to the age, body weight, and response of the individual patient. Suitable dosing regimens can be readily selected by those skilled in the art with due consideration of such factors. In general, the recommended daily dose range for the conditions described herein lie within the range of from about 1 mg to about 1000 mg per day, given as a single once-a-day dose preferably as divided doses throughout a day. More specifically, the daily dose is administered twice daily in equally divided doses. Specifically, a daily dose range should be from about 5 mg to about 500 mg per day, more specifically, between about 10 mg and about 200 mg per day. Specifically, the daily dose may be administered in 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 50 mg, or 100 mg dosage forms. In managing the patient, the therapy should be initiated at a lower dose, perhaps about 1 mg to about 25 mg, and increased if necessary up to about 200 mg to about 1000 mg per day as either a single dose or divided doses, depending on the patient's global response. Alternatively, the daily dose is from 0.01 mg/kg to 100 mg/kg.

It may be necessary to use dosages of the active ingredient outside the ranges disclosed herein in some cases, as will be apparent to those of ordinary skill in the art. Furthermore, it is noted that the clinician or treating physician will know how and when to interrupt, adjust, or terminate therapy in conjunction with individual patient response.

The phrases "therapeutically effective amount", "prophylactically effective amount" and "therapeutically or prophylactically effective amount," as used herein encompasses the above described dosage amounts and dose frequency schedules. Different therapeutically effective amounts may be applicable for different diseases and conditions, as will be readily known by those of ordinary skill in the art. Similarly, amounts sufficient to treat or prevent such disorders, but insufficient to cause, or sufficient to reduce, adverse effects associated with racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione are also encompassed by the above described dosage amounts and dose frequency schedules.

4.3. Pharmaceutical Compositions

Pharmaceutical compositions and single unit dosage forms comprising Compound A, or a pharmaceutically acceptable polymorph, prodrug, salt, solvate, hydrate, or clathrate thereof, are encompassed by the invention. Individual dosage forms of the invention may be suitable for oral, mucosal (including rectal, nasal, or vaginal), parenteral (including subcutaneous, intramuscular, bolus injection, intraarterial, or intravenous), sublingual, transdermal, buccal, or topical administration.

Pharmaceutical compositions and dosage forms of the invention comprise stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. Pharmaceutical compositions and dosage forms of the invention typically also comprise one or more pharmaceutically acceptable excipients.

A particular pharmaceutical composition encompassed by this embodiment comprises stereomerically pure Compound A, or a pharmaceutically acceptable polymorph, prodrug, salt, solvate, hydrate, or clathrate thereof, and at least one additional therapeutic agent. Examples of additional therapeutic agents include, but are not limited to: anti-cancer drugs and anti-inflammation therapies including, but not limited to, those listed above in section 4.2.

Single unit dosage forms of the invention are suitable for oral, mucosal (e.g., nasal, sublingual, vaginal, buccal, or rectal), parenteral (e.g., subcutaneous, intravenous, bolus injection, intramuscular, or intraarterial), or transdermal administration to a patient. Examples of dosage forms include, but are not limited to: tablets; caplets; capsules, such as soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suppositories; ointments; cataplasms (poultices); pastes; powders; dressings; creams; plasters; solutions; patches; aerosols (e.g., nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a patient, including suspensions (e.g., aqueous or non-aqueous liquid suspensions, oil-in-water emulsions, or a water-in-oil liquid emulsions), solutions, and elixirs; liquid dosage forms suitable for parenteral administration to a patient; and sterile solids (e.g., crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms suitable for parenteral administration to a patient.

The composition, shape, and type of dosage forms of the invention will typically vary depending on their use. For example, a dosage form used in the acute treatment of inflammation or a related disorder may contain larger amounts of one or more of the active ingredients it comprises than a dosage form used in the chronic treatment of the same disease. Similarly, a parenteral dosage form may contain smaller amounts of one or more of the active ingredients it comprises than an oral dosage form used to treat the same disease or disorder. These and other ways in which specific dosage forms encompassed by this invention will vary from one another will be readily apparent to those skilled in the art. See, e.g., *Remington's Pharmaceutical Sciences,* 18th ed., Mack Publishing, Easton Pa. (1990).

Typical pharmaceutical compositions and dosage forms comprise one or more excipients. Suitable excipients are well known to those skilled in the art of pharmacy, and non-limiting examples of suitable excipients are provided herein. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a patient. For example, oral dosage forms such as tablets may contain excipients not suited for use in parenteral dosage forms. The suitability of a particular excipient may also depend on the specific active ingredients in the dosage form.

Lactose-free compositions of the invention can comprise excipients that are well known in the art and are listed, for example, in the U.S. Pharmocopia (USP) SP (XXI)/NF (XVI). In general, lactose-free compositions comprise an active ingredient, a binder/filler, and a lubricant in pharmaceutically compatible and pharmaceutically acceptable amounts. Preferred lactose-free dosage forms comprise an active ingredient, microcrystalline cellulose, pre-gelatinized starch, and magnesium stearate.

This invention further encompasses anhydrous pharmaceutical compositions and dosage forms comprising active ingredients, since water can facilitate the degradation of some compounds. For example, the addition of water (e.g., 5%) is widely accepted in the pharmaceutical arts as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. See, e.g., Jens T. Carstensen, *Drug Stability: Principles & Practice,* 2d. Ed., Marcel Dekker, NY, N.Y., 1995, pp. 379–80. In effect, water and heat accelerate the decomposition of some compounds. Thus, the effect of water on a formulation can be of great significance since moisture and/or humidity are commonly encountered during manufacture, handling, packaging, storage, shipment, and use of formulations.

Anhydrous pharmaceutical compositions and dosage forms of the invention can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms that comprise lactose and at least one active ingredient that comprises a primary or secondary amine are preferably anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected.

An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions are preferably packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples of suitable packaging include, but are not limited to, hermetically sealed foils, plastics, unit dose containers (e.g., vials), blister packs, and strip packs.

The invention further encompasses pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will decompose. Such compounds, which are referred to herein as "stabilizers," include, but are not limited to, antioxidants such as ascorbic acid, pH buffers, or salt buffers.

Like the amounts and types of excipients, the amounts and specific types of active ingredients in a dosage form may differ depending on factors such as, but not limited to, the route by which it is to be administered to patients. However, typical dosage forms of the invention comprise compound A, or a pharmaceutically acceptable salt, solvate, clathrate, hydrate, polymoprh or prodrug thereof lie within the range of from about 1 mg to about 1000 mg per day, given as a single once-a-day dose in the morning but preferably as divided doses throughout the day taken with food. More specifically, the daily dose is administered twice daily in equally divided doses. Specifically, a daily dose range should be from about 5 mg to about 500 mg per day, more specifically, between about 10 mg and about 200 mg per day. In managing the patient, the therapy should be initiated at a lower dose, perhaps about 1 mg to about 25 mg, and increased if necessary up to about 200 mg to about 1000 mg per day as either a single dose or divided doses, depending on the patient's global response.

4.3.1. Oral Dosage Forms

Pharmaceutical compositions of the invention that are suitable for oral administration can be presented as discrete dosage forms, such as, but are not limited to, tablets (e.g., chewable tablets), caplets, capsules, and liquids (e.g., flavored syrups). Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of pharmacy well known to those skilled in the art. See generally, *Remington's Pharmaceutical Sciences,* 18th ed., Mack Publishing, Easton Pa. (1990).

Typical oral dosage forms of the invention are prepared by combining the active ingredient(s) in an intimate admixture

with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of preparation desired for administration. For example, excipients suitable for use in oral liquid or aerosol dosage forms include, but are not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents. Examples of excipients suitable for use in solid oral dosage forms (e.g., powders, tablets, capsules, and caplets) include, but are not limited to, starches, sugars, micro-crystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid excipients are employed. If desired, tablets can be coated by standard aqueous or non-aqueous techniques. Such dosage forms can be prepared by any of the methods of pharmacy. In general, pharmaceutical compositions and dosage forms are prepared by uniformly and intimately admixing the active ingredients with liquid carriers, finely divided solid carriers, or both, and then shaping the product into the desired presentation if necessary.

For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as powder or granules, optionally mixed with an excipient. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

Examples of excipients that can be used in oral dosage forms of the invention include, but are not limited to, binders, fillers, disintegrants, and lubricants. Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gelatin, natural and synthetic gums such as acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre-gelatinized starch, hydroxypropyl methyl cellulose, (e.g., Nos. 2208, 2906, 2910), microcrystalline cellulose, and mixtures thereof.

Examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof. The binder or filler in pharmaceutical compositions of the invention is typically present in from about 50 to about 99 weight percent of the pharmaceutical composition or dosage form.

Suitable forms of microcrystalline cellulose include, but are not limited to, the materials sold as AVICEL-PH-101, AVICEL-PH-103 AVICEL RC-581, AVICEL-PH-105 (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, Pa.), and mixtures thereof. An specific binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC-581. Suitable anhydrous or low moisture excipients or additives include AVICEL-PH-103™ and Starch 1500 LM.

Disintegrants are used in the compositions of the invention to provide tablets that disintegrate when exposed to an aqueous environment. Tablets that contain too much disintegrant may disintegrate in storage, while those that contain too little may not disintegrate at a desired rate or under the desired conditions. Thus, a sufficient amount of disintegrant that is neither too much nor too little to detrimentally alter the release of the active ingredients should be used to form solid oral dosage forms of the invention. The amount of disintegrant used varies based upon the type of formulation, and is readily discernible to those of ordinary skill in the art. Typical pharmaceutical compositions comprise from about 0.5 to about 15 weight percent of disintegrant, specifically from about 1 to about 5 weight percent of disintegrant.

Disintegrants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, agar-agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrilin potassium, sodium starch glycolate, potato or tapioca starch, pre-gelatinized starch, other starches, clays, other algins, other celluloses, gums, and mixtures thereof.

Lubricants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, and mixtures thereof. Additional lubricants include, for example, a syloid silica gel (AEROSIL 200, manufactured by W.R. Grace Co. of Baltimore, Md.), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Plano, Tex.), CAB-O-SIL (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, Mass.), and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about 1 weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated.

4.3.2. Delayed Release Dosage Forms

Active ingredients of the invention can be administered by controlled release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Pat. Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; and 4,008,719, 5,674,533, 5,059,595, 5,591,767, 5,120,548, 5,073,543, 5,639,476, 5,354,556, and 5,733,566, each of which is incorporated herein by reference. Such dosage forms can be used to provide slow or controlled-release of one or more active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the active ingredients of the invention. The invention thus encompasses single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled-release.

All controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition, controlled-release formulations can be used to affect the time of onset of action or other characteristics,

such as blood levels of the drug, and can thus affect the occurrence of side (e.g., adverse) effects.

Most controlled-release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled-release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, temperature, enzymes, water, or other physiological conditions or compounds.

4.3.3. Parenteral Dosage Forms

Parenteral dosage forms can be administered to patients by various routes including, but not limited to, subcutaneous, intravenous (including bolus injection), intramuscular, and intraarterial. Because their administration typically bypasses patients' natural defenses against contaminants, parenteral dosage forms are preferably sterile or capable of being sterilized prior to administration to a patient. Examples of parenteral dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions.

Suitable vehicles that can be used to provide parenteral dosage forms of the invention are well known to those skilled in the art. Examples include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

Compounds that increase the solubility of one or more of the active ingredients disclosed herein can also be incorporated into the parenteral dosage forms of the invention.

4.3.4. Transdermal, Topical, and Mucosal Dosage Forms

Transdermal, topical, and mucosal dosage forms of the invention include, but are not limited to, ophthalmic solutions, sprays, aerosols, creams, lotions, ointments, gels, solutions, emulsions, suspensions, or other forms known to one of skill in the art. See, e.g., *Remington's Pharmaceutical Sciences,* 16th and 18th eds., Mack Publishing, Easton Pa. (1980 & 1990); and *Introduction to Pharmaceutical Dosage Forms,* 4th ed., Lea & Febiger, Philadelphia (1985). Dosage forms suitable for treating mucosal tissues within the oral cavity can be formulated as mouthwashes or as oral gels. Further, transdermal dosage forms include "reservoir type" or "matrix type" patches, which can be applied to the skin and worn for a specific period of time to permit the penetration of a desired amount of active ingredients.

Suitable excipients (e.g., carriers and diluents) and other materials that can be used to provide transdermal, topical, and mucosal dosage forms encompassed by this invention are well known to those skilled in the pharmaceutical arts, and depend on the particular tissue to which a given pharmaceutical composition or dosage form will be applied. With that fact in mind, typical excipients include, but are not limited to, water, acetone, ethanol, ethylene glycol, propylene glycol, butane-1,3-diol, isopropyl myristate, isopropyl palmitate, mineral oil, and mixtures thereof to form lotions, tinctures, creams, emulsions, gels or ointments, which are non-toxic and pharmaceutically acceptable. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well known in the art. See, e.g., *Remington's Pharmaceutical Sciences,* 16th and 18th eds., Mack Publishing, Easton Pa. (1980 & 1990).

Depending on the specific tissue to be treated, additional components may be used prior to, in conjunction with, or subsequent to treatment with active ingredients of the invention. For example, penetration enhancers can be used to assist in delivering the active ingredients to the tissue. Suitable penetration enhancers include, but are not limited to: acetone; various alcohols such as ethanol, oleyl, and tetrahydrofuryl; alkyl sulfoxides such as dimethyl sulfoxide; dimethyl acetamide; dimethyl formamide; polyethylene glycol; pyrrolidones such as polyvinylpyrrolidone; Kollidon grades (Povidone, Polyvidone); urea; and various water-soluble or insoluble sugar esters such as Tween 80 (polysorbate 80) and Span 60 (sorbitan monostearate).

The pH of a pharmaceutical composition or dosage form, or of the tissue to which the pharmaceutical composition or dosage form is applied, may also be adjusted to improve delivery of one or more active ingredients. Similarly, the polarity of a solvent carrier, its ionic strength, or tonicity can be adjusted to improve delivery. Compounds such as stearates can also be added to pharmaceutical compositions or dosage forms to advantageously alter the hydrophilicity or lipophilicity of one or more active ingredients so as to improve delivery. In this regard, stearates can serve as a lipid vehicle for the formulation, as an emulsifying agent or surfactant, and as a delivery-enhancing or penetration-enhancing agent. Different salts, hydrates or solvates of the active ingredients can be used to further adjust the properties of the resulting composition.

4.3.5. Kits

Typically, active ingredients of the invention are preferably not administered to a patient at the same time or by the same route of administration. This invention therefore encompasses kits which, when used by the medical practitioner, can simplify the administration of appropriate amounts of active ingredients to a patient.

A typical kit of the invention comprises a unit dosage form of compound A, or a pharmaceutically acceptable salt, solvate, hydrate, clathrate, polymorph or prodrug thereof, and a unit dosage form of a second active ingredient. Examples of second active ingredients include, but are not limited to, those listed in section 4.2 above.

Kits of the invention can further comprise devices that are used to administer the active ingredient(s). Examples of such devices include, but are not limited to, syringes, drip bags, patches, and inhalers.

Kits of the invention can further comprise pharmaceutically acceptable vehicles that can be used to administer one or more active ingredients. For example, if an active ingredient is provided in a solid form that must be reconstituted for parenteral administration, the kit can comprise a sealed container of a suitable vehicle in which the active ingredient can be dissolved to form a particulate-free sterile solution that is suitable for parenteral administration. Examples of pharmaceutically acceptable vehicles include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chlo-

ride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

5. EXAMPLES

5.1. Example 1

Synthesis of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione

A stirred solution of 1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethylamine (1.0 g, 3.7 mmol) and 3-acetamidophthalic anhydride (751 mg, 3.66 mmol) in acetic acid (20 mL) was heated at reflux for 15 h. The solvent was removed in vacuo to yield an oil. Chromatography of the resulting oil yielded the product as a yellow solid (1.0 g, 59% yield): mp, 144° C.; ^{1}H NMR ($CDCl_3$) δ1.47 (t, J=7.0 Hz, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.88 (s, 3H, CH_3), 3.75 (dd, J=4.4, 14.3 Hz, 1H, CHH), 3.85 (s, 3H, CH3), 4.11 (q, J=7 Hz, 2H, CH2), 5.87 (dd, J=4.3, 10.5 Hz, 1H, NCH), 6.82–6.86 (m, 1H, Ar), 7.09–7.11 (m, 2H, Ar), 7.47 (d, J=7 Hz, 1H, Ar), 7.64 (t, J=8 Hz, 1H, Ar), 8.74 (d, J=8 Hz, 1H, Ar), 9.49 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ14.61, 24.85, 41.54, 48.44, 54.34, 55.85, 64.43, 111.37, 112.34, 115.04, 118.11, 120.21, 124.85, 129.17, 130.96, 136.01, 137.52, 148.54, 149.65, 167.38, 169.09, 169.40; Anal Calc'd. for $C_{22}H_{24}NO_7S$: C, 57.38; H, 5.25; N, 6.08. Found: C, 57.31; H, 5.34; N, 5.83.

5.2. Example 2

Synthesis of (+)2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione

Preparation of 3-aminopthalic Acid

10% Pd/C (2.5 g), 3-nitrophthalic acid (75.0 g, 355 mmol) and ethanol (1.5 L) were charged to a 2.5 L Parr hydrogenator, under a nitrogen atmosphere. Hydrogen was charged to the reaction vessel for up to 55 psi. The mixture was shaken for 13 hours, maintaining hydrogen pressure between 50 and 55 psi. Hydrogen was released and the mixture was purged with nitrogen 3 times. The suspension was filtered through a celite bed and rinsed with methanol. The filtrate was concentrated in vacuo. The resulting solid was reslurried in ether and isolated by vacuum filtration. The solid was dried in vacua to a constant weight, affording 54 g (84% yield) of 3-aminopthalic acid as a yellow product. ^{1}H-NMR (DMSO-d_6) δ: 3.17 (s, 2H), 6.67 (d, 1H), 6.82 (d, 1H), 7.17 (t, 1H), 8–10 (brs, 2H). ^{13}C-NMR(DMSO-d_6)δ: 112.00, 115.32, 118.20, 131.28, 135.86, 148.82, 169.15, 170.09.

Preparation of 3-acetamidophthalic Anhydride

A 1 L 3-necked round bottom flask was equipped with a mechanical stirrer, thermometer, and condenser and charged with 3-aminophthalic acid (108 g, 596 mmol) and acetic anhydride (550 mL). The reaction mixture was heated to reflux for 3 hours and cooled to ambient temperature and further to 0–5° C. for another 1 hour. The crystalline solid was collected by vacuum filtration and washed with ether. The solid product was dried in vacua at ambient temperature to a constant weight, giving 75 g (61% yield) of 3-acetamidopthalic anhydride as a white product. ^{1}H-NMR ($CDCl_3$) δ: 2.21 (s, 3H), 7.76 (d, 1H), 7.94 (t, 1H), 8.42 (d, 1H), 9.84 (s, 1H).

Resolution of 2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine

A 3 L 3-necked round bottom flask was equipped with a mechanical stirrer, thermometer, and condenser and charged with 2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine (137.0 g, 500 mmol), N-acetyl-L-leucine (52 g, 300 mmol), and methanol (1.0 L). The stirred slurry was heated to reflux for 1 hour. The stirred mixture was allowed to cool to ambient temperature and stirring was continued for another 3 hours at ambient temperature. The slurry was filtered and washed with methanol (250 mL). The solid was air-dried and then dried in vacuo at ambient temperature to a constant weight, giving 109.5 g (98% yield) of the crude product (85.8% ee). The crude solid (55.0 g) and methanol (440 mL) were brought to reflux for 1 hour, cooled to room temperature and stirred for an additional 3 hours at ambient temperature. The slurry was filtered and the filter cake was washed with methanol (200 mL). The solid was air-dried and then dried in vacuo at 30° C. to a constant weight, yielding 49.6 g (90% recovery) of (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine-N-acetyl-L-leucine salt (98.4% ee). Chiral HPLC (1/99 EtOH/20 mM KH_2PO_4 @pH 7.0, Ultron Chiral ES-OVS from Agilent Technologies, 150 mm×4.6 mm, 0.5 mL/min., @240 nm): 18.4 min (S-isomer, 99.2%), 25.5 min (R-isomer, 0.8%).

Preparation of Compound A

A 500 mL 3-necked round bottom flask was equipped with a mechanical stirrer, thermometer, and condenser. The reaction vessel was charged with (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-yl amine N-acetyl-L-leucine salt (25 g, 56 mmol, 98% ee), 3-acetamidophthalic anhydride (12.1 g 58.8 mmol), and glacial acetic acid (250 mL). The mixture was refluxed over night and then cooled to <50° C. The solvent was removed in vacuo, and the residue was dissolved in ethyl acetate. The resulting solution was washed with water (250 mL×2), saturated aqeous $NaHCO_3$ (250 mL×2), brine (250 mL×2), and dried over sodium sulphate. The solvent was evaporated in vacuo, and the residue recrystallized from a binary solvent containing ethanol (150 mL) and acetone (75 mL). The solid was isolated by vacuum filtration and washed with ethanol (100 mL×2). The product was dried in vacuo at 60° C. to a constant weight, affording 19.4 g (75% yield) of S-{2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-aminoisoindoline-1,3-dione with 98% ee. Chiral HPLC (15/85 EtOH/20 mM KH_2PO_4 @pH 3.5, Ultron Chiral ES-OVS from Agilent Technology, 150 mm×4.6 mm, 0.4 mL/min., @240 nm): 25.4 min (S-isomer, 98.7%), 29.5 min (R-isomer, 1.2%). ^{1}H-NMR ($CDCl_3$) δ: 1.47 (t, 3H), 2.26 (s, 3H), 2.87 (s, 3H), 3.68–3.75 (dd, 1H), 3.85 (s, 3H), 4.07–4.15 (q, 2H), 4.51–4.61 (dd, 1H), 5.84–5.90 (dd, 1H), 6.82–8.77 (m, 6H), 9.46 (s, 1H). ^{13}C-NMR (DMSO-d6) δ: 14.66, 24.92, 41.61, 48.53, 54.46, 55.91, 64.51, 111.44, 112.40, 115.10, 118.20, 120.28, 124.94, 129.22, 131.02, 136.09, 137.60, 148.62, 149.74, 167.46, 169.14, 169.48.

5.3. Example 3

TNF-α Inhibition

Human Whole Blood LPS-induced TNF-α Assay

The ability of compounds to inhibit LPS-induced TNF-α production by human whole blood was measured essentially as described below for the LPS-induced TNF-α assay in

human PBMC, except that freshly drawn whole blood was used instead of PBMC. (George Muller, et al. 1999, *Bioorganic & Medicinal Chemistry Letters* 9; 1625–1630.) Human whole blood LPS-induced TNF-α IC_{50}-294 nM

Mouse LPS-induced Serum TNF-α Inhibition

Compounds were tested in this animal model according to previously described methods (Corral et al. 1996, *Mol. Med* 2:506–515). Mouse LPS-induced serum TNF-α inhibition (ED_{50}, mg/kg, p.o.)=0.05.

LPS-induced TNF-α Production

Lipopolysaccharide (LPS) is an endotoxin produced by gram-negative bacteria such as *E. coli* which induces production of many pro-inflammatory cytokines, including TNF-α. In peripheral blood mononuclear cells (PBMC), the TNF-α produced in response to LPS is derived from monocytes, which comprise approximately 5–20% of the total PBMC. Compounds were tested for the ability to inhibit LPS-induced TNF-α production from human PBMC as previously described (Muller et al. 1996, *J. Med Chem.* 39:3238). PBMC from normal donors were obtained by Ficoll Hypaque (Pharmacia, Piscataway, N.J., USA) density centrifugation. Cells were cultured in RPMI (Life Technologies, Grand Island, N.Y., USA) supplemented with 10% AB± human serum (Gemini Bio-products, Woodland, Calif., USA), 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Life Technologies).

PBMC (2×10^5 cells) were plated in 96-well flat-bottom Costar tissue culture plates (Corning, N.Y., USA) in triplicate. Cells were stimulated with LPS (Sigma, St. Louis, Mo., USA) at 100 ng/ml in the absence or presence of compounds. Compounds (Celgene Corp., Warren, N.J., USA) were dissolved in DMSO (Sigma) and further dilutions were done in culture medium immediately before use. The final DMSO concentration in all samples was 0.25%. Compounds were added to cells 1 hour before LPS stimulation. Cells were incubated for 18–20 hours at 37° C. in 5% CO_2 and supernatants were then collected, diluted with culture medium and assayed for TNF-α levels by ELISA (Endogen, Boston, Mass., USA). LPS-induced TNF-α IC_{50}=77 nM.

IL-1β-induced TNF-α Production

During the course of inflammatory diseases, TNF-α production is often stimulated by the cytokine 1L-1β, rather than by bacterially derived LPS. Compounds were tested for the ability to inhibit IL-1β-induced TNF-α production from human PBMC as described above for LPS-induced TNF-α production, except that the PBMC were isolated from source leukocyte units (Sera-Tec Biologicals, North Brunswick, N.J., USA) by centrifugation on Ficoll-Paque Plus (Amersham Pharmacia, Piscataway, N.J., USA), plated in 96-well tissue culture plates at 3×10^5 cells/well in RPMI-1640 medium (BioWhittaker, Walkersville, Md., USA) containing 10% heat-inactivated fetal bovine serum (Hyclone), 2 mM L-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin (complete medium), pretreated with compounds at 10, 2, 0.4, 0.08, 0.016, 0.0032, 0.00064, and 0 μM in duplicate at a final DMSO concentration of 0.1% at 37° C. in a humidified incubator at 5% CO_2 for 1 hour, then stimulated with 50 ng/ml recombinant human IL-1β (Endogen) for 18 hours. IL-β-induced TNF-α IC_{50}=83 nM.

5.4. Example 4

PDE Selectivity

PDE1, 2, 3, 5, and 6 Enzyme Assays

The specificity of compounds for PDE4 was assessed by testing at a single concentration (10 μM) against bovine PDE1, human PDE2, PDE3, and PDE5 from human platelets (Hidaka and Asano 1976, *Biochem. Biophys. Acta* 429:485, and Nicholsen et al. 1991, *Trends Pharmaco. Sci.* 12:19), and PDE6 from bovine retinal rod outer segments (Baehr et al. 1979, J. Biol. Chem. 254:11669, and Gillespie et al. 1989, *Mol. Pharm.* 36:773). Results are listed in Table 1.

PDE7 Enzyme Assay

PDE7 is a cAMP-selective PDE expressed mainly in T cells and in skeletal muscle. T cell-derived cytokines such as IL-2 and IFN-γ are potentially regulatable via PDE7 inhibition. PDE7 was purified from Hut78 human T cells by anion exchange chromatography as previously described (Bloom and Beavo 1996, *Proc. Natl. Acad. Sci. USA* 93:14188–14192). Compounds were tested against the PDE7 preparation in the presence of 10 nM cAMP as described for PDE4 in Table 1 below.

TABLE 1

	Racemic Compound	Compound A	Compound B*
PDE Inhibition			
PDE4 IC_{50} (from U937 cells) (nM)	81.8	73.5	611
PDE1 (% inhib at 10 μM)	9%	23%	27%
PDE2 (% inhib at 10 μM)	19%	6%	10%
PDE3 (% inhib at 10 μM)	21%	20%	31%
PDE5 (% inhib at 10 μM)	3%	3%	−9%
PDE6 (% inhib at 10 μM)	ND	−6%	10%
PDE7 IC_{50} (nM)	22110	20500	ND
PDE Specificity Ratios from above data (*fold)			
PDE4/PDE1	>2700	>500	>50
PDE4/PDE2	>800	>10000	>260
PDE4/PDE3	>670	>1200	>45
PDE4/PDE5	>12000	>30000	>39000
PDE4/PDE6	ND	>40000	>250
PDE7 IC_{50}/PDE4 IC_{50}	270	279	ND

*Compound B is the opposite enantiomer of Compound A.

5.5. Example 5

PDE4 Inhibition

PDE4 (U937 Cell-derived) Enzyme Assay

PDE4 enzyme was purified from U937 human monocytic cells by gel filtration chromatography as previously described (Muller et al. 1998, *Bioorg. & Med Chem Lett* 8:2669–2674). Phosphodiesterase reactions were carried out in 50 mM Tris HCl pH 7.5, 5 mM $MgCl_2$, 1 μM cAMP, 10 nM [^{3}H]-cAMP for 30 min at 30° C., terminated by boiling, treated with 1 mg/ml snake venom, and separated using AG-1XS ion exchange resin (BioRad) as described (Muller et al. 1998, *Bioorg. & Med Chem Lett* 8:2669–2674). Reactions consumed less than 15% of available substrate. Results are listed in Table 1.

5.6. Example 6

Human T Cell Assays

SEB-induced IL-2 and IFN-γ Production

Staphylococcal Enterotoxin B (SEB) is a superantigen derived from gram-positive bacteria *Staphylococcus aureus*. SEB provides a convenient physiological stimulus specific for T cells expressing particular T cell receptor Vβ chains. Human PBMC (consisting of approximately 50% T cells)

were isolated from source leukocyte units as described above and plated in 96-well tissue culture plates at 3×10^5 cells/well in complete medium, pretreated with compounds at 10, 2, 0.4, 0.08, 0.016, 0.0032, 0.00064, and 0 μM in duplicate at a final DMSO concentration of 0.1% at 37° C. in a humidified incubator at 5% CO_2 for 1 hour, then stimulated with 100 ng/ml SEB (Sigma Chemical Co., St. Louis, Mo., USA) for 18 hours. IL-2 and IFN-γ levels were measured by ELISA (R&D Systems, Minneapolis, Minn., USA). IL-2 IC_{50}=291 nM. IFN-γ IC_{50}=46 nM.

5.7. Example 6

cAMP Elevation Assays

PGE_2-induced cAMP Elevation

Prostaglandin E_2 (PGE_2) binds to prostanoid receptors on monocytes, T cells and other leukocytes and consequently elevates intracellular cAMP levels, resulting in inhibition of cellular responses. The combination of PGE_2 and a PDE4 inhibitor synergistically elevates cAMP levels in these cell types, and the elevation of cAMP in PBMC caused by PDE4 inhibitors in the presence of PGE_2 is proportional to the inhibitory activity of that PDE4 inhibitor. Intracellular cAMP was measured in human PBMC as follows. PBMC were isolated as described above and plated in 96-well plates at 1×10^6 cells per well in RPMI-1640. The cells were pre-treated with compounds at 100, 10, 1, 0.1, 0.01, and 0 μM in a final concentration of 2% DMSO in duplicate at 37° C. in a humidified incubator at 5% CO_2 for one hour. The cells were then stimulated with PGE_2 (10 μM) (Sigma) for 1 h. The cells were lysed with HCl, 0.1N final concentration to inhibit phosphodiesterase activity and the plates were frozen at −20° C. The cAMP produced was measured using cAMP (low pH) Immunoassay kit (R&D Systems). PBMC cAMP EC_{50} for racemate is 3.09 μM. PBMC cAMP EC_{50} for Compound A is 1.58 μM.

Elevation of cAMP in human neutrophils was measured as follows. PBMC were removed from source leukocytes (Sera-Tec Biologicals) by centrifugation on Ficoll—Paque Plus (Amersham Pharmacia). The resulting erythrocyte/polymorphonuclear cell (PMN) pellet was resuspended in Hank's Balanced Salt Solution (BioWhittaker) and mixed with an equal volume of 3% Dextran T-500 (Amersham Pharmacia) in 0.9% saline. Erythrocytes were allowed to sediment for 20 minutes, and the PMN were removed and centrifuged at 120 rpm for 8 minutes at 4° C. The remaining erythrocytes were lysed in cold 0.2% saline for 30 seconds, and the cells restored to isotonicity by the addition of an equal volume of 1.6% saline. The PMN were centrifuged at 1200 rpm for 8 minutes at 4° C., then resuspended in RPMI-1640 and assayed for cAMP elevation as described for PBMC above. PMN were found to be approximately 74% $CD18/CD11b^+$, 71% $CD16^+CD9^+$ neutrophils by flow cytometzy on a FACSCalibur (Becton Dickinson, San Jose, Calif., USA). Results are shown in Table 2.

fMLF-induced LTB4 Production

N-formyl-methionine-leucine-phenylalanine (fMLF) is a bacterially derived peptide that activates neutrophils to rapidly degranulate, migrate, adhere to endothelial cells, and release leukotriene LTB4, a product of arachidonic acid metabolism and itself a neutrophil chemoattractant. Compounds were tested for the ability to block fMLF-induced neutrophil LTB4 production as previously described (Hatzelmann and Schudt 2001, *J. Pharm. Exp. Ther.* 297:267–279), with the following modifications. Neutrophils were isolated as described above and resuspended in phosphate-buffered saline without calcium or magnesium (BioWhittaker) containing 10 mM HEPES pH 7.2 and plated in 96-well tissue culture plates at a concentration of 1.7×10^6 cells/well. Cells were treated with 50 μM thimerosal (Sigma)/1 mM $CaCl_2$/1 mM $MgCl_2$ for 15 minutes at 37° C. 5% CO_2, then treated with compounds at 1000, 200, 40, 8, 1.6, 0.32, 0.064, and 0 nM in a final DMSO concentration of 0.01% in duplicate for 10 minutes. Neutrophils were stimulated with 1 μM fMLF for 30 minutes, then lysed by the addition of methanol (20% final concentration) and frozen in a dry ice/isopropanol bath for 10 minutes. Lysates were stored at −70° C. until the LTB4 content was measured by competitive LTB4 ELISA (R&D Systems). Results are shown in Table 2.

Zymosan-induced IL-8 Production

Zymosan A, or the heat-killed yeast *Saccharomyces cerevisiae*, binds to the adhesion molecule Mac-1 on the neutrophil surface and triggers phagocytosis, cell activation and IL-8 production. Zymosan-induced IL-8 production was measured as previously described (Au et al. 1998, *Brit. J. Pharm.* 123:1260–1266) with the following modifications. Human neutrophils were purified as described above, plated in 96-well tissue culture plates at 3×10^5 cells/well in complete medium, treated with compounds at 10, 2, 0.4, 0.08, 0.016, 0.0032, 0.00064, and 0 μM in duplicate in a final DMSO concentration of 0.1% for 1 hour at 37° C. 5% CO_2. Neutrophils were then stimulated with unopsonized, boiled Zymosan A (Sigma) at 2.5×10^5 particles/well for 18 hours. Supernatants were harvested and tested for IL-8 by ELISA (R&D Systems). Results are shown in Table 2.

fMLF-induced CD18/CD11b Expression

CD18/CD11b (Mac-1) expression on neutrophils was measured as previously described (Derian et al. 1995, *J. Immunol.*:154:308–317) with the following modifications. Neutrophils were isolated as described above, then resuspended in complete medium at 1×10^6 cells/ml, pretreated with compounds at 10, 1, 0.1, 0.01, and 0 μM in duplicate at a final DMSO concentration of 0.1% for 10 minutes at 37° C. 5% CO_2. Cells were then stimulated with 30 nM fMLF for 30 minutes and then chilled to 4° C. Cells were treated with rabbit IgG (Jackson ImmunoResearch Labs, West Grove, Pa., USA) (10 μg/1×10^6 cells) to block Fc receptors, stained with CD18-FITC and CD11b-PE (Becton Dickinson), and analyzed by flow cytometry on a FACS-Calibur. CD18/CD11b expression (mean fluorescence) in the absence of stimulation was subtracted from all samples to obtain inhibition curves and calculate IC_{50}s. Results are shown in Table 2.

fMLF-induced Adhesion to HUVEC

Human umbilical vein endothelial cells (HUVEC) were used as a substrate for neutrophil adhesion as previously described (Derian et al. 1995, *J. Immunol.:* 154:308–317) with the following modifications. HUVEC cells were obtained from Anthrogenesis (Cedar Knolls, N.J., USA), and neutrophils were not treated with cytochalasin B. Cells were treated with compounds at 10, 1, 0.1, 0.01, 0.001, and 0 μM in a final DMSO concentration of 0.1% in duplicate for 10 minutes, stimulated with 500 nM fMLF for 30 minutes, and washed twice with PBS before measuring fluorescence

on an FLX800 plate reader (Bio-Tek Instruments, Winooski, Vt., USA). Results are shown in Table 2.

TABLE 2

Human Neutrophil Assays (all values in nM)	Racemic Compound	Compound A
PGE_2-induced cAMP EC_{50}	12589	4570
fMLF-induced LTB4 IC_{50}	20.1	2.48
Zymosan-induced IL-8 IC_{50}	ND	94
fMLF-induced CD18 expression IC_{50}	ND	390
fMLF-induced CD11b expression IC_{50}	ND	74
fMLF-induced adhesion to HUVEC IC_{50}	ND	150

5.8. Example 8

Aqueous Solubility

Equilibrium solubilities were measured in pH 7.4 aqueous buffer. The pH 7.4 buffer was prepared by adjusting the pH of a 0.07 M NaH_2PO_4 solution to 7.4 with 10 N NaOH. The ionic strength of the solution was 0.15. At least 1 mg of powder was combined with 1 ml of buffer to make >1 mg/ml mixture. These samples were shaken for >2 hours and left to stand overnight at room temperature. The samples were then filtered through a 0.45-μm Nylon syringe filter that was first saturated with the sample. The filtrate was sampled twice, consecutively. The filtrate was assayed by HPLC against standards prepared in 50% methanol. Compound A has 3.5-fold greater aqueous solubility than the racemic mixture. Measured solubility Compound A=0.012 mg/mL; racemic mixture=0.0034 mg/mL.

5.9. Example 8

LPS-Induced Lung Neutrophilia Ferret Model

The conscious ferret model has been used to investigate anti-inflammatory, emetic and behavioral effects of PDE4 inhibitors when administered by the oral (p.o.) route. From these experiments, a therapeutic index (TI) for each PDE4 inhibitor may be determined. The TI has been calculated by dividing the threshold dose for causing emetic episodes and behavioral changes by the anti-inflammatory dose (dose that causes 50% inhibition of the LPS-induced neutrophilia).

Animal Husbandry

Male ferrets (Mustela Pulorius Euro, weighing 1–2 kg). Ferrets were supplied either by Bury Green Farm or Misay Consultancy. Following transport, the animals were allowed to acclimatize in the holding rooms for a period of not less than 7 days. The Diet comprised SDS diet C pelleted food given ad lib with Whiskers cat food given 3 times per week. Water was pasteurized animal grade drinking water and was changed daily.

Dosing with PDE4 Inhibitor

PDE4 inhibitors were administered orally (p.o.), at doses initially of 1–10 mg/kg, but subsequently up to 30 mg/kg in order to establish whether the TI was 10 or higher, and/or at lower doses to establish the minimum dose to cause 50% inhibition of neutrophilia. Ferrets were fasted overnight but allowed free access to water. The animals were orally dosed with vehicle or PDE4 inhibitor using a 15 cm dosing needle that was passed down the back of the throat into the oesophagus. After dosing, the animals were returned to holding cages fitted with Perspex doors to allow observation, and given free access to water. After dosing, the animals were constantly observed and any emesis or behavioural changes were recorded. The animals were allowed access to food 60–90 minutes after p.o. dosing

Exposure to LPS

Thirty minutes after p.o. dosing with compound or vehicle control, the ferrets were placed into sealed Perspex containers and exposed to an aerosol of LPS (100 μg/ml) for 10 minutes. Aerosols of LPS were generated by a nebulizer (DeVilbiss, USA) and this was directed into the Perspex exposure chamber. Following a 10 minute exposure period, the animals were returned to the holding cages and allowed free access to water, and at a later stage, food. Observation continued for a period of at least 2.5 hours post p.o. dosing and emetic episodes and behavioral changes were recorded.

Bronchoalveolar Lavage

Six hours after LPS exposure the animals were killed by overdose of sodium pentobarbitone administered intraperitoneally. The trachea was then cannulated with polypropylene tubing and the lungs ravaged twice with 20 ml heparinized (10 units/ml) phosphate buffered saline (PBS).

Blood Sampling/Tissue Removal

A terminal blood sample (10 ml) was removed by transthoracic cardiac puncture. The blood was spun at 2500 rpm for 15 minutes and the plasma removed and stored at −20° C. The brain also removed and frozen at −20° C. for analysis of compound content.

Cell Counts

The bronchoalveolar lavage (BAL) samples were centrifuged at 1500 rpm for 5 minutes. The supernatant was removed and the resulting cell pellet re-suspended in 1 ml PBS. A cell smear of the re-suspended fluid was prepared and stained with Leishmans stain to allow differential cell counting. A total cell count was made using the remaining re-suspended sample. From this, the total number of neutrophils in the BAL was determined.

Parameters Measured:

1. % Inhibition of LPS-induced pulmonary neutrophilia.

2. Emetic episodes—the number of vomits and retches were counted.

3. Behavioral changes—the following behavioral effects were noted: salivation, panting, mouth clawing, flattened posture, ataxia, arched back and backward walking. Any behavioral changes were semi-quantified by applying a severity rating (mild, moderate or severe).

4. The TI was calculated as the highest dose found to not cause emetic episodes divided by the lowest dose found to inhibit pulmonary neutrophilia by 50% or more.

The effect of Compound A on LPS-induced neutrophilia in the lungs of conscious ferrets is demonstrated in FIG. **1**.

Emesis and Behavioral Changes

Following p.o. dosing of the PDE4, the ferrets were observed for at least 2 hours and emetic episodes (vomits and retches) and behavioral changes were recorded.

No emetic episodes (retching or vomiting) were observed in the ferrets pre-treated p.o. with the relevant vehicle (acetone/cremophor/distilled water). In a small proportion of the control-treated animals (7/22), mild behavioral changes (lip licking and backward walking) were seen.

Compound A (0.1–3 mg/kg, p.o.), caused no emetic episodes (retching and vomiting). Some behavioral changes (flattened posture, lip licking and backward walking) were observed and classified as mild. At 10 mg/kg in 2/6 ferrets, some retching but no frank emesis was observed along with salivation and behavioral changes (scored as mild or moderate). At the highest dose tested (30 mg/kg) moderate to marked emesis was observed in 3/4 animals along with pronounced behavioral changes. These data are summarized in Table III.

TABLE III

Conscious ferret: Emetic episodes and behavioural changes following oral administration of Compound A.

Treatment/dose (mg/kg)	Vomits	Retches	Salivation	Panting	Mouth clawing	Flattened posture	Ataxia	Lip licking	Backward walking
Vehicle (acetone/cremophor/dist.H2O)	None	None	None	None	None	None	None	Mild (6/22)	Mild (7/22)
Compound A (0.1 mg/kg)	None	None	None	None	None	Mild (2/5)	None	Mild (4/5)	Mild (3/5)
Compound A (0.3 mg/kg)	None	None	None	None	None	Mild (2/6)	None	Mild (3/6)	Mild (4/6)
Compound A (1.0 mg/kg)	None	None	None	None	None	Mild (2/6)	None	Mild (6/6)	Mild (4/6)
Compound A (3.0 mg/kg)	None	None	None	None	Mild (1/8)	Marked (7/8)	None	Mild (2/8)	Moderate (5/8)
Compound A (10 mg/kg)	None	Mild (2/6)	Mild (1/6)	None	Mild (1/6)	Marked (6/6)	None	Moderate (5/6)	Marked (6/6)
Compound A (30 mg/kg)	Moderate (3/4)	Marked (3/4)	Moderate (3/4)	Mild (1/4)	Marked (4/4)	Marked (4/4)	Mild (3/4)	Moderate (4/4)	Mild (2/4)

Animals were observed for up to 3 hours following dosing. Numbers in parentheses refer to the number of animals that responded The numbers of animals in each group range from 4–22.

Therapeutic Index Calculation

From these experiments, a therapeutic index (TI) was determined for each compound by dividing the threshold dose for inducing emetic episodes by the ED_{50} value for inhibiting the pulmonary neutrophilia. The TI calculation is summarized in Table IV. Compound A had a TI of 12, causing no emetic episodes at an anti-inflammatory dose of 1 mg/kg.

TABLE IV

Summary of the effective doses (ED_{50}) for inhibition of LPS-induced pulmonary neutrophilia and induction of emesis and the therapeutic index derived from these values.

Compound	Inhibition of LPS-induced neutrophilia (ED_{50} mg/kg)	Threshold emetic dose (mg/kg)	Therapeutic index
Compound A	0.8	10	12

5.10. Example 9

200 mg Dosage Capsule

Table V illustrates a batch formulation and single dosage formulation for a 200 mg Compound A single dose unit, i.e., about 40 percent by weight, in a size #0 capsule.

TABLE V

Formulation for 200 mg capsule

Material	Percent By Weight	Quantity (mg/tablet)	Quantity (kg/batch)
Compound A	40.0%	200 mg	16.80 kg
Pregelatinized Corn Starch, NF5	9.5%	297.5 mg	24.99 kg
Magnesium Stearate	0.5%	2.5 mg	0.21 kg
Total	100.0%	500 mg	42.00 kg

The pregelatinized corn starch (SPRESS B-820) and Compound A components are passed through a 710 μm screen and then are loaded into a Diffusion Mixer with a baffle insert and blended for 15 minutes. The magnesium stearate is passed through a 210 μm screen and is added to the Diffusion Mixer. The blend is then encapsulated in a size #0 capsule, 500 mg per capsule (8400 capsule batch size) using a Dosator type capsule filling machine.

5.11. Example 10

100 mg Oral Dosage Form

Table VI illystrates a batch formulation and a single dose unit formulation containing 100 mg of Compound A.

TABLE VI

Formulation for 100 mg tablet

Material	Percent by Weight	Quantity (mg/tablet)	Quantity (kg/batch)
Compound A	40%	100.00	20.00
Microcrystalline Cellulose, NF	53.5%	133.75	26.75
Pluronic F-68	4.0%	10.00	2.00
Surfactant Croscarmellose	2.0%	5.00	1.00
Sodium Type A, NF Magnesium	0.5%	1.25	0.25
Stearate, NF Total	100.0%	250.00 mg	50.00 kg

The microcrystalline cellulose, croscarmellose sodium, and Compound A components are passed through a #30 mesh screen (about 430μ to about 655μ). The Pluronic F-68® (manufactured by JRH Biosciences, Inc. of Lenexa, Kans.) surfactant is passed through a #20 mesh screen (about 457μ to about 1041μ). The Pluronic F-68® surfactant and 0.5 kgs of croscarmellose sodium are loaded into a 16 qt. twin shell tumble blender and are mixed for about 5 minutes. The mix is then transferred to a 3 cubic foot twin shell tumble blender where the microcrystalline cellulose is added and blended for about 5 minutes. The thalidomide is added and blended for an additional 25 minutes. This pre-blend is passed through a roller compactor with a hammer mill attached at the discharge of the roller compactor and moved back to the tumble blender. The remaining croscarmellose sodium and magnesium stearate is added to the tumble blender and blended for about 3 minutes. The final mixture

is compressed on a rotary tablet press with 250 mg per tablet (200,000 tablet batch size).

5.12. Example 11

Aerosol Dosage Form

A concentrate is prepared by combining Compound A, and a 12.6 kg portion of the trichloromonofluoromethane in a sealed stainless steel vessel equipped with a high shear mixer. Mixing is carried out for about 20 minutes. The bulk suspension is then prepared in the sealed vessel by combining the concentrate with the balance of the propellants in a bulk product tank that is temperature controlled to 21° to 27° C. and pressure controlled to 2.8 to 4.0 BAR. 17 ml aerosol containers which have a metered valve which is designed to provide 100 inhalations of the composition of the invention. Each container is provided with the following:

Compound A	0.0120 g
trichloromonofluoromethane	1.6939 g
dichlorodifluoromethane	3.7175 g
dichlorotetrafluoroethane	1.5766 g
total	7.0000 g

While the invention has been described with respect to the particular embodiments, it will be apparent to those skilled in the art that various changes and modifications may be made without departing from the spirit and scope of the invention as defined in the claims. Such modifications are also intended to fall within the scope of the appended claims.

What is claimed is:

1. A method of treating diseases or disorders ameliorated by the inhibition of PDE4 in a patient which comprises administering to a patient in need of such treatment a therapeutically effective amount of stereomerically pure (+)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, or a pharmaceutically acceptable prodrug, polymorph, salt, or solvate thereof.

2. The method of claim **1** further comprising administering to a patient in need of such treatment a therapeutically effective amount of an antihistamine, anti-inflammatory drug, non-steroid anti-inflammatory drug, or steroid.

3. The method of claim **1** wherein the disease or disorder is asthma, allergic rhinitis, inflammation, or chronic pulmonary inflammatory disease.

4. The method of claim **1** wherein the disease or disorder is chronic obstructive pulmonary disease.

5. The method of claim **1** wherein the patient is a mammal.

6. The method of claim **1** wherein the stereomerically pure (+)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, or pharmaceutically acceptable prodrug, polymorph, salt, or solvate thereof is administered parenterally, transdermally, mucosally, nasally, buccally, sublingually, or orally.

7. The method of claim **6** wherein the stereomerically pure (+)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, or pharmaceutically acceptable prodrug, polymorph, salt, or solvate thereof is administered orally.

8. The method of claim **7** wherein the stereomerically pure (+)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, or pharmaceutically acceptable prodrug, polymorph, salt, or solvate thereof is administered orally in a tablet or capsule form.

9. The method of claim **1** wherein the therapeutically effective amount is from about 1 mg to about 1000 mg per day.

10. The method of claim **9** wherein the therapeutically effective amount is from about 5 mg to about 500 mg per day.

11. The method of claim **10** wherein the therapeutically effective amount is from about 10 mg to about 200 mg per day.

12. A method of treating diseases or disorders ameliorated by the inhibition of PDE4 in a patient which comprises administering to a patient in need of such treatment a therapeutically effective amount of stereomerically pure (+)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, or a pharmaceutically acceptable salt, or solvate thereof.

13. The method of claim **1**, wherein the stereomerically pure (+)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione comprises less than about 20% by weight of (−)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

14. The method of claim **13**, wherein the stereomerically pure (+)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione comprises less than about 10% by weight of (−)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

15. The method of claim **14**, wherein the stereomerically pure (+)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione comprises less than about 5% by weight of (−)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

16. The method of claim **15**, wherein the stereomerically pure (+)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione comprises less than about 3% by weight of (−)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

17. The method of claim **12**, which comprises administering stereomerically pure (+)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

18. The method of claim **12**, which comprises administering a pharmaceutically acceptable salt of stereomerically pure (+)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

19. The method of claim **12**, which comprises administering a pharmaceutically acceptable solvate of stereomerically pure (+)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

20. The method of claim **12**, which comprises administering a hydrate of stereomericaily pure (+)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

21. A method of treating asthma, allergic rhinitis, inflammation, chronic pulmonary inflammatory disease or chronic obstructive pulmonary disease in a patient, which comprises administering to a patient in need of such treatment a therapeutically effective amount of stereomericaily pure (+)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, or a pharmaceutically acceptable prodrug, polymorph, salt, or solvate thereof.

22. The method of claim **1**, **6**, **7**, **8**, **12**, or **21**, wherein said salt is a clathrate.

23. The method of claim **1**, **6**, **7**, **8**, **12**, or **21**, wherein said solvate is a hydrate.

* * * * *

EXHIBIT C

US007427638B2

(12) **United States Patent**
Muller et al.

(10) **Patent No.: US 7,427,638 B2**
(45) **Date of Patent: Sep. 23, 2008**

(54) **(+)-2-[1-(3-ETHOXY-4-METHOXYPHENYL)-2-METHYLSULFONYLETHYL]-4-ACETYL-AMINOISOINDOLINE-1,3-DIONE:, AND METHODS OF SYNTHESIS AND COMPOSITIONS THEREOF**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 446 days.

(21) Appl. No.: **11/106,142**

(22) Filed: **Apr. 13, 2005**

(65) **Prior Publication Data**

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Related U.S. Application Data

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(60) Provisional application No. 60/438,450, filed on Jan. 7, 2003, provisional application No. 60/366,515, filed on Mar. 20, 2002.

(51) **Int. Cl.**
A61K 31/40 (2006.01)

(52) **U.S. Cl.** **514/411**; 514/416; 514/417; 548/451; 548/472; 564/80

(58) **Field of Classification Search** 514/411, 514/416, 417; 548/451, 472; 564/80
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,031,450	A	4/1962	Fischer et al.
3,322,755	A	5/1967	Roch et al.
3,920,636	A	11/1975	Takahashi et al.
4,001,237	A	1/1977	Partyka et al.
4,001,238	A	1/1977	Partyka et al.
4,047,404	A	9/1977	Hayashi
4,060,615	A	11/1977	Matier et al.
4,101,548	A	7/1978	Crenshaw et al.
4,162,316	A	7/1979	Nishimura et al.
4,209,623	A	6/1980	Juby
4,880,810	A	11/1989	Lowe, III
4,885,301	A	12/1989	Coates
5,147,875	A	9/1992	Coates et al.
5,354,571	A	10/1994	Morikawa et al.
5,401,774	A	3/1995	Pamukcu et al.
5,439,895	A	8/1995	Lee et al.
5,488,055	A	1/1996	Kumar et al.
5,608,914	A	3/1997	Keesler
5,614,530	A	3/1997	Kumar et al.
5,614,627	A	3/1997	Takase et al.
5,658,940	A	8/1997	Muller et al.
5,698,579	A	12/1997	Muller
5,703,098	A	12/1997	Muller et al.
5,710,170	A	1/1998	Guay et al.
5,728,844	A	3/1998	Muller et al.
5,728,845	A	3/1998	Muller et al.
5,736,570	A	4/1998	Muller et al.
5,798,373	A	8/1998	Warrellow
5,801,195	A	9/1998	Muller et al.
5,849,770	A	12/1998	Head et al.
5,877,200	A	3/1999	Muller
5,891,896	A	4/1999	Warrellow et al.
6,011,050	A	1/2000	Muller et al.
6,020,339	A	2/2000	Perrier et al.
6,020,358	A	2/2000	Muller et al.
6,034,089	A	3/2000	Han et al.
6,046,221	A	4/2000	Muller et al.
6,069,156	A	5/2000	Oku et al.
6,162,830	A	12/2000	Connor et al.
6,166,041	A	12/2000	Cavalla et al.
6,177,471	B1	1/2001	Menander et al.
6,204,275	B1	3/2001	Friesen et al.
6,218,400	B1	4/2001	Daugan et al.
6,300,335	B1	10/2001	Campbell et al.
6,316,472	B1	11/2001	Frenette et al.
6,333,354	B1	12/2001	Schudt

FOREIGN PATENT DOCUMENTS

EP	0 347 146	6/1989
EP	0 349 239 A2	6/1989

(Continued)

OTHER PUBLICATIONS

Craven et al., 1969, CAS: 71:117431.*
Berge et al., Journal of Pharmaceutical Science, 1977, vol. 66, pp. 1-19.*
Bundgaard et al. publication, 1985, Design of prodrug, pp. 1-4.*
FDA's guidance for industry, 2000, pp. 1-20.*
Dredge et al., 2003, "Angiogenesis inhibitors in cancer therapy," Curr. Opin. Investig. Drugs 4(6):667-674.
Dredge et al., 2002, "Immunological effects of thalidomide and its chemical and functional analogs," *Crit. Rev. Immunol.* 22(5-6):425-437.
Dredge et al., 2002, "Recent developments in antiangiogenic therapy," *Expert Opin. Biol. Ther.* 2(8):953-966.
Gee et al., 2003, "Selective cytokine inhibitory drugs with enhanced antiangiogenic activity control tumor growth through vascular inhibition," *Cancer Res.* 63(23):8073-8078.

(Continued)

Primary Examiner—Rei-Tsang Shiao
(74) *Attorney, Agent, or Firm*—Jones Day

(57) **ABSTRACT**

Stereomerically pure (+)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, substantially free of its (−) isomer, and prodrugs, metabolites, polymorphs, salts, solvates, hydrates, and clathrates thereof are discussed. Also discussed are methods of using and pharmaceutical compositions comprising the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione are disclosed. The methods include methods of treating and/or preventing disorders ameliorated by the reduction of levels of TNF-α or the inhibition of PDE4.

13 Claims, 2 Drawing Sheets

FOREIGN PATENT DOCUMENTS

EP	0 351 058		6/1989
EP	0 352 960	A2	7/1989
EP	0 395 328		4/1990
EP	0 428 268	A2	10/1990
EP	0 463 756	A1	6/1991
EP	0 526 004	A1	7/1992
EP	0 607 439	A1	9/1992
EP	0 722 937	A1	1/1996
EP	0 722 943	A1	1/1996
EP	0 722 944	A1	1/1996
GB	2 063 249	A	9/1980
WO	WO 93/07149		4/1993
WO	WO 93/12095		6/1993
WO	WO 94/01728		1/1994
WO	WO 94/05661		3/1994
WO	WO 94/29277		12/1994
WO	WO 95/19978		7/1995
WO	WO 96/32379		10/1996
WO	WO 97/03070		1/1997
WO	WO 97/03675		2/1997
WO	WO 97/03985		2/1997
WO	WO 97/24334		7/1997
WO	WO 98/06722		2/1998
WO	WO 98/08848		3/1998
WO	WO 98/14448		4/1998
WO	WO 98/16521		4/1998
WO	WO 98/17668		4/1998
WO	WO 98/23597		6/1998
WO	WO 98/38168		9/1998
WO	WO 99/06041		2/1999

OTHER PUBLICATIONS

Marriott et al., 2001, "Immunotherapeutic and antitumour potential of thalidomide analogues," *Expert Opin. Biol. Ther.* 1(4):1-8.

Marriott et al., 2002, "Thalidomide and its analogues have distinct and opposing effects on TNF-alpha and TNFR2 during co-stimulation of both CD4(+) and CD8(+) T cells," *Clin. Exp. Immunol.* 130(1):75-84.

Molostvov et al., 2004, "The effects of selective cytokine inhibitory drugs (CC-10004 and CC-1088) on VEGF and IL-6 expression and apoptosis in myeloma and endothelial cell co-cultures," *Br. J. Haematol.* 124(3):366-375.

U.S. Appl. No. 60/454,155, filed Mar. 12, 2003, Muller, G. et al.

U.S. Appl. No. 60/454,149, filed Mar. 12, 2003, Muller, G. et al.

U.S. Appl. No. 60/438,448, filed Jan. 7, 2003, Muller, G. et al.

U.S. Appl. No. 60/436,975, filed Dec. 30, 2002, Muller, G. et al.

U.S. Appl. No. 60/366,515, filed Mar. 20, 2002, Muller, G. et al.

Akazome, M. et al., 1997, "Asymmetric recognition of 1-arylethylamines by (R)-phenylglycyl-(R)-phenylglycine and its mechanism," Tetrahedron: Asymmetry, Elsevier Scince Publishers, Amsterdam, NL, 8(14):2331-2336.

Au et al., 1998, *Brit. J. Pharm.* 123:1260-1266.

Baehr et al., 1979, *J. Biol. Chem.* 254:11669.

Baughman et al., 1990, *J. Lab. Clin. Med.* 115:36-42.

Beavo and Reifsnyder, *Trends in Pharm.*, 11, 150-55, 1990.

Bissonnette et al., 1989, *Inflammation* 13:329-339.

Bloom and Beavo 1996, *Proc. Natl. Acad. Sci. USA* 93:14188-14192.

Brackeen, M.F. et al., 1995, "Design and synthesis of conformationally constrained analogues of 4-(3-butoxy-4-methoxybenzyl) imidazolidin-2-one (Ro 20-1724) as potent inhibitors of cAMP-specific phosphodiesterase", *J. Med. Chem.* 38:4848-54.

Carstensen, Jens T., 1995, *Drug Stability: Principles & Practice*, 2nd ed., Marcel Dekker, New York, NY pp. 379-380.

Casini et al., 1964, Farmaco Ed. Sci. 19:563.

Clouse et al., 1989, *J. Immunol.* 142:431-438.

Derian et al., 1995, J. Immunol. 154:308-317.

Duh et al., 1989, Proc. Nat. Acad. Sci. 86:5974-5978.

Featherstone, R.L., et al., 2000, "Comparison of phosphodiesterase inhibitors of differing isoenzyme selectivity added to St. Thomas' hospital cardioplegic solution used for hypothermic preservation of rat lungs", Am. J. Respir. Crit. Care Med. 162:850-6.

Gillespie et al., 1989, *Mol. Pharm.* 36:773.

Hidaka and Asano 1976, *Biochem. Biophys. Acta* 429:485.

Hinshaw et al. 1990. *Circ. Shock* 30:2797-292.

Holler et al., 1990, *Blood* 75:1011-1016.

Johnson et al., 1989, *Endocrinology* 124:1424-1427.

List et al., 1990, *J. Clin. Oncol.* 8:1424.MDS.

Luke, G.P. et al., 1999, "Synthesis of (S)-5-(1-aminoethyl)-2-(cyclohexylmethoxy) benzamide," Tetrahedron: Asymmetry, Elsevier Science Publishers, Amsterdam, NL, 10(22):4393-4403.

Merck Manual (1999) 17th ed., 953.

Monté et al., 1990, *Blood* 79:2670.

Muller, George, et al., 1999, *Bioorganic & Medicinal Chemistry Letters* 9; pp. 1625-1630.

Muller et al., 1998, *Bioorg. & Med Chem Lett.* 8:2669-2674.

Muller, et al., 1996, *J. Med. Chem.* 39:3238.

Nicholson et al., 1991, *Trends Pharmaco. Sci.* 12:19.

Shealy et al., 1965, "D- and L-thalidomide." *Chem. Indus.* 12;24:1030-1.

Tierney, et al., ed., 1998, Current Medical Diagnosis & Treatment, 37th ed., Appleton & Lange, pp. 499.

Verghese, et al., *Journal of Pharmacology and Experimental Therapeutics*, 272(3), 1313-1320, 1995.

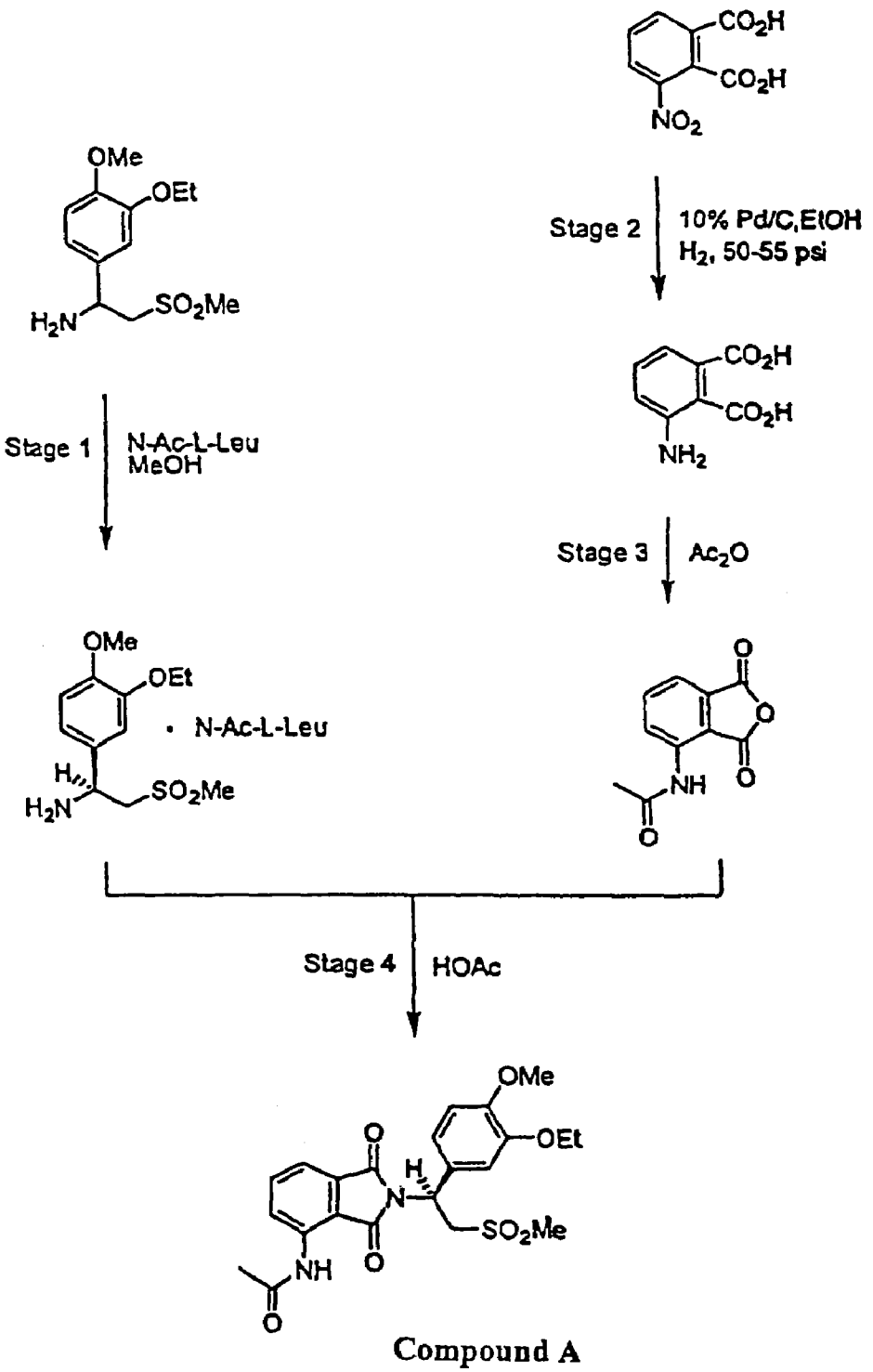
Van Dullemen et al., 1995, *Gastroenterology*, 109:129-135.

Wilen, S.H., et al., 1977, *Tetrahedron* 33:2725.

Wilen, S.H., 1972, *Tables of Resolving Agents and Optical Resoutions* (E.L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, IN) p. 268.

Wolff, Manfred E., ed., *Burger's Medicinal Chemistry and Drug Discovery*, 5th ED. 1995 172-178, 949-982.

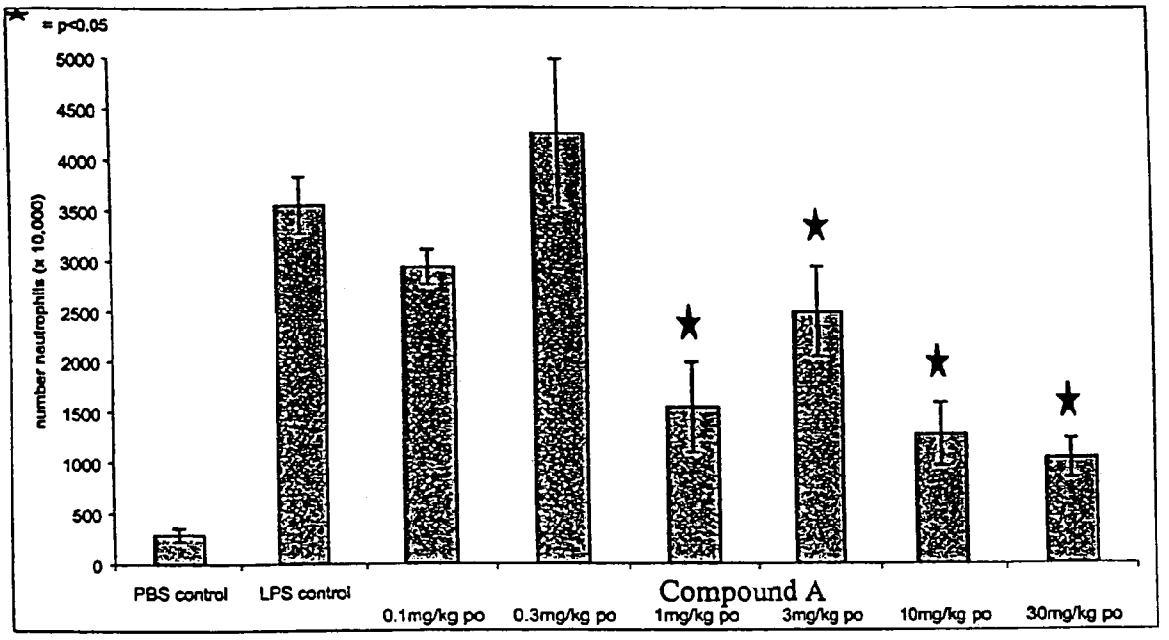
* cited by examiner



Compound A

FIG. 1

FIG 2.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4000
3500
3000
2500
2000
1500
1000
500
0
PBS control
LPS control
0.1mg/kg po
0.3mg/kg po
1mg/kg po
3mg/kg po
10mg/kg po
30mg/kg po
Compound A

(+)-2-[1-(3-ETHOXY-4-METHOXYPHENYL)-2-METHYLSULFONYLETHYL]-4-ACETYL-AMINOISOINDOLINE-1,3-DIONE:, AND METHODS OF SYNTHESIS AND COMPOSITIONS THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

This application is a divisional application of U.S. patent application Ser. No. 10/392,195, filed Mar. 19, 2003 now U.S. Pat. No. 6,962,940, which claims the benefit of U.S. Provisional Application No. 60/366,515 filed Mar. 20, 2002 and U.S. Provisional Application No. 60/438,450 filed Jan. 7, 2003, all of which are incorporated herein by reference in their entireties.

1. FIELD OF INVENTION

The invention relates to methods of using and compositions comprising the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

2. BACKGROUND OF THE INVENTION

Tumor necrosis factor alpha, (TNF-α) is a cytokine that is released primarily by mononuclear phagocytes in response to immunostimulators. TNF-α is capable of enhancing most cellular processes, such as differentiation, recruitment, proliferation, and proteolytic degradation. At low levels, TNF-α confers protection against infective agents, tumors, and tissue damage. But TNF-α also has a role in many diseases. When administered to mammals or humans, TNF-α causes or aggravates inflammation, fever, cardiovascular effects, hemorrhage, coagulation, and acute phase responses similar to those seen during acute infections and shock states. Enhanced or unregulated TNF-α production has been implicated in a number of diseases and medical conditions, for example, cancers, such as solid tumors and blood-born tumors; heart disease, such as congestive heart failure; and viral, genetic, inflammatory, allergic, and autoimmune diseases.

Adenosine 3',5'-cyclic monophosphate (cAMP) also plays a role in many diseases and conditions, such as but not limited to asthma and inflammation, and other conditions (Lowe and Cheng, *Drugs of the Future*, 17(9), 799-807, 1992). It has been shown that the elevation of cAMP in inflammatory leukocytes inhibits their activation and the subsequent release of inflammatory mediators, including TNF-α and NF-κB. Increased levels of cAMP also leads to the relaxation of airway smooth muscle.

It is believed that the primary cellular mechanism for the inactivation of cAMP is the breakdown of cAMP by a family of isoenzymes referred to as cyclic nucleotide phosphodiesterases (PDE) (Beavo and Reitsnyder, *Trends in Pharm*., 11, 150-155, 1990). There are eleven known PDE families. It is recognized, for example, that the inhibition of PDE type IV is particularly effective in both the inhibition of inflammatory mediator release and the relaxation of airway smooth muscle (Verghese, et al., *Journal of Pharmacology and Experimental Therapeutics*, 272(3), 1313-1320, 1995). Thus, compounds that inhibit PDE4 (PDE IV) specifically, may inhibit inflammation and aid the relaxation of airway smooth muscle with a minimum of unwanted side effects, such as cardiovascular or anti-platelet effects. Currently used PDE4 inhibitors lack the selective action at acceptable therapeutic doses.

Cancer is a particularly devastating disease, and increases in blood TNF-α levels are implicated in the risk of and the spreading of cancer. Normally, in healthy subjects, cancer cells fail to survive in the circulatory system, one of the reasons being that the lining of blood vessels acts as a barrier to tumor-cell extravasation. But increased levels of cytokines have been shown to substantially increase the adhesion of cancer cells to endothelium in vitro. One explanation is that cytokines, such as TNF-α, stimulate the biosynthesis and expression of a cell surface receptors called ELAM-1 (endothelial leukocyte adhesion molecule). ELAM-1 is a member of a family of calcium-dependent cell adhesion receptors, known as LEC-CAMs, which includes LECAM-1 and GMP-140. During an inflammatory response, ELAM-1 on endothelial cells functions as a "homing receptor" for leukocytes. Recently, ELAM-1 on endothelial cells was shown to mediate the increased adhesion of colon cancer cells to endothelium treated With cytokines (Rice et al., 1989, *Science* 246:1303-1306).

Inflammatory diseases such as arthritis, related arthritic conditions. (e.g., osteoarthritis and rheumatoid arthritis), inflammatory bowel disease (e.g., Crohn's disease and ulcerative colitis), sepsis, psoriasis, atopic dermatitis, contact dermatitis, and chronic obstructive pulmonary disease, chronic inflammatory pulmonary diseases are also prevalent and problematic ailments. TNF-α plays a central role in the inflammatory response and the administration of their antagonists block chronic and acute responses in animal models of inflammatory disease.

Enhanced or unregulated TNF-α production has been implicated in viral, genetic, inflammatory, allergic, and autoimmune diseases. Examples of such diseases include but are not limited to: HIV; hepatitis; adult respiratory distress syndrome; bone-resorption diseases; chronic obstructive pulmonary diseases; chronic pulmonary inflammatory diseases; asthma, dermatitis; cystic fibrosis; septic shock; sepsis; endotoxic shock; hemodynamic shock; sepsis syndrome; post ischemic reperfusion injury; meningitis; psoriasis; fibrotic disease; cachexia; graft rejection; auto-immune disease; rheumatoid spondylitis; arthritic conditions, such as rheumatoid arthritis and osteoarthritis; osteoporosis; Crohn's disease; ulcerative colitis; inflammatory-bowel disease; multiple sclerosis; systemic lupus erythrematosus; ENL in leprosy; radiation damage; asthma; and hyperoxic alveolar injury. Tracey et al., 1987, *Nature* 330:662-664 and Hinshaw et al., 1990, *Circ. Shock* 30:279-292 (endotoxic shock); Dezube et al., 1990, *Lancet*, 335:662 (cachexia); Millar et al., 1989, *Lancet* 2:712-714 and Ferrai-Baliviera et al., 1989, *Arch. Surg*. 124:1400-1405 (adult respiratory distress syndrome); Bertolini et al., 1986, *Nature* 319:516-518, Johnson et al., 1989, *Endoncrinology* 124:1424-1427, Holler et al., 1990, *Blood* 75:1011-1016, and Grau et al., 1989, *N. Engl. J. Med*. 320:1586-1591 (bone resorption diseases); Pignet et al., 1990, *Nature*, 344:245-247, Bissonnette et al., 1989, *Inflammation* 13:329-339 and Baughman et al., 1990, *J. Lab. Clin. Med*. 115:36-42 (chronic pulmonary inflammatory diseases); Elliot et al., 1995, *Int. J. Pharmac*. 17:141-145 (rheumatoid arthritis); von Dullemen et al., 1995, *Gastroenterology*, 109: 129-135 (Crohn's disease); Duh et al., 1989, *Proc. Nat. Acad. Sci*. 86:5974-5978, Poll et al., 1990, *Proc. Nat. Acad. Sci*. 87:782-785, Monto et al., 1990, *Blood* 79:2670, Clouse et al., 1989, *J. Immunol*. 142, 431-438, Poll et al., 1992, *AIDS Res. Hum. Retrovirus*, 191-197, Poli et al. 1990, Proc. *Natl. Acad. Sci*. 87:782-784, Folks et al., 1989, PNAS 86:2365-2368 (HIV and opportunistic infections resulting from HIV).

Pharmaceutical compounds that can block the activity or inhibit the production of certain cytokines, including TNF-α,

may be beneficial therapeutics. Many small-molecule inhibitors have demonstrated an ability to treat or prevent inflammatory diseases implicated by TNF-α (for a review, see Lowe, 1998 *Exp. Opin. Ther. Patents* 8:1309-1332). One such class of molecules are the substituted phenethylsulfones described in U.S. Pat. No. 6,020,358.

3. SUMMARY OF THE INVENTION

This invention relates to methods of treating diseases and disorders utilizing an enantiomer of a substituted phenethylsulfone compound and pharmaceutically acceptable salts, hydrates, solvates, clathrates, prodrugs and polymorphs thereof and methods for reducing the level of cytokines and their precursors in mammals. The invention also relates to pharmaceutical compositions comprising an enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione and a pharmaceutically acceptable carrier. The invention further relates to an enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione substantially free of its other enantiomer.

This invention particularly relates to the (+) enantiomer of2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione. This compound is believed to have increased potency and other benefits as compared to its racemate—2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl)]-4-acetylaminoisoindoline-1,3-dione.

The invention encompasses the use of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione for treating or preventing diseases or disorders ameliorated by the inhibition of TNF-α production in mammals. In certain embodiments, this treatment includes the reduction or avoidance of adverse effects. Such disorders include, but are not limited to, cancers, including, but not limited to cancer of the head, thyroid, neck, eye, skin, mouth, throat, esophagus, chest, bone, blood, bone marrow, lung, colon, sigmoid, rectum, stomach, prostate, breast, ovaries, kidney, liver, pancreas, brain, intestine, heart, adrenal, subcutaneous tissue, lymph nodes, heart, and combinations thereof. Specific cancers that can be treated by this method are multiple myeloma, malignant melanoma, malignant glioma, leukemia and solid tumors.

The invention also encompasses the use of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione in the treatment or prevention of heart disease, including, but not limited to congestive heart failure, cardiomyopathy, pulmonary edema, endotoxin-mediated septic shock, acute viral myocarditis, cardiac allograft rejection, and myocardial infarction.

The invention also encompasses the use of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione to treat diseases or disorders ameliorated by the inhibition of PDE4. For example, the compounds and compositions of the invention may be useful to treat or prevent viral, genetic, inflammatory, allergic, and autoimmune diseases. Examples of such diseases include, but are not limited to: HIV; hepatitis; adult respiratory distress syndrome; bone-resorption diseases; chronic obstructive pulmonary diseases; chronic pulmonary inflammatory diseases; dermatitis; inflammatory skin disease, atopic dermatitis, cystic fibrosis; septic shock; sepsis; endotoxic shock; hemodynamic shock; sepsis syndrome; post ischemic reperfusion injury; meningitis; psoriasis; fibrotic disease; cachexia; graft rejection including graft versus host disease; auto-immune disease; rheumatoid spondylitis; arthritic conditions, such as rheumatoid arthritis and osteoarthritis; osteoporosis; Crohn's disease; ulcerative colitis; inflammatory-bowel disease; multiple sclerosis; systemic lupus erythrematosus; erythema nodosum leprosum (ENL) in leprosy; radiation damage; asthma; and hyperoxic alveolar injury.

In yet another embodiment, the stereomerically pure (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is also useful in the treatment or prevention of microbial infections or the symptoms of microbial infections including, but not limited to, bacterial infections, fungal infections, malaria, mycobacterial infection, and opportunistic infections resulting from HIV.

The invention further encompasses pharmaceutical compositions and single unit dosage forms comprising an enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione and pharmaceutically acceptable polymorphs, prodrugs, salts, hydrates, clathrates, and solvates thereof.

In a separate embodiment, the invention encompasses the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

In a further embodiment, the invention encompasses a method of producing a stereomerically pure enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione which comprises contacting 1-(3-Ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethylamine with a chiral amino acid and contacting the product of the first step with N-(1,3-Dioxo-1,3-dihydro-isobenzofuran-4-yl)-acetamide. In a related embodiment the invention encompasses a chiral salt of 1-(3-Ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethylamine.

3.1. BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** illustrates the preparation of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

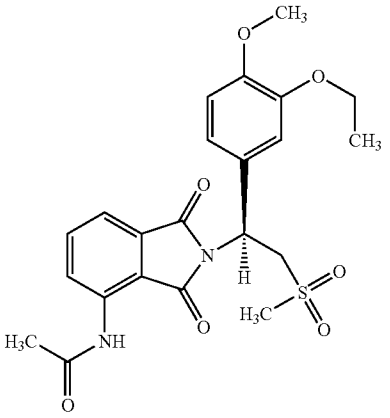
FIG. **2** illustrates the effect of the enantiomer of the invention on LPS-induced neutrophilia in the lungs of conscious ferrets.

3.2. DEFINITIONS

As used herein, term "Compound A" refers to an enantiomerically pure form of 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione which comes off of an HPLC column at about 25.4 minutes when that column is a 150 mm×4.6 mm Ultron Chiral ES-OVS chiral HPLC column (Agilent Technology), the eluent is 15:85 ethanol: 20 mM KH_2PO_4 at pH 3.5, and the observation wavelength is 240 nm. The ^1H NMR spectrum of compound A is substantially as follows: δ($CDCl_3$): 1.47 (t, 3H), 2.26 (s, 3H), 2.87 (s, 3H), 3.68-3.75 (dd, 1H), 3.85 (s, 3H), 4.07-4.15 (q, 2H), 4.51-4.61 (dd, 1H), 5.84-5.90 (dd, 1H), 6.82-8.77 (m, 6H), 9.46 (s, 1H). The ^{13}C NMR spectrum of Compound A is substantially as follows δ(DMSO-d_6): 14.66, 24.92, 41.61, 48.53, 54.46, 55.91, 64.51, 111.44, 112.40, 115.10, 118.20, 120.28, 124.94, 129.22, 131.02, 136.09, 137.60, 148.62, 149.74, 167.46, 169.14, 169.48. Compound A dissolved in methanol also rotates plane polarized light in the (+) direction.

Without being limited by theory, Compound A is believed to be S-{2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfo

nylethyl]-4-acetylaminoisoindoline-1,3-dione}, which has the following structure:



As used herein, the term "patient" refers to a mammal, particularly a human.

As used herein, the term "pharmaceutically acceptable salts" refer to salts prepared from pharmaceutically acceptable non-toxic acids or bases including inorganic acids and bases and organic acids and bases. Suitable pharmaceutically acceptable base addition salts for the compound of the present invention include metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from lysine, N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. Suitable non-toxic acids include, but are not limited to, inorganic and organic acids such as acetic, alginic, anthranilic, benzenesulfonic, benzoic, camphorsulfonic citric, ethenesulfonic, formic, fumaric, furoic, galacturonic, gluconic, glucuronic, glutamic, glycolic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phenylacetic, phosphoric, propionic, salicylic, stearic, succinic, sulfanilic, sulfric, tartaric acid, and p-toluenesulfonic acid. Specific non-toxic acids include hydrochloric, hydrobromic, phosphoric, sulfuric, and methanesulfonic acids. Examples of specific salts thus include hydrochloride and mesylate salts.

As used herein and unless otherwise indicated, the term "prodrug" means a derivative of a compound that can hydrolyze, oxidize, or otherwise react under biological conditions (in vitro or in vivo) to provide the compound. Examples of prodrugs include, but are not limited to, derivatives and metabolites of Compound A that include biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Prodrugs can typically be prepared using well-known methods, such as those described by 1 *Burger's Medicinal Chemistry and Drug Discovery*, 172-178, 949-982 (Manfred E. Wolff ed., 5th ed. 1995).

As used herein and unless otherwise indicated, the terms "biohydrolyzable amide," "biohydrolyzable ester," "biohydrolyzable carbamate," "biohydrolyzable carbonate," "biohydrolyzable ureide," "biohydrolyzable phosphate" mean an amide, ester, carbamate, carbonate, ureide, or phosphate, respectively, of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties in vivo, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted in vivo to the biologically active compound. Examples of biohydrolyzable esters include, but are not limited to, lower alkyl esters, alkoxyacyloxy esters, alkyl acylamino alkyl esters, and choline esters. Examples of biohydrolyzable amides include, but are not limited to, lower alkyl amides, α-amino acid amides, alkoxyacyl amides, and alkylaminoalkylcarbonyl amides. Examples of biohydrolyzable carbamates include, but are not limited to, lower alkylamines, substituted ethylenediamines, aminoacids, hydroxyalkylamines, heterocyclic and heteroaromatic amines, and polyether amines.

As used herein and unless otherwise indicated, the term "stereomerically pure" means a composition that comprises one stereoisomer of a compound and is substantially free of other stereoisomers of that compound. For example, a stereomerically pure composition of a compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically pure composition of a compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, more preferably greater than about 90% by weight of one stereoisomer of the compound and less than about 10% by weight of the other stereoisomers of the compound, even more preferably greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, and most preferably greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound.

As used herein and unless otherwise indicated, the term "enantiomerically pure" means a stereomerically pure composition of a compound having one chiral center.

As used herein, term "adverse effects" includes, but is not limited to gastrointestinal, renal and hepatic toxicities, leukopenia, increases in bleeding times due to, e.g., thrombocytopenia, and prolongation of gestation, nausea, vomiting, somnolence, asthenia, dizziness, teratogenicity, extra-pyramidal symptoms, akathisia, cardiotoxicity including cardiovascular disturbances, inflammation, male sexual dysfunction, and elevated serum liver enzyme levels. The term "gastrointestinal toxicities" includes but is not limited to gastric and intestinal ulcerations and erosions. The term "renal toxicities" includes but is not limited to such conditions as papillary necrosis and chronic interstitial nephritis.

As used herein and unless otherwise indicated, the phrases "reduce or avoid adverse effects" and "reducing or avoiding adverse effects" mean the reduction of the severity of one or more adverse effects as defined herein.

It should be noted that if there is a discrepancy between a depicted structure and a name given that structure, the depicted structure is to be accorded more weight. In addition, if the stereochemistry of a structure or a portion of a structure is not indicated with, for example, bold or dashed lines, the structure or portion of the structure is to be interpreted as encompassing all stereoisomers of it.

4. DETAILED DESCRIPTION OF THE INVENTION

This invention relates to stereomerically pure Compound A, which is an enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, substantially free of its other enantiomer, as well as novel methods using, and compositions comprising stereo-

merically pure Compound A. For example, the present invention encompasses the in vitro and in vivo use of Compound A, and the incorporation of Compound A into pharmaceutical compositions and single unit dosage forms useful in the treatment and prevention of a variety of diseases and disorders. Diseases and disorders which are ameliorated by the reduction of levels of TNF-α or inhibition of PDE4 are well known in the art and are described herein. Specific methods of the invention reduce or avoid the adverse effects associated with compounds used as TNF-α inhibitor. Other specific methods of the invention reduce or avoid the adverse effects associated with use of racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

Specific methods of the invention include methods of treating or preventing diseases and disorders including, but not limited to, solid tumor cancers, blood-born cancers and inflammatory diseases.

Pharmaceutical and dosage forms of the invention, which comprise Compound A or a pharmaceutically acceptable polymorph, prodrug, salt, clathrate, solvate or hydrate thereof, can be used in the methods of the invention.

Without being limited by theory, it is believed that Compound A can inhibit TNF-α production. Consequently, a first embodiment of the invention relates to a method of inhibiting TNF-α production which comprises contacting a cell exhibiting abnormal TNF-α production with an effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. In a particular embodiment, the invention relates to a method of inhibiting TNF-α production which comprises contacting a mammalian cell exhibiting abnormal TNF-α production with an effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof.

The invention also relates to a method of treating or preventing disorders ameliorated by the reduction of levels of TNF-α in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof.

A further embodiment of the invention relates to a method of treating or preventing cancer, including but not limited to, solid tumor, blood-born tumor, leukemias, and in particular, multiple myeloma in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically effective amount of stereomerically pure compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof; in particular wherein the patient is a mammal.

In another embodiment, the invention relates to a method of inhibiting PDE4 which comprises contacting PDE4 with an effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof.

In another embodiment, the invention relates to a method of controlling cAMP levels in a cell which comprises contacting a cell with an effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. As used herein the term "controlling cAMP levels" includes preventing or reducing the rate of the breakdown of Adenosine 3',5'-cyclic monophosphate (cAMP) in a cell or increasing the amount of Adenosine 3',5'-cyclic monophosphate present in a cell, preferably a mammalian cell, more preferably a human cell. In a particular method, the rate of cAMP breakdown is reduced by about 10, 25, 50, 100, 200, or 500 percent as compared to the rate in comparable cells which have not been contacted with a compound of the invention.

A further embodiment of the invention relates to a method of treating or preventing diseases or disorders ameliorated by the inhibition of PDE4 in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. Disorders ameliorated by the inhibition of PDE4 include, but are not limited to, asthma, inflammation (e.g., inflammation due to reperfusion), chronic or acute obstructive pulmonary diseases, chronic or acute pulmonary inflammatory diseases, inflammatory bowel disease, Crohn's Disease, Bechet's Disease, or colitis.

A further embodiment of the invention relates to a method of treating or preventing depression, asthma, inflammation (e.g., contact dermatitis, atopic dermatitis, psoriais, rheumatoid arthritis, osteoarthritis, inflammatory skin disease, inflammation due to reperfusion), chronic or acute obstructive pulmonary diseases, chronic or pulmonary inflammatory diseases, inflammatory bowel disease, Crohn's Disease, Bechet's Disease or colitis in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof; in particular wherein the patient is a mammal.

A separate embodiment of the invention encompasses methods of treating or preventing Myelodysplastic syndrome (MDS) which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. MDS refers to a diverse group of hematopoietic stem cell disorders. MDS is characterized by a cellular marrow with impaired morphology and maturation (dysmyelopoiesis), peripheral blood cytopenias, and a variable risk of progression to acute leukemia, resulting from ineffective blood cell production. See The Merck Manual 953 (17th ed. 1999) and List et al., 1990, *J. Clin. Oncol*. 8:1424.MDS

A separate embodiment of the invention encompasses methods of treating or preventing Myeloproliferative disease (MPD) which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. Myeloproliferative disease (MPD) refers to a group of disorders characterized by clonal abnormalities of the hematopoietic stem cell. See e.g., Current Medical Diagnosis & Treatment, pp. 499 (37th ed., Tierney et al. ed, Appleton & Lange, 1998).

The invention also encompasses a method of treating, preventing or managing complex regional pain syndrome, which comprises administering to a patient in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of a stereomerically pure Compound A, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. In a specific embodiment, the administration is before, during or after surgery or physical therapy directed at reducing or avoiding a symptom of complex regional pain syndrome in the patient.

In particular methods of the invention, stereomerically pure Compound A, or a pharmaceutically acceptable polymorph, prodrug, salt, solvate, hydrate, or clathrate thereof, is adjunctively administered with at least one additional therapeutic agent. Examples of additional therapeutic agents include, but are not limited to, anti-cancer drugs, anti-inflammatories, antihistamines and decongestants.

4.1. Synthesis and Preparation

Racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is readily prepared using the methods in U.S. Pat. No. 6,020,358, which is incorporated herein by reference.

Compound A can be isolated from the racemic compound by techniques known in the art. Examples include, but are not limited to, the formation of chiral salts and the use of chiral or high performance liquid chromatography "HPLC" and the formation and crystallization of chiral salts. See, e.g., Jacques, J., et al., *Enantiomers, Racemates and Resolutions* (Wiley-Interscience, New York, 1981); Wilen, S. H., et al., *Tetrahedron* 33:2725 (1977); Eliel, E. L., *Stereochemistry of Carbon Compounds* (McGraw-Hill, NY, 1962); and Wilen, S. H., *Tables of Resolving Agents and Optical Resolutions* p. 268 (E. L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, Ind., 1972).

In a specific method, Compound A is synthesized from 3-acetamidophthalic anhydride and a chiral amino acid salt of (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine. Chiral amino acid salts of (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine include, but not limited to salts formed with the L isomers of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, ornithine, 4-aminobutyric acid, 2 amino isobutyric acid, 3 amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, and N-acetyl-leucine. A specific chiral amino acid salt is (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine N-acetyl-L-leucine salt, which is resolved from 2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine and N-acetyl-L-leucine in methanol.

4.2. Methods of Treatment

The invention encompasses methods of treating and preventing diseases or disorders ameliorated by the reduction of levels of TNF-α in a patient which comprise administering to a patient in need of such treatment or prevention a therapeutically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof.

Disorders ameliorated by the inhibition of TNF-α but are not limited to: heart disease, such as congestive heart failure, cardiomyopathy, pulmonary edema, endotoxin-mediated septic shock, acute viral myocarditis, cardiac allograft rejection, and myocardial infarction; solid tumors, including but not limited to, sarcoma, carcinomas, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, Kaposi's sarcoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma; and blood-born tumors including but not limited to, acute lymphoblastic leukemia "ALL", acute lymphoblastic B-cell leukemia, acute lymphoblastic T-cell leukemia, acute myeloblastic leukemia "AML", acute promyelocytic leukemia "APL", acute monoblastic leukemia, acute erythroleukemic leukemia, acute megakaryoblastic leukemia, acute myelomonocytic leukemia, acute nonlymphocyctic leukemia, acute undifferentiated leukemia, chronic myelocytic leukemia "CML", chronic lymphocytic leukemia "CLL", hairy cell leukemia, multiple myeloma and acute and chronic leukemias, for example, lymphoblastic, myelogenous, lymphocytic, and myelocytic leukemias.

Specific methods of the invention further comprise the administration of an additional therapeutic agent (i.e., a therapeutic agent other than Compound A). Examples of additional therapeutic agents include, but are not limited to, anti-cancer drugs such as, but are not limited to: alkylating agents, nitrogen mustards, ethylenimines, methylmelamines, alkyl sulfonates, nitrosoureas, triazenes, folic acid analofs, pyrimidine analogs, purine analogs, vinca alkaloids, epipodophyllotoxins, antibiotics, topoisomerase inhibitors and anti-cancer vaccines.

Specific additional therapeutic agents include, but are not limited to: acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimnastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cisplatin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; dactinomycin; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; docetaxel; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; flurocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; ilmofosine; interleukin II (including recombinant interleukin II, or rIL2), interferon alfa-2a; interferon alfa-2b; interferon alfa-n1; interferon alfa-n3; interferon beta-I a; interferon gamma-I b; iproplatin; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedepa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane;

mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; paclitaxel; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; rogletimide; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; triciribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin hydrochloride. Other anti-cancer drugs include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; antidorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-aminotriazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorlns; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl spiromustine; docetaxel; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamnine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritreximn; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnsyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymnes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mnimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine;

tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalaamer.

The invention further encompasses a method of treating or preventing diseases or disorders ameliorated by the inhibition of PDE4 in a patient which comprise administering to a patient in need of such treatment or prevention a therapeutically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. Disorders ameliorated by the inhibition of PDE4 include, but are not limited to, asthma, inflammation, chronic or acute obstructive pulmonary disease, chronic or acute pulmonary inflammatory disease, inflammatory bowel disease, Crohn's Disease, Bechet's Disease, colitis, ulcerative colitis and arthritis or inflammation due to reperfusion. In a preferred embodiment, the disease or disorder to be treated or prevented is chronic obstructive pulmonary disease.

Specific methods of the invention can comprise the administration of an additional therapeutic agent such as, but not limited to, anti-inflammatory drugs, antihistamines and decongestants. Examples of such additional therapeutic agents include, but are not limited to: antihistamines including, but not limited to, ethanolamines, ethylenediamines, piperazines, and phenothiazines; antinflammatory drugs; NSAIDS, including, but not limited to, aspirin, salicylates, acetominophen, indomethacin, sulindac, etodolac, fenamates, tolmetin, ketorolac, diclofenac, ibuprofen, naproxen, fenoprofen, ketoprofen, flurbiprofen, oxaprozin, piroxicam, meloxicamn, pyrazolon derivatives; and steriods including, but not limited to, cortical steroids and adrenocortical steroids.

Specific methods of the invention avoid or reduce drug-drug interactions and other adverse effects associated with agents used in the treatment of such disorders, including racemic substituted phenylethylsulfones. Without being limited by any theory, stereomerically pure Compound A may further provide an overall improved therapeutic effectiveness, or therapeutic index, over racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione. For example, a smaller amount of the drug may in some circumstances be administered to attain the same level of effectiveness.

As stated above, the active compound of the invention (i.e., Compound A) may be used in the treatment or prevention of a wide range of diseases and conditions. The magnitude of a prophylactic or therapeutic dose of a particular active ingredient of the invention in the acute or chronic management of a disease or condition will vary, however, with the nature and severity of the disease or condition, and the route by which the active ingredient is administered. The dose, and perhaps the dose frequency, will also vary according to the age, body weight, and response of the individual patient. Suitable dosing regimens can be readily selected by those skilled in the art with due consideration of such factors. In general, the recommended daily dose range for the conditions described herein lie within the range of from about 1 mg to about 1000 mg per day, given as a single once-a-day dose preferably as divided doses throughout a day. More specifically, the daily dose is administered twice daily in equally divided doses. Specifically, a daily dose range should be from about 5 mg to about 500 mg per day, more specifically, between about 10 mg and about 200 mg per day. Specifically, the daily dose may be administered in 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 50 mg, or 100 mg dosage forms. In managing the patient, the therapy should be initiated at a lower dose, perhaps about 1 mg to about 25 mg, and increased if necessary up to about 200 mg to about 1000 mg per day as either a single dose or divided doses, depending on the patient's global response. Alternatively, the daily dose is from 0.01 mg/kg to 100 mg/kg.

It may be necessary to use dosages of the active ingredient outside the ranges disclosed herein in some cases, as will be apparent to those of ordinary skill in the art. Furthermore, it is noted that the clinician or treating physician will know how and when to interrupt, adjust, or terminate therapy in conjunction with individual patient response.

The phrases "therapeutically effective amount", "prophylactically effective amount" and "therapeutically or prophylactically effective amount," as used herein encompasses the above described dosage amounts and dose frequency schedules. Different therapeutically effective amounts may be applicable for different diseases and conditions, as will be readily known by those of ordinary skill in the art. Similarly, amounts sufficient to treat or prevent such disorders, but insufficient to cause, or sufficient to reduce, adverse effects associated with racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione are also encompassed by the above described dosage amounts and dose frequency schedules.

4.3. Pharmaceutical Compositions

Pharmaceutical compositions and single unit dosage forms comprising Compound A, or a pharmaceutically acceptable polymorph, prodrug, salt, solvate, hydrate, or clathrate thereof, are encompassed by the invention. Individual dosage forms of the invention may be suitable for oral, mucosal (including rectal, nasal, or vaginal), parenteral (including subcutaneous, intramuscular, bolus injection, intraarterial, or intravenous), sublingual, transdermal, buccal, or topical administration.

Pharmaceutical compositions and dosage forms of the invention comprise stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. Pharmaceutical compositions and dosage forms of the invention typically also comprise one or more pharmaceutically acceptable excipients.

A particular pharmaceutical composition encompassed by this embodiment comprises stereomerically pure Compound A, or a pharmaceutically acceptable polymorph, prodrug, salt, solvate, hydrate, or clathrate thereof, and at least one additional therapeutic agent. Examples of additional therapeutic agents include, but are not limited to: anti-cancer drugs and anti-inflammation therapies including, but not limited to, those listed above in section 4.2.

Single unit dosage forms of the invention are suitable for oral, mucosal (e.g., nasal, sublingual, vaginal, buccal, or rectal), parenteral (e.g., subcutaneous, intravenous, bolus injection, intramuscular, or intraarterial), or transdermal administration to a patient. Examples of dosage forms include, but are not limited to: tablets; caplets; capsules, such as soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; sup-

positories; ointments; cataplasms (poultices); pastes; powders; dressings; creams; plasters; solutions; patches; aerosols (e.g., nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a patient, including suspensions (e.g. aqueous or non-aqueous liquid suspensions, oil-in-water emulsions, or a water-in-oil liquid emulsions), solutions, and elixirs; liquid dosage forms suitable for parenteral administration to a patient; and sterile solids (e.g., crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms suitable for parenteral administration to a patient.

The composition, shape, and type of dosage forms of the invention will typically vary depending on their use. For example, a dosage form used in the acute treatment of inflammation or a related disorder may contain larger amounts of one or more of the active ingredients it comprises than a dosage form used in the chronic treatment of the same disease. Similarly, a parenteral dosage form may contain smaller amounts of one or more of the active ingredients it comprises than an oral dosage form used to treat the same disease or disorder. These and other ways in which specific dosage forms encompassed by this invention will vary from one another will be readily apparent to those skilled in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 18th ed., Mack Publishing, Easton Pa. (1990).

Typical pharmaceutical compositions and dosage forms comprise one or more excipients. Suitable excipients are well known to those skilled in the art of pharmacy, and non-limiting examples of suitable excipients are provided herein. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a patient. For example, oral dosage forms such as tablets may contain excipients not suited for use in parenteral dosage forms. The suitability of a particular excipient may also depend on the specific active ingredients in the dosage form.

Lactose-free compositions of the invention can comprise excipients that are well known in the art and are listed, for example, in the U.S. Pharmocopia (USP) SP (XXI)/NF (XVI). In general, lactose-free compositions comprise an active ingredient, a binder/filler, and a lubricant in pharmaceutically compatible and pharmaceutically acceptable amounts. Preferred lactose-free dosage forms comprise an active ingredient, microcrystalline cellulose, pre-gelatinized starch, and magnesium stearate.

This invention further encompasses anhydrous pharmaceutical compositions and dosage forms comprising active ingredients, since water can facilitate the degradation of some compounds. For example, the addition of water (e.g., 5%) is widely accepted in the pharmaceutical arts as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. See, e.g., Jens T. Carstensen, *Drug Stability: Principles & Practice*, 2d. Ed., Marcel Dekker, NY, N.Y., 1995, pp. 379-80. In effect, water and heat accelerate the decomposition of some compounds. Thus, the effect of water on a formulation can be of great significance since moisture and/or humidity are commonly encountered during manufacture, handling, packaging, storage, shipment, and use of formulations.

Anhydrous pharmaceutical compositions and dosage forms of the invention can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms that comprise lactose and at least one active ingredient that comprises a primary or secondary amine are preferably anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected.

An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions are preferably packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples of suitable packaging include, but are not limited to, hermetically sealed foils, plastics, unit dose containers (e.g., vials), blister packs, and strip packs.

The invention further encompasses pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will decompose. Such compounds, which are referred to herein as "stabilizers," include, but are not limited to, antioxidants such as ascorbic acid, pH buffers, or salt buffers.

Like the amounts and types of excipients, the amounts and specific types of active ingredients in a dosage form may differ depending on factors such as, but not limited to, the route by which it is to be administered to patients. However, typical dosage forms of the invention comprise compound A, or a pharmaceutically acceptable salt, solvate, clathrate, hydrate, polymoprh or prodrug thereof lie within the range of from about 1 mg to about 1000 mg per day, given as a single once-a-day dose in the morning but preferably as divided doses throughout the day taken with food. More specifically, the daily dose is administered twice daily in equally divided doses. Specifically, a daily dose range should be from about 5 mg to about 500 mg per day, more specifically, between about 10 mg and about 200 mg per day. In managing the patient, the therapy should be initiated at a lower dose, perhaps about 1 mg to about 25 mg, and increased if necessary up to about 200 mg to about 1000 mg per day as either a single dose or divided doses, depending on the patient's global response.

4.3.1. Oral Dosage Forms

Pharmaceutical compositions of the invention that are suitable for oral administration can be presented as discrete dosage forms, such as, but are not limited to, tablets (e.g., chewable tablets), caplets, capsules, and liquids (e.g., flavored syrups). Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of pharmacy well known to those skilled in the art. See generally, *Remington's Pharmaceutical Sciences,* 18th ed., Mack Publishing, Easton Pa. (1990).

Typical oral dosage forms of the invention are prepared by combining the active ingredient(s) in an intimate admixture with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of preparation desired for administration. For example, excipients suitable for use in oral liquid or aerosol dosage forms include, but are not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives and coloring agents. Examples of excipients suitable for use in solid oral dosage forms (e.g., powders, tablets, capsules, and caplets) include, but are not limited to, starches, sugars, micro-crystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid excipients are employed. If desired, tablets can be coated by standard aqueous or nonaqueous techniques. Such dosage forms can be prepared by any of the methods of pharmacy. In general, pharmaceutical compositions and dosage forms are prepared by uniformly and intimately admixing the active ingredients with liquid carriers,

finely divided solid carriers, or both, and then shaping the product into the desired presentation if necessary.

For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as powder or granules, optionally mixed with an excipient. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

Examples of excipients that can be used in oral dosage forms of the invention include, but are not limited to, binders, fillers, disintegrants, and lubricants. Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gelatin, natural and synthetic gums such as acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre-gelatinized starch, hydroxypropyl methyl cellulose, (e.g., Nos. 2208, 2906, 2910), microcrystalline cellulose, and mixtures thereof.

Examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof. The binder or filler in pharmaceutical compositions of the invention is typically present in from about 50 to about 99 weight percent of the pharmaceutical composition or dosage form.

Suitable forms of microcrystalline cellulose include, but are not limited to, the materials sold as AVICEL-PH-101, AVICEL-PH-103 AVICEL RC-581, AVICEL-PH-105 (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, Pa.), and mixtures thereof. An specific binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC-581. Suitable anhydrous or low moisture excipients or additives include AVICEL-PH-103™ and Starch 1500 LM.

Disintegrants are used in the compositions of the invention to provide tablets that disintegrate when exposed to an aqueous environment. Tablets that contain too much disintegrant may disintegrate in storage, while those that contain too little may not disintegrate at a desired rate or under the desired conditions. Thus, a sufficient amount of disintegrant that is neither too much nor too little to detrimentally alter the release of the active ingredients should be used to form solid oral dosage forms of the invention. The amount of disintegrant used varies based upon the type of formulation, and is readily discernible to those of ordinary skill in the art. Typical pharmaceutical compositions comprise from about 0.5 to about 15 weight percent of disintegrant, specifically from about 1 to about 5 weight percent of disintegrant.

Disintegrants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, agar-agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrilin potassium, sodium starch glycolate, potato or tapioca starch, pre-gelatinized starch, other starches, clays, other algins, other celluloses, gums, and mixtures thereof.

Lubricants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g. peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, and mixtures thereof. Additional lubricants include, for example, a syloid silica gel (AEROSIL 200, manufactured by W.R. Grace Co. of Baltimore, Md.), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Plano, Tex.), CAB-O-SIL (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, Mass.), and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about 1 weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated.

4.3.2. Delayed Release Dosage Forms

Active ingredients of the invention can be administered by controlled release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Pat. Nos.: 3,845,770; 3,916,899; 3,536,809; 3,598,123; and 4,008,719, 5,674,533, 5,059,595, 5,591,767, 5,120,548, 5,073,543, 5,639,476, 5,354,556, and 5,733,566, each of which is incorporated herein by reference. Such dosage forms can be used to provide slow or controlled-release of one or more active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the active ingredients of the invention. The invention thus encompasses single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled-release.

All controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition, controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the drug, and can thus affect the occurrence of side (e.g., adverse) effects.

Most controlled-release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled-release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, temperature, enzymes, water, or other physiological conditions or compounds.

4.3.3. Parenteral Dosage Forms

Parenteral dosage forms can be administered to patients by various routes including, but not limited to, subcutaneous, intravenous (including bolus injection), intramuscular, and intraarterial. Because their administration typically bypasses patients' natural defenses against contaminants, parenteral dosage forms are preferably sterile or capable of being sterilized prior to administration to a patient. Examples of

parenteral dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions.

Suitable vehicles that can be used to provide parenteral dosage forms of the invention are well known to those skilled in the art. Examples include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

Compounds that increase the solubility of one or more of the active ingredients disclosed herein can also be incorporated into the parenteral dosage forms of the invention.

4.3.4. Transdermal, Topical, and Mucosal Dosage Forms

Transdermal, topical, and mucosal dosage forms of the invention include, but are not limited to, ophthalmic solutions, sprays, aerosols, creams, lotions, ointments, gels, solutions, emulsions, suspensions, or other forms known to one of skill in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 16th and 18th eds., Mack Publishing, Easton Pa. (1980 & 1990); and *Introduction to Pharmaceutical Dosage Forms*, 4th ed., Lea & Febiger, Philadelphia (1985). Dosage forms suitable for treating mucosal tissues within the oral cavity can be formulated as mouthwashes or as oral gels. Further, transdermal dosage forms include "reservoir type" or "matrix type" patches, which can be applied to the skin and worn for a specific period of time to permit the penetration of a desired amount of active ingredients.

Suitable excipients (e.g., carriers and diluents) and other materials that can be used to provide transdermal, topical, and mucosal dosage forms encompassed by this invention are well known to those skilled in the pharmaceutical arts, and depend on the particular tissue to which a given pharmaceutical composition or dosage form will be applied. With that fact in mind, typical excipients include, but are not limited to, water, acetone, ethanol, ethylene glycol, propylene glycol, butane-1,3-diol, isopropyl myristate, isopropyl palmitate, mineral oil, and mixtures thereof to form lotions, tinctures, creams, emulsions, gels or ointments, which are non-toxic and pharmaceutically acceptable. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well known in the art. See, e.g., *Remington's Pharmaceutical Sciences*, 16th and 18th eds., Mack Publishing, Easton Pa. (1980 & 1990).

Depending on the specific tissue to be treated, additional components may be used prior to, in conjunction with, or subsequent to treatment with active ingredients of the invention. For example, penetration enhancers can be used to assist in delivering the active ingredients to the tissue. Suitable penetration enhancers include, but are not limited to: acetone; various alcohols such as ethanol, oleyl, and tetrahydrofuryl; alkyl sulfoxides such as dimethyl sulfoxide; dimethyl acetamide; dimethyl formamide; polyethylene glycol; pyrrolidones such as polyvinylpyrrolidone; Kollidon grades (Povidone, Polyvidone); urea; and various water-soluble or insoluble sugar esters such as Tween 80 (polysorbate 80) and Span 60 (sorbitan monostearate).

The pH of a pharmaceutical composition or dosage form, or of the tissue to which the pharmaceutical composition or dosage form is applied, may also be adjusted to improve delivery of one or more active ingredients. Similarly, the polarity of a solvent carrier, its ionic strength, or tonicity can be adjusted to improve delivery. Compounds such as stearates can also be added to pharmaceutical compositions or dosage forms to advantageously alter the hydrophilicity or lipophilicity of one or more active ingredients so as to improve delivery. In this regard, stearates can serve as a lipid vehicle for the formulation, as an emulsifying agent or surfactant, and as a delivery-enhancing or penetration-enhancing agent. Different salts, hydrates or solvates of the active ingredients can be used to further adjust the properties of the resulting composition.

4.3.5. Kits

Typically, active ingredients of the invention are preferably not administered to a patient at the same time or by the same route of administration. This invention therefore encompasses kits which, when used by the medical practitioner, can simplify the administration of appropriate amounts of active ingredients to a patient.

A typical kit of the invention comprises a unit dosage form of compound A, or a pharmaceutically acceptable salt, solvate, hydrate, clathrate, polymorph or prodrug thereof, and a unit dosage form of a second active ingredient. Examples of second active ingredients include, but are not limited to, those listed in section 4.2 above.

Kits of the invention can further comprise devices that are used to administer the active ingredient(s). Examples of such devices include, but are not limited to, syringes, drip bags, patches, and inhalers.

Kits of the invention can further comprise pharmaceutically acceptable vehicles that can be used to administer one or more active ingredients. For example, if an active ingredient is provided in a solid form that must be reconstituted for parenteral administration, the kit can comprise a sealed container of a suitable vehicle in which the active ingredient can be dissolved to form a particulate-free sterile solution that is suitable for parenteral administration. Examples of pharmaceutically acceptable vehicles include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

5. EXAMPLES

5.1. Example 1

Synthesis of 2-[1-(3-Ethoxy4-Methoxyphenyl)-2-Methylsulfonylethyl]-4-Acetylaminoisoindoline-1,3-Dione

A stirred solution of 1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethylamine (1.0 g, 3.7 mmol) and 3-acetamidophthalic anhydride (751 mg, 3.66 mmol) in acetic acid (20 mL) was heated at reflux for 15 h. The solvent was removed in vacuo to yield an oil. Chromatography of the resulting oil yielded the product as a yellow solid (1.0 g, 59% yield): mp, 144° C.; ^{1}H NMR ($CDCl_3$) δ1.47 (t, J=7.0 Hz, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.88 (s,3H, CH_3), 3.75 (dd, J=4.4, 14.3 Hz, 1H, CHH), 3.85 (s, 3H, CH3), 4.11 (q, J=7 Hz, 2H, CH2), 5.87 (dd, J=4.3, 10.5 Hz, 1H, NCH), 6.82-6.86 (m, 1H, Ar), 7.09-7.11 (m, 2H, Ar), 7.47 (d, J=7 Hz, 1H., Ar), 7.64 (t, J=8 Hz, 1H, Ar), 8.74 (d, J=8 Hz, 1H, Ar), 9.49 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ14.61, 24.85, 41.54, 48.44, 54.34, 55.85,

64.43, 111.37, 112.34, 115.04, 118.11, 120.21, 124.85, 129.17, 130.96, 136.01, 137.52, 148.54, 149.65, 167.38, 169.09, 169.40; Anal Calc'd. for $C_{22}H_{24}NO_7S$: C, 57.38; H, 5.25; N, 6.08. Found: C, 57.31; H, 5.34; N, 5.83.

5.2. Example 2

Synthesis of (+)2-[1-(3-Ethoxy-4-Methoxyphenyl)-2-Methylsulfonylethyl]-4-Acetylaminoisoindoline-1,3-Dione

Preparation of 3-aminopthalic acid

10% Pd/C (2.5 g), 3-nitrophthalic acid (75.0 g, 355 mmol) and ethanol (1.5 L) were charged to a 2.5 L Parr hydrogenator, under a nitrogen atmosphere. Hydrogen was charged to the reaction vessel for up to 55 psi. The mixture was shaken for 13 hours, maintaining hydrogen pressure between 50 and 55 psi. Hydrogen was released and the mixture was purged with nitrogen 3 times. The suspension was filtered through a celite bed and rinsed with methanol. The filtrate was concentrated in vacuo. The resulting solid was reslurried in ether and isolated by vacuum filtration. The solid was dried in vacua to a constant weight, affording 54 g (84% yield) of 3-aminopthalic acid as a yellow product. ^{1}H-NMR (DMSO-d6) δ: 3.17 (s, 2H), 6.67 (d, 1H), 6.82 (d, 1H), 7.17 (t, 1H), 8-10 (brs, 2H). ^{13}C-NMR DMSO-d6)δ: 112.00, 115.32, 118.20, 131.28, 135.86, 148.82, 169.15, 170.09.

Preparation of 3-acetamidophthalic anhydride

A 1 L 3-necked round bottom flask was equipped with a mechanical stirrer, thermometer, and condenser and charged with 3-aminophthalic acid (108 g, 596 mmol) and acetic anhydride (550 mL). The reaction mixture was heated to reflux for 3 hours and cooled to ambient temperature and further to 0-5° C. for another 1 hour. The crystalline solid was collected by vacuum filtration and washed with ether. The solid product was dried in vacua at ambient temperature to a constant weight, giving 75 g (61% yield) of 3-acetamidopthalic anhydride as a white product. ^{1}H-NMR ($CDCl_3$) δ: 2.21 (s, 3H), 7.76 (d, 1H), 7.94 (t, 1H), 8.42 (d, 1H), 9.84 (s, 1H).

Resolution of

2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine

A 3 L 3-necked round bottom flask was equipped with a mechanical stirrer, thermometer, and condenser and charged with 2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine (137.0 g, 500 mmol), N-acetyl-L-leucine (52 g, 300 mmol), and methanol (1.0 L). The stirred slurry was heated to reflux for 1 hour. The stirred mixture was allowed to cool to ambient temperature and stirring was continued for another 3 hours at ambient temperature. The slurry was filtered and washed with methanol (250 mL). The solid was air-dried and then dried in vacuo at ambient temperature to a constant weight, giving 109.5 g (98% yield) of the crude product (85.8% ee). The crude solid (55.0 g) and methanol (440 mL) were brought to reflux for 1 hour, cooled to room temperature and stirred for an additional 3 hours at ambient temperature. The slurry was filtered and the filter cake was washed with methanol (200 mL). The solid was air-dried and then dried in vacuo at 30° C. to a constant weight, yielding 49.6 g (90% recovery) of (S)-2-(3-ethoxy4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine-N-acetyl-L-leucine salt (98.4% ee). Chiral HPLC (1/99 EtOH/20 mM KH_2PO_4 @pH 7.0, Ultron Chiral ES-OVS from Agilent Technologies, 150 mm×4.6 mm, 0.5 mL/min., @240 nm): 18.4 min (S-isomer, 99.2%), 25.5 min (R-isomer, 0.8%).

Preparation of Compound A

A 500 mL 3-necked round bottom flask was equipped with a mechanical stirrer, thermometer, and condenser. The reaction vessel was charged with (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-yl amine N-acetyl-L-leucine salt (25 g, 56 mmol, 98% ee), 3-acetamidophthalic anhydride (12.1 g 58.8 mmol), and glacial acetic acid (250 mL). The mixture was refluxed over night and then cooled to <50° C. The solvent was removed in vacuo, and the residue was dissolved in ethyl acetate. The resulting solution was washed with water (250 mL×2), saturated aqeous $NaHCO_3$ (250 mL×2), brine (250 mL×2), and dried over sodium sulphate. The solvent was evaporated in vacuo, and the residue recrystallized from a binary solvent containing ethanol (150 mL) and acetone (75 mL). The solid was isolated by vacuum filtration and washed with ethanol (100 mL×2). The product was dried in vacuo at 60° C. to a constant weight, affording 19.4 g (75% yield) of S-{2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-aminoisoindoline-1,3-dione with 98% ee. Chiral HPLC (15/85 EtOH/20 mM KH_2PO_4 @pH 3.5, Ultron Chiral ES-OVS from Agilent Technology, 150 mm×4.6 mm, 0.4 mL/min., @240 nm): 25.4 min (S-isomer, 98.7%), 29.5 min (R-isomer, 1.2%). ^{1}H-NMR ($CDCl_3$) δ:1.47 (t, 3H), 2.26 (s, 3H), 2.87 (s, 3H), 3.68-3.75 (dd, 1H), 3.85 (s, 3H), 4.07-4.15 (q, 2H), 4.51-4.61 (dd, 1H), 5.84-5.90 (dd, 1H), 6.82-8.77 (m, 6H), 9.46 (s, 1H). ^{13}C-NMR (DMSO-d6) δ: 14.66, 24.92, 41.61, 48.53, 54.46, 55.91, 64.51, 111.44, 112.40, 115.10, 118.20, 120.28, 124.94, 129.22, 131.02, 136.09, 137.60, 148.62, 149.74, 167.46, 169.14, 169.48.

5.3. Example 3

TNF-α Inhibition

Human Whole Blood LPS-induced TNF-α assay

The ability of compounds to inhibit LPS-induced TNF-α production by human whole blood was measured essentially as described below for the LPS-induced TNF-α assay in human PBMC, except that freshly drawn whole blood was used instead of PBMC. (George Muller, et al. 1999, *Bioorganic & Medicinal Chemistry Letters* 9; 1625-1630.) Human whole blood LPS-induced TNF-α IC_{50}-294 nM

Mouse LPS-induced serum TNF-α inhibition

Compounds were tested in this animal model according to previously described methods (Corral et al. 1996, *Mol. Med* 2:506-515). Mouse LPS-induced serum TNF-α inhibition (ED_{50}, mg/kg, p.o.)=0.05.

LPS-induced TNF-α production

Lipopolysaccharide (LPS) is an endotoxin produced by gram-negative bacteria such as *E. coli* which induces production of many pro-inflammatory cytokines, including TNF-α. In peripheral blood mononuclear cells (PBMC), the TNF-α produced in response to LPS is derived from monocytes, which comprise approximately 5-20% of the total PBMC. Compounds were tested for the ability to inhibit LPS-induced TNF-α production from human PBMC as previously described (Muller et al. 1996, *J. Med Chem.* 39:3238). PBMC from normal donors were obtained by Ficoll Hypaque (Pharmacia, Piscataway, N.J., USA) density centrifugation. Cells were cultured in RPMI (Life Technologies, Grand Island, N.Y., USA) supplemented with 10% AB±human serum (Gemini Bio-products, Woodland, Calif., USA), 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Life Technologies).

PBMC (2×10^5 cells) were plated in 96-well flat-bottom Costar tissue culture plates (Corning, N.Y., USA) in triplicate. Cells were stimulated with LPS (Sigma, St. Louis, Mo.,

USA) at 100 ng/ml in the absence or presence of compounds. Compounds (Celgene Corp., Warren, N.J., USA) were dissolved in DMSO (Sigma) and further dilutions were done in culture medium immediately before use. The final DMSO concentration in all samples was 0.25%. Compounds were added to cells 1 hour before LPS stimulation. Cells were incubated for 18-20 hours at 37° C. in 5% CO_2 and supernatants were then collected, diluted with culture medium and assayed for TNF-α levels by ELISA (Endogen, Boston, Mass., USA). LPS-induced TNF-α IC_{50}=77 nM.

IL-1β-induced TNF-α production

During the course of inflammatory diseases, TNF-α production is often stimulated by the cytokine 1L-1β, rather than by bacterially derived LPS. Compounds were tested for the ability to inhibit IL-1β-induced TNFα production from human PBMC as described above for LPS-induced TNFα production, except that the PBMC were isolated from source leukocyte units (Sera-Tec Biologicals, North Brunswick, N.J., USA) by centrifugation on Ficoll-Paque Plus (Amersham Pharmacia, Piscataway, N.J., USA), plated in 96-well tissue culture plates at 3×10^5 cells/well in RPMI-1640 medium (BioWhittaker, Walkersville, Md., USA) containing 10% heat-inactivated fetal bovine serum (Hyclone), 2 mM L-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin (complete medium), pretreated with compounds at 10, 2, 0.4, 0.08, 0.016, 0.0032, 0.00064, and 0 μM in duplicate at a final DMSO concentration of 0.1% at 37° C. in a humidified incubator at 5% CO_2 for 1 hour, then stimulated with 50 ng/ml recombinant human IL-1β (Endogen) for 18 hours. IL-β-induced TNF-α IC_{50}=83 nM.

5.4. Example 4

PDE Selectivity

PDE1, 2, 3, 5, and 6 enzyme assays

The specificity of compounds for PDE4 was assessed by testing at a single concentration (10 μM) against bovine PDE1, human PDE2, PDE3, and PDE5 from human platelets (Hidaka and Asano 1976, *Biochem. Biophys. Acta* 429:485, and Nicholsen et al. 1991, *Trends Pharmaco. Sci*. 12:19), and PDE6 from bovine retinal rod outer segments (Baehr et al. 1979, *J. Biol. Chem*. 254:11669, and Gillespie et al. 1989, *Mol. Pharm.* 36:773). Results are listed in Table 1.

PDE7 enzyme assay

PDE7 is a cAMP-selective PDE expressed mainly in T cells and in skeletal muscle. T cell-derived cytokines such as IL-2 and IFN-γ are potentially regulatable via PDE7 inhibition. PDE7 was purified from Hut78 human T cells by anion exchange chromatography as previously described (Bloom and Beavo 1996, *Proc. Natl. Acad Sci. USA* 93:14188-14192). Compounds were tested against the PDE7 preparation in the presence of 10 nM cAMP as described for PDE4 in Table 1 below.

TABLE 1

	Racemic Compound	Compound A	Compound B*
PDE Inhibition			
PDE4 IC_{50} (from U937 cells) (nM)	81.8	73.5	611
PDE1 (% inhib at 10 μM)	9%	23%	27%
PDE2 (% inhib at 10 μM)	19%	6%	10%
PDE3 (% inhib at 10 μM)	21%	20%	31%
PDE5 (% inhib at 10 μM)	3%	3%	−9%
PDE6 (% inhib at 10 μM)	ND	−6%	10%
PDE7 IC_{50} (nM)	22110	20500	ND
PDE Specificity Ratios from above data (*fold)			
PDE4/PDE1	>2700	>500	>50
PDE4/PDE2	>800	>10000	>260
PDE4/PDE3	>670	>1200	>45
PDE4/PDE5	>12000	>30000	>39000
PDE4/PDE6	ND	>40000	>250
PDE7 IC_{50}/PDE4 IC_{50}	270	279	ND

*Compound B is the opposite enantiomer of Compound A.

5.5. Example 5

PDE4 Inhibition

PDE4 (U937 cell-derived) enzyme assay

PDE4 enzyme was purified from U937 human monocytic cells by gel filtration chromatography as previously described (Muller et al. 1998, *Bioorg. & Med Chem Lett* 8:2669-2674). Phosphodiesterase reactions were carried out in 50 mM Tris HCl pH 7.5, 5 mM $MgCl_2$, 1 μM cAMP, 10 nM [^{3}H]-cAMP for 30 min at 30° C., terminated by boiling, treated with 1 mg/ml snake venom, and separated using AG-1XS ion exchange resin (BioRad) as described (Muller et al. 1998, *Bioorg. & Med Chem Lett* 8:2669-2674). Reactions consumed less than 15% of available substrate. Results are listed in Table 1.

5.6. Example 6

Human T Cell Assays

SEB -induced IL-2 and IFN-γ production

Staphylococcal Enterotoxin B (SEB) is a superantigen derived from gram-positive bacteria *Staphylococcus aureus*. SEB provides a convenient physiological stimulus specific for T cells expressing particular T cell receptor Vβ chains. Human PBMC (consisting of approximately 50% T cells) were isolated from source leukocyte units as described above and plated in 96-well tissue culture plates at 3×10^5 cells/well in complete medium, pretreated with compounds at 10, 2, 0.4, 0.08, 0.016, 0.0032, 0.00064, and 0 μM in duplicate at a final DMSO concentration of 0.1% at 37° C. in a humidified incubator at 5% CO_2 for 1 hour, then stimulated with 100 ng/ml SEB (Sigma Chemical Co., St. Louis, Mo., USA) for 18 hours. IL-2 and IFN-γ levels were measured by ELISA (R&D Systems, Minneapolis, Minn., USA). IL-2 IC_{50}=291 nM. IFN-γ IC_{50}=46 nM.

5.7. Example 6

cAMP Elevation Assays

PGE_2-induced cAMP elevation

Prostaglandin E_2 (PGE_2) binds to prostanoid receptors on monocytes, T cells and other leukocytes and consequently elevates intracellular cAMP levels, resulting in inhibition of cellular responses. The combination of PGE_2 and a PDE4 inhibitor synergistically elevates cAMP levels in these cell types, and the elevation of cAMP in PBMC caused by PDE4 inhibitors in the presence of PGE_2 is proportional to the

inhibitory activity of that PDE4 inhibitor. Intracellular cAMP was measured in human PBMC as follows. PBMC were isolated as described above and plated in 96-well plates at 1×10^6 cells per well in RPMI-1640. The cells were pre-treated with compounds at 100, 10, 1, 0.1, 0.01, and 0 μM in a final concentration of 2% DMSO in duplicate at 37° C. in a humidified incubator at 5% CO_2 for one hour. The cells were then stimulated with PGE_2 (10 μM) (Sigma) for 1 h. The cells were lysed with HCl, 0.1N final concentration to inhibit phosphodiesterase activity and the plates were frozen at −20° C. The cAMP produced was measured using cAMP (low pH) Immunoassay kit (R&D Systems). PBMC cAMP EC_{50} for racemate is 3.09 μM. PBMC cAMP EC_{50} for Compound A is 1.58 μM.

Elevation of cAMP in human neutrophils was measured as follows. PBMC were removed from source leukocytes (SeraTec Biologicals) by centrifugation on Ficoll—Paque Plus (Amersham Pharmacia). The resulting erythrocyte/polymorphonuclear cell (PMN) pellet was resuspended in Hank's Balanced Salt Solution (BioWhittaker) and mixed with an equal volume of 3% Dextran T-500 (Amersham Pharmacia) in 0.9% saline. Erythrocytes were allowed to sediment for 20 minutes, and the PMN were removed and centrifuged at 120 rpm for 8 minutes at 4° C. The remaining erythrocytes were lysed in cold 0.2% saline for 30 seconds, and the cells restored to isotonicity by the addition of an equal volume of 1.6% saline. The PMN were centrifuged at 1200 rpm for 8 minutes at 4° C., then resuspended in RPMI-1640 and assayed for cAMP elevation as described for PBMC above. PMN were found to be approximately 74% $CD18/CD11b^+$, 71% $CD16^+$ $CD9^+$ neutrophils by flow cytometzy on a FACSCalibur (Becton Dickinson, San Jose, Calif., USA). Results are shown in Table 2.

fMLF-induced LTB4 production

N-formyl-methionine-leucine-phenylalanine (fMLF) is a bacterially derived peptide that activates neutrophils to rapidly degranulate, migrate, adhere to endothelial cells, and release leukotriene LTB4, a product of arachidonic acid metabolism and itself a neutrophil chemoattractant. Compounds were tested for the ability to block fMLF-induced neutrophil LTB4 production as previously described (Hatzelmann and Schudt 2001, *J. Pharm. Exp. Ther.* 297:267-279), with the following modifications. Neutrophils were isolated as described above and resuspended in phosphate-buffered saline without calcium or magnesium (BioWhittaker) containing 10 mM HEPES pH7.2 and plated in 96-well tissue culture plates at a concentration of 1.7×10^6 cells/well. Cells were treated with 50 μM thimerosal (Sigma)/1 mM $CaCl_2$/1 MM $MgCl_2$ for 15 minutes at 37° C. 5% CO_2, then treated with compounds at 1000, 200, 40, 8, 1.6, 0.32, 0.064, and 0 nM in a final DMSO concentration of 0.01% in duplicate for 10 minutes. Neutrophils were stimulated with 1 μM fMLF for 30 minutes, then lysed by the addition of methanol (20% final concentration) and frozen in a dry ice/isopropanol bath for 10 minutes. Lysates were stored at −70° C. until the LTB4 content was measured by competitive LTB4 ELISA (R&D Systems). Results are shown in Table 2.

Zymosan-induced IL-8 production

Zymosan A, or the heat-killed yeast *Saccharomyces cerevisiae*, binds to the adhesion molecule Mac-1 on the neutrophil surface and triggers phagocytosis, cell activation and IL-8 production. Zymosan-induced IL-8 production was measured as previously described (Au et al. 1998, *Brit. J. Pharm.* 123:1260-1266) with the following modifications. Human neutrophils were purified as described above, plated in 96-well tissue culture plates at 3×10^5 cells/well in complete medium, treated with compounds at 10, 2, 0.4, 0.08, 0.016, 0.0032, 0.00064, and 0 μM in duplicate in a final DMSO concentration of 0.1% for 1 hour at 37° C. 5%CO_2. Neutrophils were then stimulated with unopsonized, boiled Zymosan A (Sigma) at 2.5×10^5 particles/well for 18 hours. Supernatants were harvested and tested for IL-8 by ELISA (R&D Systems). Results are shown in Table 2.

fMLF-induced CD18/CD11b expression

CD18/CD 11b (Mac-1) expression on neutrophils was measured as previously described (Derian et al. 1995, *J. Immunol.*: 154:308-317) with the following modifications. Neutrophils were isolated as described above, then resuspended in complete medium at 1×10^6cells/ml, pretreated with compounds at 10, 1, 0.1, 0.01, and 0 μM in duplicate at a final DMSO concentration of 0.1% for 10 minutes at 37° C. 5%CO_2. Cells were then stimulated with 30 nM fMLF for 30 minutes and then chilled to 4° C. Cells were treated with rabbit IgG (Jackson ImmunoResearch Labs, West Grove, Pa., USA) (10 μg/1×10^6 cells) to block Fc receptors, stained with CD18-FITC and CD11b-PE (Becton Dickinson), and analyzed by flow cytometry on a FACSCalibur. CD18/CD11b expression (mean fluorescence) in the absence of stimulation was subtracted from all samples to obtain inhibition curves and calculate IC_{50}s. Results are shown in Table 2.

fMLF-induced adhesion to HUVEC

Human umbilical vein endothelial cells (HUVEC) were used as a substrate for neutrophil adhesion as previously described (Derian et al. 1995, *J. Immunol.*: 154:308-317) with the following modifications. HUVEC cells were obtained from Anthrogenesis (Cedar Knolls, N.J., USA), and neutrophils were not treated with cytochalasin B. Cells were treated with compounds at 10, 1, 0.1, 0.01, 0.001, and 0 μM in a final DMSO concentration of 0.1% in duplicate for 10 minutes, stimulated with 500 nM fMLF for 30 minutes, and washed twice with PBS before measuring fluorescence on an FLX800 plate reader (Bio-Tek Instruments, Winooski, Vt., USA). Results are shown in Table 2.

TABLE 2

Human Neutrophil Assays (all values in nM)	Racemic Compound	Compound A
PGE_2-induced cAMP EC_{50}	12589	4570
fMLF-induced LTB4 IC_{50}	20.1	2.48
Zymosan-induced IL-8 IC_{50}	ND	94
fMLF-induced CD18 expression IC_{50}	ND	390
fMLF-induced CD11b expression IC_{50}	ND	74
fMLF-induced adhesion to HUVEC IC_{50}	ND	150

5.8. Example 8

Aqueous Solubility

Equilibrium solubilities were measured in pH 7.4 aqueous buffer. The pH 7.4 buffer was prepared by adjusting the pH of a 0.07 M NaH_2PO_4 solution to 7.4 with 10 N NaOH. The ionic strength of the solution was 0.15. At least 1 mg of powder was combined with 1 ml of buffer to make >1 mg/ml mixture. These samples were shaken for >2 hours and left to stand overnight at room temperature. The samples were then filtered through a 0.45-μm Nylon syringe filter that was first saturated with the sample. The filtrate was sampled twice, consecutively. The filtrate was assayed by HPLC against standards prepared in 50% methanol. Compound A has 3.5-fold greater aqueous solubility than the racemic mixture. Measured solubility Compound A=0.012 mg/ML; racemic mixture=0.0034 mg/mL.

5.9. Example 8

LPS-Induced Lung

Neutrophilia Ferret Model

The conscious ferret model has been used to investigate anti-inflammatory, emetic and behavioral effects of PDE4 inhibitors when administered by the oral (p.o.) route. From these experiments, a therapeutic index (TI) for each PDE4 inhibitor may be determined. The TI has been calculated by dividing the threshold dose for causing emetic episodes and behavioral changes by the anti-inflammatory dose (dose that causes 50% inhibition of the LPS-induced neutrophilia).

Animal husbandry

Male ferrets (Mustela Pulorius Euro, weighing 1-2 kg). Ferrets were supplied either by Bury Green Farm or Misay Consultancy. Following transport, the animals were allowed to acclimatize in the holding rooms for a period of not less than 7 days. The Diet comprised SDS diet C pelleted food given ad lib with Whiskers cat food given 3 times per week. Water was pasteurized animal grade drinking water and was changed daily.

Dosing with PDE4 inhibitor

PDE4 inhibitors were administered orally (p.o.), at doses initially of 1-10 mg/kg, but subsequently up to 30 mg/kg in order to establish whether the TI was 10 or higher, and/or at lower doses to establish the minimum dose to cause 50% inhibition of neutrophilia. Ferrets were fasted overnight but allowed free access to water. The animals were orally dosed with vehicle or PDE4 inhibitor using a 15 cm dosing needle that was passed down the back of the throat into the oesophagus. After dosing, the animals were returned to holding cages fitted with Perspex doors to allow observation, and given free access to water. After dosing, the animals were constantly observed and any emesis or behavioural changes were recorded. The animals were allowed access to food 60-90 minutes after p.o. dosing

Exposure to LPS

Thirty minutes after p.o. dosing with compound or vehicle control, the ferrets were placed into sealed Perspex containers and exposed to an aerosol of LPS (100 μg/ml) for 10 minutes. Aerosols of LPS were generated by a nebulizer (DeVilbiss, USA) and this was directed into the Perspex exposure chamber. Following a 10 minute exposure period, the animals were returned to the holding cages and allowed free access to water, and at a later stage, food. Observation continued for a period of at least 2.5 hours post p.o. dosing and emetic episodes and behavioral changes were recorded.

Bronchoalveolar lavage

Six hours after LPS exposure the animals were killed by overdose of sodium pentobarbitone administered intraperitoneally. The trachea was then cannulated with polypropylene tubing and the lungs lavaged twice with 20 ml heparinized (10 units/ml) phosphate buffered saline (PBS).

Blood sampling/tissue removal

A terminal blood sample (10 ml) was removed by transthoracic cardiac puncture. The blood was spun at 2500 rpm for 15 minutes and the plasma removed and stored at −20° C. The brain also removed and frozen at −20° C. for analysis of compound content.

Cell counts

The bronchoalveolar lavage (BAL) samples were centrifuged at 1500 rpm for 5 minutes. The supernatant was removed and the resulting cell pellet re-suspended in 1 ml PBS. A cell smear of the re-suspended fluid was prepared and stained with Leishmans stain to allow differential cell counting. A total cell count was made using the remaining re-suspended sample. From this, the total number of neutrophils in the BAL-was determined.

Parameters measured:

1. % Inhibition of LPS-induced pulmonary neutrophilia.

2. Emetic episodes—the number of vomits and retches were counted.

3. Behavioral changes—the following behavioral effects were noted: salivation, panting, mouth clawing, flattened posture, ataxia, arched back and backward walking. Any behavioral changes were semi-quantified by applying a severity rating (mild, moderate or severe).

4. The TI was calculated as the highest dose found to not cause emetic episodes divided by the lowest dose found to inhibit pulmonary neutrophilia by 50% or more.

The effect of Compound A on LPS-induced neutrophilia in the lungs of conscious ferrets is demonstrated in FIG. **1**.

Emesis and behavioral changes

Following p.o. dosing of the PDE4, the ferrets were observed for at least 2 hours and emetic episodes (vomits and retches) and behavioral changes were recorded.

No emetic episodes (retching or vomiting) were observed in the ferrets pre-treated p.o. with the relevant vehicle (acetone/cremophor/distilled water). In a small proportion of the control-treated animals (7/22), mild behavioral changes (lip licking and backward walking) were seen.

Compound A (0.1-3 mg/kg, p.o.), caused no emetic episodes (retching and vomiting). Some behavioral changes (flattened posture, lip licking and backward walking) were observed and classified as mild. At 10 mg/kg in 2/6 ferrets, some retching but no frank emesis was observed along with salivation and behavioral changes (scored as mild or moderate). At the highest dose tested (30 mg/kg) moderate to marked emesis was observed in ¾ animals along with pronounced behavioral changes. These data are summarized in Table III.

TABLE III

Conscious ferret: Emetic episodes and behavioural changes following oral administration of Compound A.

Treatment/dose (mg/kg)	Vomits	Retches	Salivation	Panting	Mouth clawing	Flattened posture	Ataxia	Lip licking	Backward walking
Vehicle (acetone/ cremophor/ dist.H2O)	None	None	None	None	None	None	None	Mild (6/22)	Mild (7/22)
Compound A (0.1 mg/kg)	None	None	None	None	None	Mild (2/5)	None	Mild (4/5)	Mild (3/5)
Compound A (0.3 mg/kg)	None	None	None	None	None	Mild (2/6)	None	Mild (3/6)	Mild (4/6)

TABLE III-continued

Conscious ferret: Emetic episodes and behavioural changes following oral administration of Compound A.

Treatment/dose (mg/kg)	Vomits	Retches	Salivation	Panting	Mouth clawing	Flattened posture	Ataxia	Lip licking	Backward walking
Compound A (1.0 mg/kg)	None	None	None	None	None	Mild (2/6)	None	Mild (6/6)	Mild (4/6)
Compound A (3.0 mg/kg)	None	None	None	None	Mild (1/8)	Marked (7/8)	None	Mild (2/8)	Moderate (5/8)
Compound A (10 mg/kg)	None	Mild (2/6)	Mild (1/6)	None	Mild (1/6)	Marked (6/6)	None	Moderate (5/6)	Marked (6/6)
Compound A (30 mg/kg)	Moderate (3/4)	Marked (3/4)	Moderate (3/4)	Mild (1/4)	Marked (4/4)	Marked (4/4)	Mild (3/4)	Moderate (4/4)	Mild (2/4)

Animals were observed for up to 3 hours following dosing. Numbers in parentheses refer to the number of animals that responded The numbers of animals in each group range from 4-22.

Therapeutic Index Calculation

From these experiments, a therapeutic index (TI) was determined for each compound by dividing the threshold dose for inducing emetic episodes by the ED_{50} value for inhibiting the pulmonary neutrophilia. The TI calculation is summarized in Table IV. Compound A had a TI of 12, causing no emetic episodes at an anti-inflammatory dose of 1 mg/kg.

TABLE IV

Summary of the effective doses (ED_{50}) for inhibition of LPS-induced pulmonary neutrophilia and induction of emesis and the therapeutic index derived from these values.

Compound	Inhibition of LPS-induced neutrophilia (ED_{50} mg/kg)	Threshold emetic dose (mg/kg)	Therapeutic index
Compound A	0.8	10	12

5.10. Example 9

200MG Dosage Capsule

Table V illustrates a batch formulation and single dosage formulation for a 200 mg Compound A single dose unit, i.e., about 40 percent by weight, in a size #0 capsule.

TABLE V

Formulation for 200 mg capsule

Material	Percent By Weight	Quantity (mg/tablet)	Quantity (kg/batch)
Compound A	40.0%	200 mg	16.80 kg
Pregelatinized Corn Starch, NF5	9.5%	297.5 mg	24.99 kg
Magnesium Stearate	0.5%	2.5 mg	0.21 kg
Total	100.0%	500 mg	42.00 kg

The pregelatinized corn starch (SPRESS B-820) and Compound A components are passed through a 710 μm screen and then are loaded into a Diffusion Mixer with a baffle insert and blended for 15 minutes. The magnesium stearate is passed through a 210 μm screen and is added to the Diffusion Mixer. The blend is then encapsulated in a size #0 capsule, 500 mg per capsule (8400 capsule batch size) using a Dosator type capsule filling machine.

5.11. Example 10

100 MG Oral Dosage Form

Table VI illustrates a batch formulation and a single dose unit formulation containing 100 mg of Compound A.

TABLE VI

Formulation for 100 mg tablet

Material	Percent by Weight	Quantity (mg/tablet)	Quantity (kg/batch)
Compound A	40%	100.00	20.00
Microcrystalline Cellulose, NF	53.5%	133.75	26.75
Pluronic F-68 Surfactant	4.0%	10.00	2.00
Croscarmellose Sodium Type A, NF	2.0%	5.00	1.00
Magnesium Stearate, NF	0.5%	1.25	0.25
Total	100.0%	250.00 mg	50.00 kg

The microcrystalline cellulose, croscarmellose sodium, and Compound A components are passed through a #30 mesh screen (about 430μ to about 655μ). The Pluronic F-68® (manufactured by JRH Biosciences, Inc. of Lenexa, Kans.) surfactant is passed through a #20 mesh screen (about 457μ to about 1041μ). The Pluronic F-68® surfactant and 0.5 kgs of croscarmellose sodium are loaded into a 16 qt. twin shell tumble blender and are mixed for about 5 minutes. The mix is then transferred to a 3 cubic foot twin shell tumble blender where the microcrystalline cellulose is added and blended for about 5 minutes. The thalidomide is added and blended for an additional 25 minutes. This pre-blend is passed through a roller compactor with a hammer mill attached at the discharge of the roller compactor and moved back to the tumble blender. The remaining croscarmellose sodium and magnesium stearate is added to the tumble blender and blended for about 3 minutes. The final mixture is compressed on a rotary tablet press with 250 mg per tablet (200,000 tablet batch size).

5.12. Example 11

Aerosol Dosage Form

A concentrate is prepared by combining Compound A, and a 12.6 kg portion of the trichloromonofluoromethane in a

sealed stainless steel vessel equipped with a high shear mixer. Mixing is carried out for about 20 minutes. The bulk suspension is then prepared in the sealed vessel by combining the concentrate with the balance of the propellants in a bulk product tank that is temperature controlled to 21° to 27° C. and pressure controlled to 2.8 to 4.0 BAR. 17 ml aerosol containers which have a metered valve which is designed to provide 100 inhalations of the composition of the invention. Each container is provided with the following:

Compound A	0.0120 g
trichloromonofluoromethane	1.6939 g
dichlorodifluoromethane	3.7175 g
dichlorotetrafluoroethane	1.5766 g
total	7.0000 g

While the invention has been described with respect to the particular embodiments, it will be apparent to those skilled in the art that various changes and modifications may be made without departing from the spirit and scope of the invention as defined in the claims. Such modifications are also intended to fall within the scope of the appended claims.

What is claimed is:

1. A pharmaceutical composition comprising stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, or a pharmaceutically acceptable salt, solvate or hydrate, thereof; and a pharmaceutically acceptable carrier, excipient or diluent.

2. The pharmaceutical composition of claim **1** wherein said pharmaceutical composition is suitable for parenteral, transdermal, mucosal, nasal, buccal, sublingual, or oral administration to a patient.

3. The pharmaceutical composition of claim **2** wherein said pharmaceutical composition is suitable for oral administration to a patient.

4. The pharmaceutical composition of claim **2** wherein the amount of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonyl-ethyl]-4-acetylaminoisoindoline-1,3-dione is from 1 mg to 1000 mg.

5. The pharmaceutical composition of claim **4** wherein the amount of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonyl-ethyl]-4-acetylaminoisoindoline-1,3-dione is from 5 mg to 500 mg.

6. The pharmaceutical composition of claim **5** wherein the amount of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonyl-ethyl]-4-acetylaminoisoindoline-1 ,3-dione is from 10 mg to 200 mg.

7. A single unit dosage form which comprises about 1 mg to about 1000 mg of a stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylamino-isoindoline-1,3-dione or a pharmaceutically acceptable salt, solvate or hydrate, thereof; and a pharmaceutically acceptable carrier, excipient or diluent.

8. The dosage form of claim **7** wherein said dosage form is suitable for parenteral, transdermal, mucosal, nasal, buccal, sublingual, or oral administration to a patient.

9. The dosage form of claim **8** wherein said dosage form is a capsule or a tablet.

10. The dosage form of claim **9** wherein said dosage form is an aerosol.

11. The dosage form of claim **7** wherein the amount of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is from about 5 mg to about 500 mg.

12. The dosage form of claim **11** wherein the amount of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is from about 10 mg to about 200 mg.

13. Stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfo- nylethyl]-4-acetylaminoisoindoline-1,3-dione, substantially free of its (−) isomer, or a pharmaceutically acceptable metabolite, salt, solvate or hydrate, thereof.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,427,638 B2
APPLICATION NO. : 11/106142
DATED : September 23, 2008
INVENTOR(S) : Muller et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title Pg, Item (54)

In the title, replace "(+)-2-[1-(3-ETHOXY-4-METHOXYPHENYL)-2-METHYLSULFONYLETHYL]-4-ACETYLAMINOISOINDOLINE-1,3-DIONE:, AND METHODS OF SYNTHESIS AND COMPOSITIONS THEREOF"

with -- (+)-2-[1-(3-ETHOXY-4-METHOXYPHENYL)-2-METHYLSULFONYLETHYL]-4-ACETYLAMINOISOINDOLINE-1,3-DIONE: METHODS OF USING AND COMPOSITIONS THEREOF --

In claim 4, at column 32, line 3, "methylsulfonyl-ethyl" should be -- methylsulfonylethyl --

In claim 5, at column 32, line 7, "methylsulfonyl-ethyl" should be -- methylsulfonylethyl --

In claim 6, at column 32, line 11, "methylsulfonyl-ethyl" should be -- methylsulfonylethyl --

In claim 13, at column 32, line 35, "methylsulfo- nylethyl" should be -- methylsulfonylethyl --

Signed and Sealed this

Twentieth Day of January, 2009

JON W. DUDAS
Director of the United States Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,427,638 B2
APPLICATION NO. : 11/106142
DATED : September 23, 2008
INVENTOR(S) : Muller et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title Pg, Item (54) and Column 1, lines 1-5

In the title, replace "(+)-2-[1-(3-ETHOXY-4-METHOXYPHENYL)-2-METHYLSULFONYLETHYL]-4-ACETYLAMINOISOINDOLINE-1,3-DIONE:, AND METHODS OF SYNTHESIS AND COMPOSITIONS THEREOF"

with -- (+)-2-[1-(3-ETHOXY-4-METHOXYPHENYL)-2-METHYLSULFONYLETHYL]-4-ACETYLAMINOISOINDOLINE-1,3-DIONE: METHODS OF USING AND COMPOSITIONS THEREOF --

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In claim 13, at column 32, line 35, "methylsulfo- nylethyl" should be -- methylsulfonylethyl --

This certificate supersedes the Certificate of Correction issued January 20, 2009.

Signed and Sealed this

Seventeenth Day of February, 2009

John Doll

JOHN DOLL
Acting Director of the United States Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,427,638 B2
APPLICATION NO. : 11/106142
DATED : September 23, 2008
INVENTOR(S) : Muller et al.

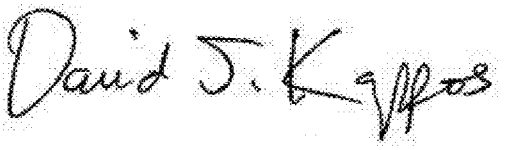
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the Title Page:

The first or sole Notice should read --

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 609 days.

Signed and Sealed this
Twenty-sixth Day of July, 2011



David J. Kappos
Director of the United States Patent and Trademark Office

EXHIBIT D

US007659302B2

(12) **United States Patent**
Muller et al.

(10) **Patent No.: US 7,659,302 B2**
(45) **Date of Patent: Feb. 9, 2010**

(54) **METHODS OF USING (+)-2-[1-(3-ETHOXY-4 METHOXYPHENYL)-2-METHYLSULFONYL-ETHYL]-4 ACETYLAMINOISOINDOLINE 1,3-DIONE**

(75) Inventors: **George W. Muller**, Bridgewater, NJ (US); **Peter H. Schafer**, Somerset, NJ (US); **Hon-Wah Man**, Princeton, NJ (US); **Chuansheng Ge**, Belle Mead, NJ (US)

(73) Assignee: **Celgene Corporation**, Summit, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **12/069,282**

(22) Filed: **Feb. 8, 2008**

(65) **Prior Publication Data**

US 2008/0207730 A1 Aug. 28, 2008

Related U.S. Application Data

(62) Division of application No. 11/170,308, filed on Jun. 28, 2005, now Pat. No. 7,358,272, which is a division of application No. 10/392,195, filed on Mar. 19, 2003, now Pat. No. 6,962,940.

(60) Provisional application No. 60/366,515, filed on Mar. 20, 2002, provisional application No. 60/438,450, filed on Jan. 7, 2003.

(51) **Int. Cl.**
A61K 31/4035 (2006.01)
C07D 209/44 (2006.01)

(52) **U.S. Cl.** **514/417**; 548/469; 548/478

(58) **Field of Classification Search** 548/469, 548/478; 514/417
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,031,450	A	4/1962	Fischer et al.	
3,322,755	A	5/1967	Roch et al.	
3,920,636	A	11/1975	Takahashi et al.	
4,001,237	A	1/1977	Partyka et al.	
4,001,238	A	1/1977	Partyka et al.	
4,047,404	A	9/1977	Hayashi	
4,060,615	A	11/1977	Matier et al.	
4,101,548	A	7/1978	Crenshaw et al.	
4,162,316	A	7/1979	Nishimura et al.	
4,209,623	A	6/1980	Juby	
4,880,810	A	11/1989	Lowe, III	
4,885,301	A	12/1989	Coates	
5,147,875	A	9/1992	Coates et al.	
5,354,571	A	10/1994	Morikawa et al.	
5,401,774	A	3/1995	Pamukcu et al.	
5,439,895	A	8/1995	Lee et al.	
5,488,055	A	1/1996	Kumar et al.	
5,608,914	A	3/1997	Keesler	
5,614,530	A	3/1997	Kumar et al.	
5,614,627	A	3/1997	Takase et al.	
5,658,940	A	8/1997	Muller et al.	
5,698,579	A	12/1997	Muller	
5,703,098	A	12/1997	Muller et al.	
5,710,170	A	1/1998	Guay et al.	
5,728,844	A	3/1998	Muller et al.	
5,728,845	A	3/1998	Muller et al.	
5,736,570	A	4/1998	Muller et al.	
5,798,373	A	8/1998	Warrellow	
5,801,195	A	9/1998	Muller et al.	
5,849,770	A	12/1998	Head et al.	
5,877,200	A	3/1999	Muller	
5,891,896	A	4/1999	Warrellow et al.	
6,011,050	A	1/2000	Muller et al.	
6,020,339	A	2/2000	Perrier et al.	
6,020,358	A	2/2000	Muller et al.	
6,034,089	A	3/2000	Han et al.	
6,046,221	A	4/2000	Muller et al.	
6,069,156	A	5/2000	Oku et al.	
6,162,830	A	12/2000	Connor et al.	
6,166,041	A	12/2000	Cavalla et al.	
6,177,471	B1	1/2001	Menander et al.	
6,204,275	B1	3/2001	Friesen et al.	
6,218,400	B1	4/2001	Daugan et al.	
6,300,335	B1	10/2001	Campbell et al.	
6,316,472	B1	11/2001	Frenette et al.	
6,333,354	B1	12/2001	Schudt	
6,962,940	B2 *	11/2005	Muller et al.	514/417
7,208,516	B2 *	4/2007	Muller et al.	514/417
7,276,529	B2 *	10/2007	Muller et al.	514/417
7,358,272	B2 *	4/2008	Muller et al.	514/417
7,427,638	B2 *	9/2008	Muller et al.	514/411
7,507,759	B2 *	3/2009	Muller et al.	514/417

FOREIGN PATENT DOCUMENTS

EP	0 347 146		6/1989
EP	0 349 239	A2	6/1989
EP	0 351 058		6/1989
EP	0 352 960	A2	7/1989
EP	0 395 328		4/1990
EP	0 428 268	A2	10/1990
EP	0 463 756	A1	6/1991
EP	0 526 004	A1	7/1992

(Continued)

OTHER PUBLICATIONS

U.S. Appl. No. 60/454,155, filed Mar. 12, 2003, Muller, G. et al.

(Continued)

Primary Examiner—Golam M Shameem
(74) *Attorney, Agent, or Firm*—Jones Day

(57) **ABSTRACT**

Stereomerically pure (+)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, substantially free of its (−) isomer, and prodrugs, metabolites, polymorphs, salts, solvates, hydrates, and clathrates thereof are discussed. Also discussed are methods of using and pharmaceutical compositions comprising the (+) enantiomer of 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione are disclosed. The methods include methods of treating and/or preventing disorders ameliorated by the reduction of levels of TNF-α or the inhibition of PDE4.

20 Claims, 2 Drawing Sheets

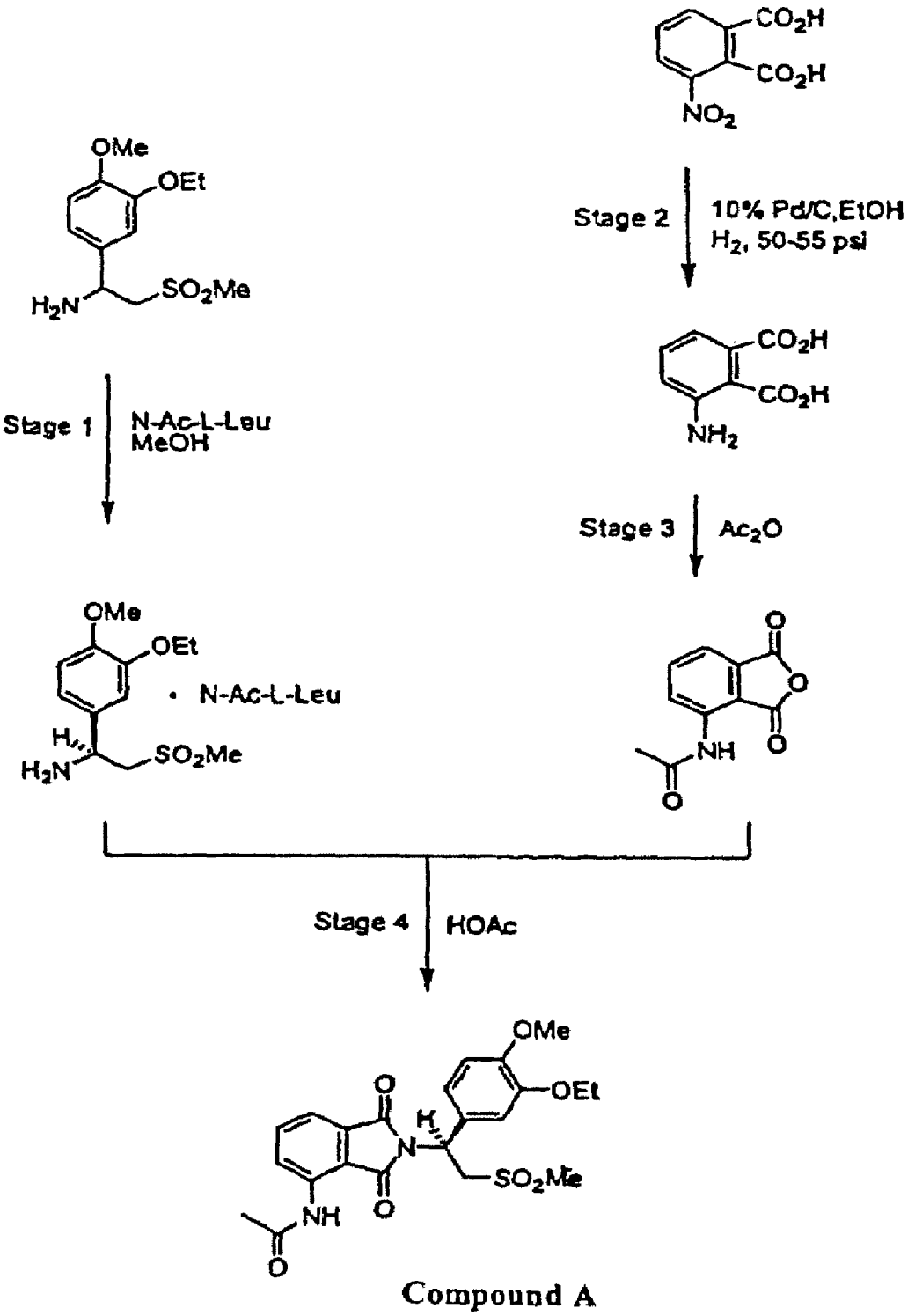
FOREIGN PATENT DOCUMENTS

EP	0 607 439	A1	9/1992
EP	0 722 937	A1	1/1996
EP	0 722 943	A1	1/1996
EP	0 722 944	A1	1/1996
GB	2 063 249	A	9/1980
WO	WO 93/07149		4/1993
WO	WO 93/12095		6/1993
WO	WO 94/01728		1/1994
WO	WO 94/05661		3/1994
WO	WO 94/29277		12/1994
WO	WO 95/19978		7/1995
WO	WO 96/32379		10/1996
WO	WO 97/03070		1/1997
WO	WO 97/03675		2/1997
WO	WO 97/03985		2/1997
WO	WO 97/24334		7/1997
WO	WO 98/06722		2/1998
WO	WO 98/08848		3/1998
WO	WO 98/14448		4/1998
WO	WO 98/16521		4/1998
WO	WO 98/17668		4/1998
WO	WO 98/23597		6/1998
WO	WO 98/38168		9/1998
WO	WO 99/06041		2/1999

OTHER PUBLICATIONS

U.S. Appl. No. 60/454,149, filed Mar. 12, 2003, Muller, G. et al.
U.S. Appl. No. 60/438,448, filed Jan. 7, 2003, Muller, G. et al.
U.S. Appl. No. 60/436,975, filed Dec. 30, 2002, Muller, G. et al.
U.S. Appl. No. 60/366,515, filed Mar. 20, 2002, Muller, G. et al.
Akazome, M. et al., 1997, "Asymmetric recognition of 1-arylethylamines by (R)-phenylglycyl-(R)-phenylglycine and its mechanism," Tetrahedron: Asymmetry, Elsevier Scince Publishers, Amsterdam, NL, 8(14):2331-2336.
Au et al., 1998, *Brit. J. Pharm*. 123:1260-1266.
Baehr et al., 1979, *J. Biol. Chem*. 254:11669.
Baughman et al., 1990, *J. Lab. Clin. Med*. 115:36-42.
Beavo and Reifsnyder, *Trends in Pharm*., 11, 150-55, 1990.
Bissonnette et al., 1989, *Inflammation* 13:329-339.
Bloom and Beavo 1996, *Proc. Natl. Acad Sci. USA* 93:14188-14192.
Brackeen, M.F. et al., 1995, "Design and synthesis of conformationally constrained analogues of 4-(3-butoxy-4-methoxybenzyl) imidazolidin-2-one (Ro 20-1724) as potent inhibitors of cAMP-specific phosphodiesterase", *J. Med. Chem*. 38:4848-54.
Carstensen, Jens T., 1995, *Drug Stability: Principles & Practice*, 2nd ed., Marcel Dekker, New York, NY pp. 379-380.
Casini et al., 1964, Farmaco Ed. Sci. 19:563.
Clouse et al., 1989, *J. Immunol*. 142:431-438.
Derian et al., 1995, J. Immunol. 154:308-317.
Duh et al., 1989, *Proc. Nat. Acad. Sci*. 86:5974-5978.
Featherstone, R.L., et al., 2000, "Comparison of phosphodiesterase inhibitors of differing isoenzyme selectivity added to St. Thomas' hospital cardioplegic solution used for hypothermic preservation of rat lungs", Am. J. Respir. Crit. Care Med. 162:850-6.
Gillespie et al., 1989, *Mol. Pharm*. 36:773.
Hidaka and Asano 1976, *Biochem. Biophys. Acta* 429:485.
Hinshaw et al. 1990, *Circ. Shock* 30:2797-292.
Holler et al., 1990, *Blood* 75:1011-1016.
Johnson et al., 1989, *Endocrinology* 124:1424-1427.
List et al., 1990, *J. Clin. Oncol*. 8:1424.MDS.
Luke, G.P. et al., 1999, "Synthesis of (S)-5-(1-aminoethyl)-2-(cyclohexylmethoxy) benzamide," Tetrahedron: Asymmetry, Elsevier Science Publishers, Amsterdam, NL, 10(22):4393-4403.
Merck Manual (1999) 17th ed., 953.
Monté et al., 1990, *Blood* 79:2670.
Muller, George, et al., 1999, *Bioorganic & Medicinal Chemistry Letters* 9; pp. 1625-1630.
Muller et al., 1998, *Bioorg. & Med Chem Lett*. 8:2669-2674.
Muller, et al., 1996, *J. Med. Chem*. 39:3238.
Nicholson et al., 1991, *Trends Pharmaco. Sci*. 12:19.
Shealy et al., 1965, "D- and L-thalidomide." *Chem. Indus*. 12;24:1030-1.
Tierney, et al., ed., 1998, Current Medical Diagnosis & Treatment, 37th ed., Appleton & Lange, pp. 499.
Verghese, et al., *Journal of Pharmacology and Experimental Therapeutics*, 272(3), 1313-1320, 1995.
Van Dullemen et al., 1995, *Gastroenterology*, 109:129-135.
Wilen, S.H., et al., 1977, *Tetrahedron* 33:2725.
Wilen, S.H., 1972, *Tables of Resolving Agents and Optical Resoutions* (E.L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, IN) p. 268.
Wolff, Manfred E., *ed., Burger's Medicinal Chemistry and Drug Discovery*, 5th ed. 1995 172-178, 949-982.
Dredge et al., 2003, "Angiogenesis inhibitors in cancer therapy," *Curr. Opin. Investig. Drugs* 4(6):667-674.
Dredge et al., 2002, "Immunological effects of thalidomide and its chemical and functional analogs," *Crit. Rev. Immunol*. 22(5-6):425-437.
Dredge et al., 2002, "Recent developments in antiangiogenic therapy," *Expert Opin. Biol. Ther*. 2(8):953-966.
Gee et al., 2003, "Selective cytokine inhibitory drugs with enhanced antiangiogenic activity control tumor growth through vascular inhibition," *Cancer Res*. 63(23):8073-8078.
Marriott et al., 2001, "Immunotherapeutic and antitumour potential of thalidomide analogues," *Expert Opin. Biol. Ther*. 1(4):1-8.
Marriott et al., 2002, "Thalidomide and its analogues have distinct and opposing effects on TNF-alpha and TNFR2 during co-stimulation of both CD4(+) and CD8(+) T cells," *Clin. Exp. Immunol*. 130(1):75-84.
Molostvov et al., 2004, "The effects of selective cytokine inhibitory drugs (CC-10004 and CC-1088) on VEGF and IL-6 expression and apoptosis in myeloma and endothelial cell co-cultures," *Br. J. Haematol*. 124(3):366-375.
Molostvov, G. et al., "Interactions between myeloma and endothelial cells and the effects of thalidomide and its analogues," Database Caplus [Online] Chemical Abstracts Service, Aug. 21, 2003, XP-002526541, AN 2003:649755.
Molostvov, G. et al, "Interactions Between Myeloma and Endothelial Cells and the Effects of Thalidomide and Its Analogues," *Annual Meeting of the European Haematology Association*, 7th Florence, Italy, Jun. 6-9, 2002, pp. 263-266.
Database Registry [Online] Chemical Abstracts Service, Oct. 23, 2003, XP-002526542, AN 608141-41-9.
European Search Report in corresponding EP Patent Application No. 09 00 3138.

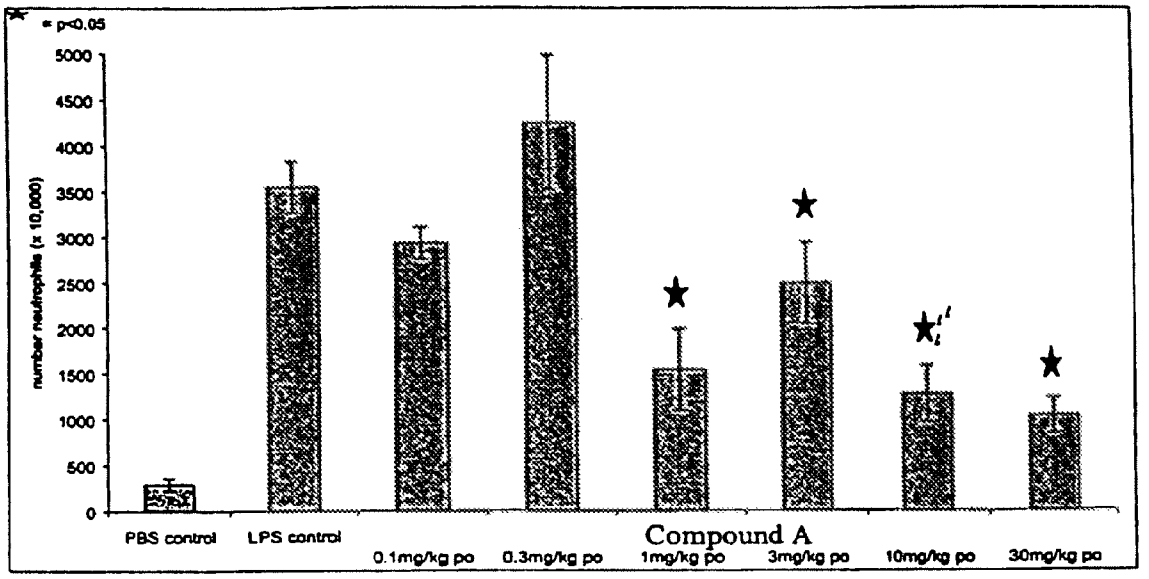
* cited by examiner



Compound A

FIG. 1

FIG 2.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4500
4000
3500
3000
2500
2000
1500
1000
500
0
PBS control
LPS control
0.1mg/kg po
0.3mg/kg po
1mg/kg po
3mg/kg po
10mg/kg po
30mg/kg po
Compound A

METHODS OF USING (+)-2-[1-(3-ETHOXY-4 METHOXYPHENYL)-2-METHYLSULFONYL-ETHYL]-4 ACETYLAMINOISOINDOLINE 1,3-DIONE

CROSS-REFERENCE TO RELATED APPLICATION

This application is a divisional application of U.S. patent application Ser. No. 11/170,308, filed Jun. 28, 2005, now U.S. Pat. No. 7,358,272 which is a divisional application of U.S. patent application Ser. No. 10/392,195, filed Mar. 19, 2003. issued U.S. Pat. No. 6,962,940, which claims the benefit of U.S. Provisional Application No. 60/366,515 filed Mar. 20, 2002 and U.S. Provisional Application No. 60/438,450 filed Jan. 7, 2003, all of which are incorporated herein by reference in their entireties.

1. FIELD OF INVENTION

The invention relates to methods of using and compositions comprising the (+)enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

2. BACKGROUND OF THE INVENTION

Tumor necrosis factor alpha, (TNF-α) is a cytokine that is released primarily by mononuclear phagocytes in response to immunostimulators. TNF-α is capable of enhancing most cellular processes, such as differentiation, recruitment, proliferation, and proteolytic degradation. At low levels, TNF-α confers protection against infective agents, tumors, and tissue damage. But TNF-α also has a role in many diseases. When administered to mammals or humans, TNF-α causes or aggravates inflammation, fever, cardiovascular effects, hemorrhage, coagulation, and acute phase responses similar to those seen during acute infections and shock states. Enhanced or unregulated TNF-α production has been implicated in a number of diseases and medical conditions, for example, cancers, such as solid tumors and blood-born tumors; heart disease, such as congestive heart failure; and viral, genetic, inflammatory, allergic, and autoimmune diseases.

Adenosine 3',5'-cyclic monophosphate (cAMP) also plays a role in many diseases and conditions, such as but not limited to asthma and inflammation, and other conditions (Lowe and Cheng, *Drugs of the Future,* 17(9), 799-807, 1992). It has been shown that the elevation of cAMP in inflammatory leukocytes inhibits their activation and the subsequent release of inflammatory mediators, including TNF-α and NF-κB. Increased levels of cAMP also leads to the relaxation of airway smooth muscle.

It is believed that the primary cellular mechanism for the inactivation of cAMP is the breakdown of cAMP by a family of isoenzymes referred to as cyclic nucleotide phosphodiesterases (PDE) (Beavo and Reitsnyder, *Trends in Pharm.,* 11, 150-155, 1990). There are eleven known PDE families. It is recognized, for example, that the inhibition of PDE type IV is particularly effective in both the inhibition of inflammatory mediator release and the relaxation of airway smooth muscle (Verghese, et al., *Journal of Pharmacology and Experimental Therapeutics,* 272(3), 1313-1320, 1995). Thus, compounds that inhibit PDE4 (PDE IV) specifically, may inhibit inflammation and aid the relaxation of airway smooth muscle with a minimum of unwanted side effects, such as cardiovascular or anti-platelet effects. Currently used PDE4 inhibitors lack the selective action at acceptable therapeutic doses.

Cancer is a particularly devastating disease, and increases in blood TNF-α levels are implicated in the risk of and the spreading of cancer. Normally, in healthy subjects, cancer cells fail to survive in the circulatory system, one of the reasons being that the lining of blood vessels acts as a barrier to tumor-cell extravasation. But increased levels of cytokines have been shown to substantially increase the adhesion of cancer cells to endothelium in vitro. One explanation is that cytokines, such as TNF-α, stimulate the biosynthesis and expression of a cell surface receptors called ELAM-1 (endothelial leukocyte adhesion molecule). ELAM-1 is a member of a family of calcium-dependent cell adhesion receptors, known as LEC-CAMs, which includes LECAM-1 and GMP-140. During an inflammatory response, ELAM-1 on endothelial cells functions as a "homing receptor" for leukocytes. Recently, ELAM-1 on endothelial cells was shown to mediate the increased adhesion of colon cancer cells to endothelium treated with cytokines (Rice et al., 1989, *Science* 246:1303-1306).

Inflammatory diseases such as arthritis, related arthritic conditions (e.g., osteoarthritis and rheumatoid arthritis), inflammatory bowel disease (e.g., Crohn's disease and ulcerative colitis), sepsis, psoriasis, atopic dermatitis, contact dermatitis, and chronic obstructive pulmonary disease, chronic inflammatory pulmonary diseases are also prevalent and problematic ailments. TNF-α plays a central role in the inflammatory response and the administration of their antagonists block chronic and acute responses in animal models of inflammatory disease.

Enhanced or unregulated TNF-α production has been implicated in viral, genetic, inflammatory, allergic, and autoimmune diseases. Examples of such diseases include but are not limited to: HIV; hepatitis; adult respiratory distress syndrome; bone-resorption diseases; chronic obstructive pulmonary diseases; chronic pulmonary inflammatory diseases; asthma, dermatitis; cystic fibrosis; septic shock; sepsis; endotoxic shock; hemodynamic shock; sepsis syndrome; post ischemic reperfusion injury; meningitis; psoriasis; fibrotic disease; cachexia; graft rejection; auto-immune disease; rheumatoid spondylitis; arthritic conditions, such as rheumatoid arthritis and osteoarthritis; osteoporosis; Crohn's disease; ulcerative colitis; inflammatory-bowel disease; multiple sclerosis; systemic lupus erythrematosus; ENL in leprosy; radiation damage; asthma; and hyperoxic alveolar injury. Tracey et al., 1987, *Nature* 330:662-664 and Hinshaw et al., 1990, *Circ. Shock* 30:279-292 (endotoxic shock); Dezube et al., 1990, *Lancet,* 335:662 (cachexia); Millar et al., 1989, *Lancet* 2:712-714 and Ferrai-Baliviera et al., 1989, *Arch. Surg.* 124:1400-1405 (adult respiratory distress syndrome); Bertolini et al., 1986, *Nature* 319:516-518, Johnson et al., 1989, *Endocrinology* 124:1424-1427, Holler et al., 1990, *Blood* 75:1011-1016, and Grau et al., 1989, *N. Engl. J. Med.* 320:1586-1591 (bone resorption diseases); Pignet et al., 1990, *Nature,* 344:245-247, Bissonnette et al., 1989, *Inflammation* 13:329-339 and Baughman et al., 1990, *J. Lab. Clin. Med.* 115:36-42 (chronic pulmonary inflammatory diseases); Elliot et al., 1995, *Int. J. Pharmac.* 17:141-145 (rheumatoid arthritis); von Dullemen et al., 1995, *Gastroenterology,* 109: 129-135 (Crohn's disease); Duh et al., 1989, *Proc. Nat. Acad. Sci.* 86:5974-5978, Poll et al., 1990, *Proc. Nat. Acad. Sci.* 87:782-785, Monto et al., 1990, *Blood* 79:2670, Clouse et al., 1989, *J. Immunol.* 142, 431-438, Poll et al., 1992, *AIDS Res. Hum. Retrovirus,* 191-197, Poll et al. 1990, Proc. *Natl. Acad. Sci.* 87:782-784, Folks et al., 1989, PNAS 86:2365-2368 (HIV and opportunistic infections resulting from HIV).

Pharmaceutical compounds that can block the activity or inhibit the production of certain cytokines, including TNF-α,

may be beneficial therapeutics. Many small-molecule inhibitors have demonstrated an ability to treat or prevent inflammatory diseases implicated by TNF-α (for a review, see Lowe, 1998 *Exp. Opin. Ther. Patents* 8:1309-1332). One such class of molecules are the substituted phenethylsulfones described in U.S. Pat. No. 6,020,358.

3. SUMMARY OF THE INVENTION

This invention relates to methods of treating diseases and disorders utilizing an enantiomer of a substituted phenethylsulfone compound and pharmaceutically acceptable salts, hydrates, solvates, clathrates, prodrugs and polymorphs thereof and methods for reducing the level of cytokines and their precursors in mammals. The invention also relates to pharmaceutical compositions comprising an enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione and a pharmaceutically acceptable carrier. The invention further relates to an enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione substantially free of its other enantiomer.

This invention particularly relates to the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione. This compound is believed to have increased potency and other benefits as compared to its racemate—2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

The invention encompasses the use of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione for treating or preventing diseases or disorders ameliorated by the inhibition of TNF-α production in mammals. In certain embodiments, this treatment includes the reduction or avoidance of adverse effects. Such disorders include, but are not limited to, cancers, including, but not limited to cancer of the head, thyroid, neck, eye, skin, mouth, throat, esophagus, chest, bone, blood, bone marrow, lung, colon, sigmoid, rectum, stomach, prostate, breast, ovaries, kidney, liver, pancreas, brain, intestine, heart, adrenal, subcutaneous tissue, lymph nodes, heart, and combinations thereof. Specific cancers that can be treated by this method are multiple myeloma, malignant melanoma, malignant glioma, leukemia and solid tumors.

The invention also encompasses the use of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione in the treatment or prevention of heart disease, including, but not limited to congestive heart failure, cardiomyopathy, pulmonary edema, endotoxin-mediated septic shock, acute viral myocarditis, cardiac allograft rejection, and myocardial infarction.

The invention also encompasses the use of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione to treat diseases or disorders ameliorated by the inhibition of PDE4. For example, the compounds and compositions of the invention may be useful to treat or prevent viral, genetic, inflammatory, allergic, and autoimmune diseases. Examples of such diseases include, but are not limited to: HIV; hepatitis; adult respiratory distress syndrome; bone-resorption diseases; chronic obstructive pulmonary diseases; chronic pulmonary inflammatory diseases; dermatitis; inflammatory skin disease, atopic dermatitis, cystic fibrosis; septic shock; sepsis; endotoxic shock; hemodynamic shock; sepsis syndrome; post ischemic reperfusion injury; meningitis; psoriasis; fibrotic disease; cachexia; graft rejection including graft versus host disease; auto-immune disease; rheumatoid spondylitis; arthritic conditions, such as rheumatoid arthritis and osteoarthritis; osteoporosis; Crohn's disease; ulcerative colitis; inflammatory-bowel disease; multiple sclerosis; systemic lupus erythrematosus; erythema nodosum leprosum (ENL) in leprosy; radiation damage; asthma; and hyperoxic alveolar injury.

In yet another embodiment, the stereomerically pure (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is also useful in the treatment or prevention of microbial infections or the symptoms of microbial infections including, but not limited to, bacterial infections, fungal infections, malaria, mycobacterial infection, and opportunistic infections resulting from HIV.

The invention further encompasses pharmaceutical compositions and single unit dosage forms comprising an enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione and pharmaceutically acceptable polymorphs, prodrugs, salts, hydrates, clathrates, and solvates thereof.

In a separate embodiment, the invention encompasses the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

In a further embodiment, the invention encompasses a method of producing a stereomerically pure enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione which comprises contacting 1-(3-Ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethylamine with a chiral amino acid and contacting the product of the first step with N-(1,3-Dioxo-1,3-dihydro-isobenzofuran-4-yl)-acetamide. In a related embodiment the invention encompasses a chiral salt of 1-(3-Ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethylamine.

3.1. BRIEF DESCRIPTION OF THE FIGURES

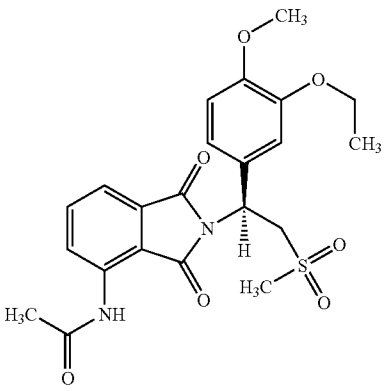
FIG. **1**. illustrates the preparation of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

FIG. **2**. illustrates the effect of the enantiomer of the invention on LPS-induced neutrophilia in the lungs of conscious ferrets.

3.2. Definitions

As used herein, term "Compound A" refers to an enantiomerically pure form of 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione which comes off of an HPLC column at about 25.4 minutes when that column is a 150 mm×4.6 mm Ultron Chiral ES-OVS chiral HPLC column (Agilent Technology), the eluent is 15:85 ethanol: 20 mM KH_2PO_4 at pH 3.5, and the observation wavelength is 240 nm. The ^1H NMR spectrum of compound A is substantially as follows: δ($CDCl_3$): 1.47 (t, 3H), 2.26 (s, 3H), 2.87 (s, 3H), 3.68-3.75 (dd, 1H), 3.85 (s, 3H), 4.07-4.15 (q, 2H), 4.51-4.61 (dd, 1H), 5.84-5.90 (dd, 1H), 6.82-8.77 (m, 6H), 9.46 (s, 1H). The ^{13}C NMR spectrum of Compound A is substantially as follows δ(DMSO-d_6): 14.66, 24.92, 41.61, 48.53, 54.46, 55.91, 64.51, 111.44, 112.40, 115.10, 118.20, 120.28, 124.94, 129.22, 131.02, 136.09, 137.60, 148.62, 149.74, 167.46, 169.14, 169.48. Compound A dissolved in methanol also rotates plane polarized light in the (+) direction.

Without being limited by theory, Compound A is believed to be S-{2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione}, which has the following structure:



As used herein, the term "patient" refers to a mammal, particularly a human.

As used herein, the term "pharmaceutically acceptable salts" refer to salts prepared from pharmaceutically acceptable non-toxic acids or bases including inorganic acids and bases and organic acids and bases. Suitable pharmaceutically acceptable base addition salts for the compound of the present invention include metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from lysine, N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. Suitable non-toxic acids include, but are not limited to, inorganic and organic acids such as acetic, alginic, anthranilic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethenesulfonic, formic, fumaric, furoic, galacturonic, gluconic, glucuronic, glutamic, glycolic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phenylacetic, phosphoric, propionic, salicylic, stearic, succinic, sulfanilic, sulfuric, tartaric acid, and p-toluenesulfonic acid. Specific non-toxic acids include hydrochloric, hydrobromic, phosphoric, sulfuric, and methanesulfonic acids. Examples of specific salts thus include hydrochloride and mesylate salts.

As used herein and unless otherwise indicated, the term "prodrug" means a derivative of a compound that can hydrolyze, oxidize, or otherwise react under biological conditions (in vitro or in vivo) to provide the compound. Examples of prodrugs include, but are not limited to, derivatives and metabolites of Compound A that include biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Prodrugs can typically be prepared using well-known methods, such as those described by 1 *Burger's Medicinal Chemistry and Drug Discovery,* 172-178, 949-982 (Manfred E. Wolff ed., 5th ed. 1995).

As used herein and unless otherwise indicated, the terms "biohydrolyzable amide," "biohydrolyzable ester," "biohydrolyzable carbamate," "biohydrolyzable carbonate," "biohydrolyzable ureide," "biohydrolyzable phosphate" mean an amide, ester, carbamate, carbonate, ureide, or phosphate, respectively, of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties in vivo, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted in vivo to the biologically active compound. Examples of biohydrolyzable esters include, but are not limited to, lower alkyl esters, alkoxyacyloxy esters, alkyl acylamino alkyl esters, and choline esters. Examples of biohydrolyzable amides include, but are not limited to, lower alkyl amides, α-amino acid amides, alkoxyacyl amides, and alkylaminoalkylcarbonyl amides. Examples of biohydrolyzable carbamates include, but are not limited to, lower alkylamines, substituted ethylenediamines, aminoacids, hydroxyalkylamines, heterocyclic and heteroaromatic amines, and polyether amines.

As used herein and unless otherwise indicated, the term "stereomerically pure" means a composition that comprises one stereoisomer of a compound and is substantially free of other stereoisomers of that compound. For example, a stereomerically pure composition of a compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically pure composition of a compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, more preferably greater than about 90% by weight of one stereoisomer of the compound and less than about 10% by weight of the other stereoisomers of the compound, even more preferably greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, and most preferably greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound.

As used herein and unless otherwise indicated, the term "enantiomerically pure" means a stereomerically pure composition of a compound having one chiral center.

As used herein, term "adverse effects" includes, but is not limited to gastrointestinal, renal and hepatic toxicities, leukopenia, increases in bleeding times due to, e.g., thrombocytopenia, and prolongation of gestation, nausea, vomiting, somnolence, asthenia, dizziness, teratogenicity, extra-pyramidal symptoms, akathisia, cardiotoxicity including cardiovascular disturbances, inflammation, male sexual dysfunction, and elevated serum liver enzyme levels. The term "gastrointestinal toxicities" includes but is not limited to gastric and intestinal ulcerations and erosions. The term "renal toxicities" includes but is not limited to such conditions as papillary necrosis and chronic interstitial nephritis.

As used herein and unless otherwise indicated, the phrases "reduce or avoid adverse effects" and "reducing or avoiding adverse effects" mean the reduction of the severity of one or more adverse effects as defined herein.

It should be noted that if there is a discrepancy between a depicted structure and a name given that structure, the depicted structure is to be accorded more weight. In addition, if the stereochemistry of a structure or a portion of a structure is not indicated with, for example, bold or dashed lines, the structure or portion of the structure is to be interpreted as encompassing all stereoisomers of it.

4. DETAILED DESCRIPTION OF THE INVENTION

This invention relates to stereomerically pure Compound A, which is an enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, substantially free of its other enantiomer, as well as novel methods using, and compositions comprising stereomerically pure Compound A. For example, the present invention encompasses the in vitro and in vivo use of Compound A, and the incorporation of Compound A into pharmaceutical

compositions and single unit dosage forms useful in the treatment and prevention of a variety of diseases and disorders. Diseases and disorders which are ameliorated by the reduction of levels of TNF-α or inhibition of PDE4 are well known in the art and are described herein. Specific methods of the invention reduce or avoid the adverse effects associated with compounds used as TNF-α inhibitor. Other specific methods of the invention reduce or avoid the adverse effects associated with use of racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

Specific methods of the invention include methods of treating or preventing diseases and disorders including, but not limited to, solid tumor cancers, blood-born cancers and inflammatory diseases.

Pharmaceutical and dosage forms of the invention, which comprise Compound A or a pharmaceutically acceptable polymorph, prodrug, salt, clathrate, solvate or hydrate thereof, can be used in the methods of the invention.

Without being limited by theory, it is believed that Compound A can inhibit TNF-α production. Consequently, a first embodiment of the invention relates to a method of inhibiting TNF-α production which comprises contacting a cell exhibiting abnormal TNF-α production with an effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. In a particular embodiment, the invention relates to a method of inhibiting TNF-α production which comprises contacting a mammalian cell exhibiting abnormal TNF-α production with an effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof.

The invention also relates to a method of treating or preventing disorders ameliorated by the reduction of levels of TNF-α in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof.

A further embodiment of the invention relates to a method of treating or preventing cancer, including but not limited to, solid tumor, blood-born tumor, leukemias, and in particular, multiple myeloma in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically effective amount of stereomerically pure compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof; in particular wherein the patient is a mammal.

In another embodiment, the invention relates to a method of inhibiting PDE4 which comprises contacting PDE4 with an effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof.

In another embodiment, the invention relates to a method of controlling cAMP levels in a cell which comprises contacting a cell with an effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. As used herein the term "controlling cAMP levels" includes preventing or reducing the rate of the breakdown of Adenosine 3',5'-cyclic monophosphate (cAMP) in a cell or increasing the amount of Adenosine 3',5'-cyclic monophosphate present in a cell, preferably a mammalian cell, more preferably a human cell. In a particular method, the rate of cAMP breakdown is reduced by about 10, 25, 50, 100, 200, or 500 percent as compared to the rate in comparable cells which have not been contacted with a compound of the invention.

A further embodiment of the invention relates to a method of treating or preventing diseases or disorders ameliorated by the inhibition of PDE4 in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. Disorders ameliorated by the inhibition of PDE4 include, but are not limited to, asthma, inflammation (e.g., inflammation due to reperfusion), chronic or acute obstructive pulmonary diseases, chronic or acute pulmonary inflammatory diseases, inflammatory bowel disease, Crohn's Disease, Bechet's Disease, or colitis.

A further embodiment of the invention relates to a method of treating or preventing depression, asthma, inflammation (e.g., contact dermatitis, atopic dermatitis, psoriais, rheumatoid arthritis, osteoarthritis, inflammatory skin disease, inflammation due to reperfusion), chronic or acute obstructive pulmonary diseases, chronic or pulmonary inflammatory diseases, inflammatory bowel disease, Crohn's Disease, Bechet's Disease or colitis in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof; in particular wherein the patient is a mammal.

A separate embodiment of the invention encompasses methods of treating or preventing Myelodysplastic syndrome (MDS) which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. MDS refers to a diverse group of hematopoietic stem cell disorders. MDS is characterized by a cellular marrow with impaired morphology and maturation (dysmyelopoiesis), peripheral blood cytopenias, and a variable risk of progression to acute leukemia, resulting from ineffective blood cell production. See The Merck Manual 953 (17th ed. 1999) and List et al., 1990, *J. Clin. Oncol.* 8:1424.MDS

A separate embodiment of the invention encompasses methods of treating or preventing Myeloproliferative disease (MPD) which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. Myeloproliferative disease (MPD) refers to a group of disorders characterized by clonal abnormalities of the hematopoietic stem cell. See e.g., Current Medical Diagnosis & Treatment, pp. 499 (37th ed., Tierney et al. ed, Appleton & Lange, 1998).

The invention also encompasses a method of treating, preventing or managing complex regional pain syndrome, which comprises administering to a patient in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of a stereomerically pure Compound A, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. In a specific embodiment, the administration is before, during or after surgery or physical therapy directed at reducing or avoiding a symptom of complex regional pain syndrome in the patient.

In particular methods of the invention, stereomerically pure Compound A, or a pharmaceutically acceptable polymorph, prodrug, salt, solvate, hydrate, or clathrate thereof, is

adjunctively administered with at least one additional therapeutic agent. Examples of additional therapeutic agents include, but are not limited to, anti-cancer drugs, anti-inflammatories, antihistamines and decongestants.

4.1. Synthesis and Preparation

Racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is readily prepared using the methods in U.S. Pat. No. 6,020,358, which is incorporated herein by reference.

Compound A can be isolated from the racemic compound by techniques known in the art. Examples include, but are not limited to, the formation of chiral salts and the use of chiral or high performance liquid chromatography "HPLC" and the formation and crystallization of chiral salts. See, e.g., Jacques, J., et al., *Enantiomers, Racemates and Resolutions* (Wiley-Interscience, New York, 1981); Wilen, S. H., et al., *Tetrahedron* 33:2725 (1977); Eliel, E. L., *Stereochemistry of Carbon Compounds* (McGraw-Hill, N.Y., 1962); and Wilen, S. H., *Tables of Resolving Agents and Optical Resolutions* p. 268 (E. L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, Ind., 1972).

In a specific method, Compound A is synthesized from 3-acetamidophthalic anhydride and a chiral amino acid salt of (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine. Chiral amino acid salts of (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine include, but not limited to salts formed with the L isomers of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, ornithine, 4-aminobutyric acid, 2 amino isobutyric acid, 3 amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, and N-acetyl-leucine. A specific chiral amino acid salt is (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine N-acetyl-L-leucine salt, which is resolved from 2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine and N-acetyl-L-leucine in methanol.

4.2. Methods of Treatment

The invention encompasses methods of treating and preventing diseases or disorders ameliorated by the reduction of levels of TNF-α in a patient which comprise administering to a patient in need of such treatment or prevention a therapeutically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof.

Disorders ameliorated by the inhibition of TNF-α include, but are not limited to: heart disease, such as congestive heart failure, cardiomyopathy, pulmonary edema, endotoxin-mediated septic shock, acute viral myocarditis, cardiac allograft rejection, and myocardial infarction; solid tumors, including but not limited to, sarcoma, carcinomas, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, Kaposi's sarcoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma; and blood-born tumors including but not limited to, acute lymphoblastic leukemia "ALL", acute lymphoblastic B-cell leukemia, acute lymphoblastic T-cell leukemia, acute myeloblastic leukemia "AML", acute promyelocytic leukemia "APL", acute monoblastic leukemia, acute erythroleukemic leukemia, acute megakaryoblastic leukemia, acute myelomonocytic leukemia, acute nonlymphocyctic leukemia, acute undifferentiated leukemia, chronic myelocytic leukemia "CML", chronic lymphocytic leukemia "CLL", hairy cell leukemia, multiple myeloma and acute and chronic leukemias, for example, lymphoblastic, myelogenous, lymphocytic, and myelocytic leukemias.

Specific methods of the invention further comprise the administration of an additional therapeutic agent (i.e., a therapeutic agent other than Compound A). Examples of additional therapeutic agents include, but are not limited to, anti-cancer drugs such as, but are not limited to: alkylating agents, nitrogen mustards, ethylenimines, methylmelamines, alkyl sulfonates, nitrosoureas, triazenes, folic acid analofs, pyrimidine analogs, purine analogs, vinca alkaloids, epipodophyllotoxins, antibiotics, topoisomerase inhibitors and anti-cancer vaccines.

Specific additional therapeutic agents include, but are not limited to: acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cisplatin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; dactinomycin; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; docetaxel; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; flurocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; ilmofosine; interleukin II (including recombinant interleukin II, or rIL2), interferon alfa-2a; interferon alfa-2b; interferon alfa-n1; interferon alfa-n3; interferon beta-I a; interferon gamma-I b; iproplatin; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedepa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; paclitaxel; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; piposulfan; piroxantrone

hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; rogletimide; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; triciribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin hydrochloride. Other anti-cancer drugs include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; antidorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-aminotriazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorlns; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl spiromustine; docetaxel; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflomithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptoistatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin

mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

The invention further encompasses a method of treating or preventing diseases or disorders ameliorated by the inhibition of PDE4 in a patient which comprise administering to a patient in need of such treatment or prevention a therapeutically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. Disorders ameliorated by the inhibition of PDE4 include, but are not limited to, asthma, inflammation, chronic or acute obstructive pulmonary disease, chronic or acute pulmonary inflammatory disease, inflammatory bowel disease, Crohn's Disease, Bechet's Disease, colitis, ulcerative colitis and arthritis or inflammation due to reperfusion. In a preferred embodiment, the disease or disorder to be treated or prevented is chronic obstructive pulmonary disease.

Specific methods of the invention can comprise the administration of an additional therapeutic agent such as, but not limited to, anti-inflammatory drugs, antihistamines and decongestants. Examples of such additional therapeutic agents include, but are not limited to: antihistamines including, but not limited to, ethanolamines, ethylenediamines, piperazines, and phenothiazines; antinflammatory drugs; NSAIDS, including, but not limited to, aspirin, salicylates, acetominophen, indomethacin, sulindac, etodolac, fenamates, tolmetin, ketorolac, diclofenac, ibuprofen, naproxen, fenoprofen, ketoprofen, flurbiprofen, oxaprozin, piroxicam, meloxicam, pyrazolon derivatives; and steriods including, but not limited to, cortical steroids and adrenocortical steroids.

Specific methods of the invention avoid or reduce drug-drug interactions and other adverse effects associated with agents used in the treatment of such disorders, including racemic substituted phenylethylsulfones. Without being limited by any theory, stereomerically pure Compound A may further provide an overall improved therapeutic effectiveness, or therapeutic index, over racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione. For example, a smaller amount of the drug may in some circumstances be administered to attain the same level of effectiveness.

As stated above, the active compound of the invention (i.e., Compound A) may be used in the treatment or prevention of a wide range of diseases and conditions. The magnitude of a prophylactic or therapeutic dose of a particular active ingredient of the invention in the acute or chronic management of a disease or condition will vary, however, with the nature and severity of the disease or condition, and the route by which the active ingredient is administered. The dose, and perhaps the dose frequency, will also vary according to the age, body weight, and response of the individual patient. Suitable dosing regimens can be readily selected by those skilled in the art with due consideration of such factors. In general, the recommended daily dose range for the conditions described herein lie within the range of from about 1 mg to about 1000 mg per day, given as a single once-a-day dose preferably as divided doses throughout a day. More specifically, the daily dose is administered twice daily in equally divided doses. Specifically, a daily dose range should be from about 5 mg to about 500 mg per day, more specifically, between about 10 mg and about 200 mg per day. Specifically, the daily dose may be administered in 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 50 mg, or 100 mg dosage forms. In managing the patient, the therapy should be initiated at a lower dose, perhaps about 1 mg to about 25 mg, and increased if necessary up to about 200 mg to about 1000 mg per day as either a single dose or divided doses, depending on the patient's global response. Alternatively, the daily dose is from 0.01 mg/kg to 100 mg/kg.

It may be necessary to use dosages of the active ingredient outside the ranges disclosed herein in some cases, as will be apparent to those of ordinary skill in the art. Furthermore, it is noted that the clinician or treating physician will know how and when to interrupt, adjust, or terminate therapy in conjunction with individual patient response.

The phrases "therapeutically effective amount", "prophylactically effective amount" and "therapeutically or prophylactically effective amount," as used herein encompasses the above described dosage amounts and dose frequency schedules. Different therapeutically effective amounts may be applicable for different diseases and conditions, as will be readily known by those of ordinary skill in the art. Similarly, amounts sufficient to treat or prevent such disorders, but insufficient to cause, or sufficient to reduce, adverse effects associated with racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione are also encompassed by the above described dosage amounts and dose frequency schedules.

4.3. Pharmaceutical Compositions

Pharmaceutical compositions and single unit dosage forms comprising Compound A, or a pharmaceutically acceptable polymorph, prodrug, salt, solvate, hydrate, or clathrate thereof, are encompassed by the invention. Individual dosage forms of the invention may be suitable for oral, mucosal (including rectal, nasal, or vaginal), parenteral (including subcutaneous, intramuscular, bolus injection, intraarterial, or intravenous), sublingual, transdermal, buccal, or topical administration.

Pharmaceutical compositions and dosage forms of the invention comprise stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. Pharmaceutical compositions and dosage forms of the invention typically also comprise one or more pharmaceutically acceptable excipients.

A particular pharmaceutical composition encompassed by this embodiment comprises stereomerically pure Compound A, or a pharmaceutically acceptable polymorph, prodrug, salt, solvate, hydrate, or clathrate thereof, and at least one additional therapeutic agent. Examples of additional therapeutic agents include, but are not limited to: anti-cancer drugs and anti-inflammation therapies including, but not limited to, those listed above in section 4.2.

Single unit dosage forms of the invention are suitable for oral, mucosal (e.g., nasal, sublingual, vaginal, buccal, or rectal), parenteral (e.g., subcutaneous, intravenous, bolus injection, intramuscular, or intraarterial), or transdermal administration to a patient. Examples of dosage forms include, but are not limited to: tablets; caplets; capsules, such as soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suppositories; ointments; cataplasms (poultices); pastes; powders; dressings; creams; plasters; solutions; patches; aerosols (e.g., nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a patient, including suspensions (e.g., aqueous or non-aqueous liquid

suspensions, oil-in-water emulsions, or a water-in-oil liquid emulsions), solutions, and elixirs; liquid dosage forms suitable for parenteral administration to a patient; and sterile solids (e.g., crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms suitable for parenteral administration to a patient.

The composition, shape, and type of dosage forms of the invention will typically vary depending on their use. For example, a dosage form used in the acute treatment of inflammation or a related disorder may contain larger amounts of one or more of the active ingredients it comprises than a dosage form used in the chronic treatment of the same disease. Similarly, a parenteral dosage form may contain smaller amounts of one or more of the active ingredients it comprises than an oral dosage form used to treat the same disease or disorder. These and other ways in which specific dosage forms encompassed by this invention will vary from one another will be readily apparent to those skilled in the art. See, e.g., *Remington's Pharmaceutical Sciences,* 18th ed., Mack Publishing, Easton Pa. (1990).

Typical pharmaceutical compositions and dosage forms comprise one or more excipients. Suitable excipients are well known to those skilled in the art of pharmacy, and non-limiting examples of suitable excipients are provided herein. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a patient. For example, oral dosage forms such as tablets may contain excipients not suited for use in parenteral dosage forms. The suitability of a particular excipient may also depend on the specific active ingredients in the dosage form.

Lactose-free compositions of the invention can comprise excipients that are well known in the art and are listed, for example, in the U.S. Pharmocopia (USP) SP (XXI)/NF (XVI). In general, lactose-free compositions comprise an active ingredient, a binder/filler, and a lubricant in pharmaceutically compatible and pharmaceutically acceptable amounts. Preferred lactose-free dosage forms comprise an active ingredient, microcrystalline cellulose, pre-gelatinized starch, and magnesium stearate.

This invention further encompasses anhydrous pharmaceutical compositions and dosage forms comprising active ingredients, since water can facilitate the degradation of some compounds. For example, the addition of water (e.g., 5%) is widely accepted in the pharmaceutical arts as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. See, e.g., Jens T. Carstensen, *Drug Stability: Principles & Practice,* 2d. Ed., Marcel Dekker, NY, N.Y., 1995, pp. 379-80. In effect, water and heat accelerate the decomposition of some compounds. Thus, the effect of water on a formulation can be of great significance since moisture and/or humidity are commonly encountered during manufacture, handling, packaging, storage, shipment, and use of formulations.

Anhydrous pharmaceutical compositions and dosage forms of the invention can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms that comprise lactose and at least one active ingredient that comprises a primary or secondary amine are preferably anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected.

An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions are preferably packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples of suitable packaging include, but are not limited to, hermetically sealed foils, plastics, unit dose containers (e.g., vials), blister packs, and strip packs.

The invention further encompasses pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will decompose. Such compounds, which are referred to herein as "stabilizers," include, but are not limited to, antioxidants such as ascorbic acid, pH buffers, or salt buffers.

Like the amounts and types of excipients, the amounts and specific types of active ingredients in a dosage form may differ depending on factors such as, but not limited to, the route by which it is to be administered to patients. However, typical dosage forms of the invention comprise compound A, or a pharmaceutically acceptable salt, solvate, clathrate, hydrate, polymoprh or prodrug thereof lie within the range of from about 1 mg to about 1000 mg per day, given as a single once-a-day dose in the morning but preferably as divided doses throughout the day taken with food. More specifically, the daily dose is administered twice daily in equally divided doses. Specifically, a daily dose range should be from about 5 mg to about 500 mg per day, more specifically, between about 10 mg and about 200 mg per day. In managing the patient, the therapy should be initiated at a lower dose, perhaps about 1 mg to about 25 mg, and increased if necessary up to about 200 mg to about 1000 mg per day as either a single dose or divided doses, depending on the patient's global response.

4.3.1. Oral Dosage Forms

Pharmaceutical compositions of the invention that are suitable for oral administration can be presented as discrete dosage forms, such as, but are not limited to, tablets (e.g., chewable tablets), caplets, capsules, and liquids (e.g., flavored syrups). Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of pharmacy well known to those skilled in the art. See generally, *Remington's Pharmaceutical Sciences,* 18th ed., Mack Publishing, Easton Pa. (1990).

Typical oral dosage forms of the invention are prepared by combining the active ingredient(s) in an intimate admixture with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of preparation desired for administration. For example, excipients suitable for use in oral liquid or aerosol dosage forms include, but are not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents. Examples of excipients suitable for use in solid oral dosage forms (e.g., powders, tablets, capsules, and caplets) include, but are not limited to, starches, sugars, micro-crystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid excipients are employed. If desired, tablets can be coated by standard aqueous or nonaqueous techniques. Such dosage forms can be prepared by any of the methods of pharmacy. In general, pharmaceutical compositions and dosage forms are prepared by uniformly and intimately admixing the active ingredients with liquid carriers, finely divided solid carriers, or both, and then shaping the product into the desired presentation if necessary.

For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compress-

ing in a suitable machine the active ingredients in a free-flowing form such as powder or granules, optionally mixed with an excipient. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

Examples of excipients that can be used in oral dosage forms of the invention include, but are not limited to, binders, fillers, disintegrants, and lubricants. Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gelatin, natural and synthetic gums such as acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre-gelatinized starch, hydroxypropyl methyl cellulose, (e.g., Nos. 2208, 2906, 2910), microcrystalline cellulose, and mixtures thereof.

Examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof. The binder or filler in pharmaceutical compositions of the invention is typically present in from about 50 to about 99 weight percent of the pharmaceutical composition or dosage form.

Suitable forms of microcrystalline cellulose include, but are not limited to, the materials sold as AVICEL-PH-101, AVICEL-PH-103 AVICEL RC-581, AVICEL-PH-105 (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, Pa.), and mixtures thereof. An specific binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC-581. Suitable anhydrous or low moisture excipients or additives include AVICEL-PH-103™ and Starch 1500 LM.

Disintegrants are used in the compositions of the invention to provide tablets that disintegrate when exposed to an aqueous environment. Tablets that contain too much disintegrant may disintegrate in storage, while those that contain too little may not disintegrate at a desired rate or under the desired conditions. Thus, a sufficient amount of disintegrant that is neither too much nor too little to detrimentally alter the release of the active ingredients should be used to form solid oral dosage forms of the invention. The amount of disintegrant used varies based upon the type of formulation, and is readily discernible to those of ordinary skill in the art. Typical pharmaceutical compositions comprise from about 0.5 to about 15 weight percent of disintegrant, specifically from about 1 to about 5 weight percent of disintegrant.

Disintegrants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, agar-agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrilin potassium, sodium starch glycolate, potato or tapioca starch, pre-gelatinized starch, other starches, clays, other algins, other celluloses, gums, and mixtures thereof Lubricants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, and mixtures thereof Additional lubricants include, for example, a syloid silica gel (AEROSIL 200, manufactured by W.R. Grace Co. of Baltimore, Md.), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Plano, Tex.), CAB-O-SIL (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, Mass.), and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about 1 weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated.

4.3.2. Delayed Release Dosage Forms

Active ingredients of the invention can be administered by controlled release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Pat. Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; and 4,008,719, 5,674,533, 5,059,595, 5,591,767, 5,120,548, 5,073,543, 5,639,476, 5,354,556, and 5,733,566, each of which is incorporated herein by reference. Such dosage forms can be used to provide slow or controlled-release of one or more active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the active ingredients of the invention. The invention thus encompasses single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled-release.

All controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition, controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the drug, and can thus affect the occurrence of side (e.g., adverse) effects.

Most controlled-release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled-release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, temperature, enzymes, water, or other physiological conditions or compounds.

4.3.3. Parenteral Dosage Forms

Parenteral dosage forms can be administered to patients by various routes including, but not limited to, subcutaneous, intravenous (including bolus injection), intramuscular, and intraarterial. Because their administration typically bypasses patients' natural defenses against contaminants, parenteral dosage forms are preferably sterile or capable of being sterilized prior to administration to a patient. Examples of parenteral dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions.

Suitable vehicles that can be used to provide parenteral dosage forms of the invention are well known to those skilled in the art. Examples include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

Compounds that increase the solubility of one or more of the active ingredients disclosed herein can also be incorporated into the parenteral dosage forms of the invention.

4.3.4. Transdermal, Topical, and Mucosal Dosage Forms

Transdermal, topical, and mucosal dosage forms of the invention include, but are not limited to, ophthalmic solutions, sprays, aerosols, creams, lotions, ointments, gels, solutions, emulsions, suspensions, or other forms known to one of skill in the art. See, e.g., *Remington's Pharmaceutical Sciences,* 16th and 18th eds., Mack Publishing, Easton Pa. (1980 & 1990); and *Introduction to Pharmaceutical Dosage Forms,* 4th ed., Lea & Febiger, Philadelphia (1985). Dosage forms suitable for treating mucosal tissues within the oral cavity can be formulated as mouthwashes or as oral gels. Further, transdermal dosage forms include "reservoir type" or "matrix type" patches, which can be applied to the skin and worn for a specific period of time to permit the penetration of a desired amount of active ingredients.

Suitable excipients (e.g., carriers and diluents) and other materials that can be used to provide transdermal, topical, and mucosal dosage forms encompassed by this invention are well known to those skilled in the pharmaceutical arts, and depend on the particular tissue to which a given pharmaceutical composition or dosage form will be applied. With that fact in mind, typical excipients include, but are not limited to, water, acetone, ethanol, ethylene glycol, propylene glycol, butane-1,3-diol, isopropyl myristate, isopropyl palmitate, mineral oil, and mixtures thereof to form lotions, tinctures, creams, emulsions, gels or ointments, which are non-toxic and pharmaceutically acceptable. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well known in the art. See, e.g., *Remington's Pharmaceutical Sciences,* 16th and 18th eds., Mack Publishing, Easton Pa. (1980 & 1990).

Depending on the specific tissue to be treated, additional components may be used prior to, in conjunction with, or subsequent to treatment with active ingredients of the invention. For example, penetration enhancers can be used to assist in delivering the active ingredients to the tissue. Suitable penetration enhancers include, but are not limited to: acetone; various alcohols such as ethanol, oleyl, and tetrahydrofuryl; alkyl sulfoxides such as dimethyl sulfoxide; dimethyl acetamide; dimethyl formamide; polyethylene glycol; pyrrolidones such as polyvinylpyrrolidone; Kollidon grades (Povidone, Polyvidone); urea; and various water-soluble or insoluble sugar esters such as Tween 80 (polysorbate 80) and Span 60 (sorbitan monostearate).

The pH of a pharmaceutical composition or dosage form, or of the tissue to which the pharmaceutical composition or dosage form is applied, may also be adjusted to improve delivery of one or more active ingredients. Similarly, the polarity of a solvent carrier, its ionic strength, or tonicity can be adjusted to improve delivery. Compounds such as stearates can also be added to pharmaceutical compositions or dosage forms to advantageously alter the hydrophilicity or lipophilicity of one or more active ingredients so as to improve delivery. In this regard, stearates can serve as a lipid vehicle for the formulation, as an emulsifying agent or surfactant, and as a delivery-enhancing or penetration-enhancing agent. Different salts, hydrates or solvates of the active ingredients can be used to further adjust the properties of the resulting composition.

4.3.5. Kits

Typically, active ingredients of the invention are preferably not administered to a patient at the same time or by the same route of administration. This invention therefore encompasses kits which, when used by the medical practitioner, can simplify the administration of appropriate amounts of active ingredients to a patient.

A typical kit of the invention comprises a unit dosage form of compound A, or a pharmaceutically acceptable salt, solvate, hydrate, clathrate, polymorph or prodrug thereof, and a unit dosage form of a second active ingredient. Examples of second active ingredients include, but are not limited to, those listed in section 4.2 above.

Kits of the invention can further comprise devices that are used to administer the active ingredient(s). Examples of such devices include, but are not limited to, syringes, drip bags, patches, and inhalers.

Kits of the invention can further comprise pharmaceutically acceptable vehicles that can be used to administer one or more active ingredients. For example, if an active ingredient is provided in a solid form that must be reconstituted for parenteral administration, the kit can comprise a sealed container of a suitable vehicle in which the active ingredient can be dissolved to form a particulate-free sterile solution that is suitable for parenteral administration. Examples of pharmaceutically acceptable vehicles include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

5. EXAMPLES

5.1. Example 1

Synthesis of 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione

A stirred solution of 1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethylamine (1.0 g, 3.7 mmol) and 3-acetamidophthalic anhydride (751 mg, 3.66 mmol) in acetic acid (20 mL) was heated at reflux for 15 h. The solvent was removed in vacuo to yield an oil. Chromatography of the resulting oil yielded the product as a yellow solid (1.0 g, 59% yield): mp, 144° C.; ^{1}H NMR ($CDCl_3$) δ1.47 (t, J=7.0 Hz, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.88 (s, 3H, CH_3), 3.75 (dd, J=4.4, 14.3 Hz, 1H, CHH), 3.85 (s, 3H, CH3), 4.11 (q, J=7 Hz, 2H, CH2), 5.87 (dd, J=4.3, 10.5 Hz, 1H, NCH), 6.82-6.86 (m, 1H, Ar), 7.09-7.11 (m, 2H, Ar), 7.47 (d, J=7 Hz, 1H, Ar), 7.64 (t, J=8 Hz, 1H, Ar), 8.74 (d, J=8 Hz, 1H, Ar), 9.49 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ14.61, 24.85, 41.54, 48.44, 54.34, 55.85, 64.43, 111.37, 112.34, 115.04, 118.11, 120.21, 124.85, 129.17, 130.96, 136.01, 137.52, 148.54, 149.65, 167.38, 169.09, 169.40; Anal Calc'd. for $C_{22}H_{24}NO_7S$: C, 57.38; H, 5.25; N, 6.08. Found: C, 57.31; H, 5.34; N, 5.83.

5.2. Example 2

Synthesis of (+)2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione

Preparation of 3-aminopthalic Acid

10% Pd/C (2.5 g), 3-nitrophthalic acid (75.0 g, 355 mmol) and ethanol (1.5 L) were charged to a 2.5 L Parr hydrogenator, under a nitrogen atmosphere. Hydrogen was charged to the reaction vessel for up to 55 psi. The mixture was shaken for 13 hours, maintaining hydrogen pressure between 50 and 55 psi. Hydrogen was released and the mixture was purged with nitrogen 3 times. The suspension was filtered through a celite bed and rinsed with methanol. The filtrate was concentrated in vacuo. The resulting solid was reslurried in ether and isolated by vacuum filtration. The solid was dried in vacua to a constant weight, affording 54 g (84% yield) of 3-aminopthalic acid as a yellow product. ^{1}H-NMR (DMSO-d6) δ: 3.17 (s, 2H), 6.67 (d, 1H), 6.82 (d, 1H), 7.17 (t, 1H), 8-10 (brs, 2H). ^{13}C-NMR(DMSO-d6)δ: 112.00, 115.32, 118.20, 131.28, 135.86, 148.82, 169.15, 170.09.

Preparation of 3-acetamidophthalic Anhydride

A 1 L 3-necked round bottom flask was equipped with a mechanical stirrer, thermometer, and condenser and charged with 3-aminophthalic acid (108 g, 596 mmol) and acetic anhydride (550 mL). The reaction mixture was heated to reflux for 3 hours and cooled to ambient temperature and further to 0-5° C. for another 1 hour. The crystalline solid was collected by vacuum filtration and washed with ether. The solid product was dried in vacua at ambient temperature to a constant weight, giving 75 g (61% yield) of 3-acetamidopthalic anhydride as a white product. ^{1}H-NMR ($CDCl_3$) δ: 2.21 (s, 3H), 7.76 (d, ^{1}H), 7.94 (t, 1H), 8.42 (d, 1H), 9.84 (s, 1H).

Resolution of 2-(3-ethoxy-4-methoxphenyl)-1-(methylsulphonyl)-eth-2-ylamine

A 3 L 3-necked round bottom flask was equipped with a mechanical stirrer, thermometer, and condenser and charged with 2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine (137.0 g, 500 mmol), N-acetyl-L-leucine (52 g, 300 mmol), and methanol (1.0 L). The stirred slurry was heated to reflux for 1 hour. The stirred mixture was allowed to cool to ambient temperature and stirring was continued for another 3 hours at ambient temperature. The slurry was filtered and washed with methanol (250 mL). The solid was air-dried and then dried in vacuo at ambient temperature to a constant weight, giving 109.5 g (98% yield) of the crude product (85.8% ee). The crude solid (55.0 g) and methanol (440 mL) were brought to reflux for 1 hour, cooled to room temperature and stirred for an additional 3 hours at ambient temperature. The slurry was filtered and the filter cake was washed with methanol (200 mL). The solid was air-dried and then dried in vacuo at 30° C. to a constant weight, yielding 49.6 g (90% recovery) of (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine-N-acetyl-L-leucine salt (98.4% ee). Chiral HPLC (1/99 EtOH/20 mM KH_2PO_4@pH 7.0, Ultron Chiral ES-OVS from Agilent Technologies, 150 mm×4.6 mm, 0.5 mL/min., @240 nm): 18.4 min (S-isomer, 99.2%), 25.5 min (R-isomer, 0.8%).

Preparation of Compound A

A 500 mL 3-necked round bottom flask was equipped with a mechanical stirrer, thermometer, and condenser. The reaction vessel was charged with (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-yl amine N-acetyl-L-leucine salt (25 g, 56 mmol, 98% ee), 3-acetamidophthalic anhydride (12.1 g 58.8 mmol), and glacial acetic acid (250 mL). The mixture was refluxed over night and then cooled to <50° C. The solvent was removed in vacuo, and the residue was dissolved in ethyl acetate. The resulting solution was washed with water (250 mL×2), saturated aqeous $NaHCO_3$ (250 mL×2), brine (250 mL×2), and dried over sodium sulphate. The solvent was evaporated in vacuo, and the residue recrystallized from a binary solvent containing ethanol (150 mL) and acetone (75 mL). The solid was isolated by vacuum filtration and washed with ethanol (100 mL×2). The product was dried in vacuo at 60° C. to a constant weight, affording 19.4 g (75% yield) of S-{2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-aminoisoindoline-1,3-dione with 98% ee. Chiral HPLC (15/85 EtOH/20 mM KH_2PO_4@pH 3.5, Ultron Chiral ES-OVS from Agilent Technology, 150 mm×4.6 mm, 0.4 mL/min., @240 nm): 25.4 min (S-isomer, 98.7%), 29.5 min (R-isomer, 1.2%). ^{1}H-NMR ($CDCl_3$) δ:1.47 (t, 3H), 2.26 (s, 3H), 2.87 (s, 3H), 3.68-3.75 (dd, 1H), 3.85 (s, 3H), 4.07-4.15 (q, 2H), 4.51-4.61 (dd, 1H), 5.84-5.90 (dd, 1H), 6.82-8.77 (m, 6H), 9.46 (s, 1H). ^{13}C-NMR (DMSO-d6) δ: 14.66, 24.92, 41.61, 48.53, 54.46, 55.91, 64.51, 111.44, 112.40, 115.10, 118.20, 120.28, 124.94, 129.22, 131.02, 136.09, 137.60, 148.62, 149.74, 167.46, 169.14, 169.48.

5.3. Example 3

TNF-α Inhibition

Human Whole Blood LPS-induced TNF-α Assay

The ability of compounds to inhibit LPS-induced TNF-α production by human whole blood was measured essentially as described below for the LPS-induced TNF-α assay in human PBMC, except that freshly drawn whole blood was used instead of PBMC. (George Muller, et al. 1999, *Bioorganic & Medicinal Chemistry Letters* 9; 1625-1630.) Human whole blood LPS-induced TNF-α IC_{50}-294 nM

Mouse LPS-Induced Serum TNF-α Inhibition

Compounds were tested in this animal model according to previously described methods (Corral et al. 1996, *Mol. Med* 2:506-515). Mouse LPS-induced serum TNF-α inhibition (ED_{50}, mg/kg, p.o.)=0.05.

LPS-Induced TNF-α Production

Lipopolysaccharide (LPS) is an endotoxin produced by gram-negative bacteria such as *E. coli* which induces production of many pro-inflammatory cytokines, including TNF-α. In peripheral blood mononuclear cells (PBMC), the TNF-α produced in response to LPS is derived from monocytes, which comprise approximately 5-20% of the total PBMC. Compounds were tested for the ability to inhibit LPS-induced TNF-α production from human PBMC as previously described (Muller et al. 1996, *J. Med Chem.* 39:3238). PBMC from normal donors were obtained by Ficoll Hypaque (Pharmacia, Piscataway, N.J., USA) density centrifugation. Cells were cultured in RPMI (Life Technologies, Grand Island, N.Y., USA) supplemented with 10% AB±human serum (Gemini Bio-products, Woodland, Calif., USA), 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Life Technologies).

PBMC (2×10^5 cells) were plated in 96-well flat-bottom Costar tissue culture plates (Corning, N.Y., USA) in triplicate. Cells were stimulated with LPS (Sigma, St. Louis, Mo., USA) at 100 ng/ml in the absence or presence of compounds. Compounds (Celgene Corp., Warren, N.J., USA) were dissolved in DMSO (Sigma) and further dilutions were done in culture medium immediately before use. The final DMSO concentration in all samples was 0.25%. Compounds were added to cells 1 hour before LPS stimulation. Cells were incubated for 18-20 hours at 37° C. in 5% CO_2 and superna-

tants were then collected, diluted with culture medium and assayed for TNF-α levels by ELISA (Endogen, Boston, Mass., USA). LPS-induced TNF-α IC_{50}=77 nM.

IL-1β-Induced TNF-α Production

During the course of inflammatory diseases, TNF-α production is often stimulated by the cytokine IL-1β, rather than by bacterially derived LPS. Compounds were tested for the ability to inhibit IL-1βP-induced TNF-α production from human PBMC as described above for LPS-induced TNF-α production, except that the PBMC were isolated from source leukocyte units (Sera-Tec Biologicals, North Brunswick, N.J., USA) by centrifugation on Ficoll-Paque Plus (Amersham Pharmacia, Piscataway, N.J., USA), plated in 96-well tissue culture plates at 3×10^5 cells/well in RPMI-1640 medium (Bio Whittaker, Walkersville, Md., USA) containing 10% heat-inactivated fetal bovine serum (Hyclone), 2 mM L-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin (complete medium), pretreated with compounds at 10, 2, 0.4, 0.08, 0.016, 0.0032, 0.00064, and 0 μM in duplicate at a final DMSO concentration of 0.1% at 37° C. in a humidified incubator at 5% CO_2 for 1 hour, then stimulated with 50 ng/ml recombinant human IL-1β (Endogen) for 18 hours. IL-β-induced TNF-α IC_{50}=83 nM.

5.4. Example 4

PDE Selectivity

PDE1, 2, 3, 5, and 6 Enzyme Assays

The specificity of compounds for PDE4 was assessed by testing at a single concentration (10 μM) against bovine PDE1, human PDE2, PDE3, and PDE5 from human platelets (Hidaka and Asano 1976, *Biochem. Biophys. Acta* 429:485, and Nicholsen et al. 1991, *Trends Pharmaco. Sci.* 12:19), and PDE6 from bovine retinal rod outer segments (Baehr et al. 1979, J. Biol. Chem. 254:11669, and Gillespie et al. 1989, *Mol. Pharm.* 36:773). Results are listed in Table 1.

PDE7 Enzyme Assay

PDE7 is a cAMP-selective PDE expressed mainly in T cells and in skeletal muscle. T cell-derived cytokines such as IL-2 and IFN-γ are potentially regulatable via PDE7 inhibition. PDE7 was purified from Hut78 human T cells by anion exchange chromatography as previously described (Bloom and Beavo 1996, *Proc. Natl. Acad Sci. USA* 93:14188-14192). Compounds were tested against the PDE7 preparation in the presence of 10 nM cAMP as described for PDE4 in Table 1 below.

TABLE 1

	Racemic Compound	Compound A	Compound B*
	PDE Inhibition		
PDE4 IC_{50} (from U937 cells) (nM)	81.8	73.5	611
PDE1 (% inhib at 10 μM)	9%	23%	27%
PDE2(% inhib at 10 μM)	19%	6%	10%
PDE3 (% inhib at 10 μM)	21%	20%	31%
PDE5 (% inhib at 10 μM)	3%	3%	−9%
PDE6 (% inhib at 10 μM)	ND	−6%	10%
PDE7 IC_{50} (nM)	22110	20500	ND
	PDE Specificity Ratios from above data (*fold)		
PDE4/PDE1	>2700	>500	>50
PDE4/PDE2	>800	>10000	>260
PDE4/PDE3	>670	>1200	>45
PDE4/PDE5	>12000	>30000	>39000
PDE4/PDE6	ND	>40000	>250
PDE7 IC_{50}/PDE4 IC_{50}	270	279	ND

*Compound B is the opposite enantiomer of Compound A.

5.5. Example 5

PDE4 Inhibition

PDE4 (U937 Cell-Derived) Enzyme Assay

PDE4 enzyme was purified from U937 human monocytic cells by gel filtration chromatography as previously described (Muller et al. 1998, *Bioorg. & Med Chem Lett* 8:2669-2674). Phosphodiesterase reactions were carried out in 50 mM Tris HCl pH 7.5, 5 mM $MgCl_2$, 1 μM cAMP, 10 nM [^{3}H]-cAMP for 30 min at 30° C., terminated by boiling, treated with 1 mg/ml snake venom, and separated using AG-IXS ion exchange resin (BioRad) as described (Muller et al. 1998, *Bioorg. & Med Chem Lett* 8:2669-2674). Reactions consumed less than 15% of available substrate. Results are listed in Table 1.

5.6. Example 6

Human T Cell Assays

SEB-Induced IL-2 and IFN-γ Production

Staphylococcal Enterotoxin B (SEB) is a superantigen derived from gram-positive bacteria *Staphylococcus aureus*. SEB provides a convenient physiological stimulus specific for T cells expressing particular T cell receptor Vβ chains. Human PBMC (consisting of approximately 50% T cells) were isolated from source leukocyte units as described above and plated in 96-well tissue culture plates at 3×10^5 cells/well in complete medium, pretreated with compounds at 10, 2, 0.4, 0.08, 0.016, 0.0032, 0.00064, and 0 μM in duplicate at a final DMSO concentration of 0.1% at 37° C. in a humidified incubator at 5% CO_2 for 1 hour, then stimulated with 100 ng/ml SEB (Sigma Chemical Co., St. Louis, Mo., USA) for 18 hours. IL-2 and IFN-γ levels were measured by ELISA (R&D Systems, Minneapolis, Minn., USA). IL-2 IC_{50}=291 nM. IFN-γ IC_{50}=46 nM.

5.7. Example 6

cAMP Elevation Assays

PGE_2-Induced cAMP Elevation

Prostaglandin E_2 (PGE_2) binds to prostanoid receptors on monocytes, T cells and other leukocytes and consequently elevates intracellular cAMP levels, resulting in inhibition of cellular responses. The combination of PGE_2 and a PDE4 inhibitor synergistically elevates cAMP levels in these cell types, and the elevation of cAMP in PBMC caused by PDE4 inhibitors in the presence of PGE_2 is proportional to the inhibitory activity of that PDE4 inhibitor. Intracellular cAMP was measured in human PBMC as follows. PBMC were isolated as described above and plated in 96-well plates at 1×10^6 cells per well in RPMI-1640. The cells were pretreated with compounds at 100, 10, 1, 0.1, 0.01, and 0 μM in a final concentration of 2% DMSO in duplicate at 37° C. in a humidified incubator at 5% CO_2 for one hour. The cells were

then stimulated with PGE_2 (10 μM) (Sigma) for 1 h. The cells were lysed with HCl, 0.1N final concentration to inhibit phosphodiesterase activity and the plates were frozen at −20° C. The cAMP produced was measured using cAMP (low pH) Immunoassay kit (R&D Systems). PBMC cAMP EC_{50} for racemate is 3.09 μM. PBMC cAMP EC_{50} for Compound A is 1.58 μM.

Elevation of cAMP in human neutrophils was measured as follows. PBMC were removed from source leukocytes (Sera-Tec Biologicals) by centrifugation on Ficoll—Paque Plus (Amersham Pharmacia). The resulting erythrocyte/polymorphonuclear cell (PMN) pellet was resuspended in Hank's Balanced Salt Solution (Bio Whittaker) and mixed with an equal volume of 3% Dextran T-500 (Amersham Pharmacia) in 0.9% saline. Erythrocytes were allowed to sediment for 20 minutes, and the PMN were removed and centrifuged at 120 rpm for 8 minutes at 4° C. The remaining erythrocytes were lysed in cold 0.2% saline for 30 seconds, and the cells restored to isotonicity by the addition of an equal volume of 1.6% saline. The PMN were centrifuged at 1200 rpm for 8 minutes at 4° C., then resuspended in RPMI-1640 and assayed for cAMP elevation as described for PBMC above. PMN were found to be approximately 74% $CD18/CD11b^+$, 71% $CD16^+$ $CD9^+$ neutrophils by flow cytometzy on a FACSCalibur (Becton Dickinson, San Jose, Calif., USA). Results are shown in Table 2.

fMLF-Induced LTB4 Production

N-formyl-methionine-leucine-phenylalanine (fMLF) is a bacterially derived peptide that activates neutrophils to rapidly degranulate, migrate, adhere to endothelial cells, and release leukotriene LTB4, a product of arachidonic acid metabolism and itself a neutrophil chemoattractant. Compounds were tested for the ability to block fMLF-induced neutrophil LTB4 production as previously described (Hatzelmann and Schudt 2001, *J. Pharm. Exp. Ther.* 297:267-279), with the following modifications. Neutrophils were isolated as described above and resuspended in phosphate-buffered saline without calcium or magnesium (Bio Whittaker) containing 10 mM HEPES pH7.2 and plated in 96-well tissue culture plates at a concentration of 1.7×10^6 cells/well. Cells were treated with 50 μM thimerosal (Sigma)/1 mM $CaCl_2$/1 mM $MgCl_2$ for 15 minutes at 37° C. 5% CO_2, then treated with compounds at 1000, 200, 40, 8, 1.6, 0.32, 0.064, and 0 nM in a final DMSO concentration of 0.01% in duplicate for 10 minutes. Neutrophils were stimulated with 1 μM fMLF for 30 minutes, then lysed by the addition of methanol (20% final concentration) and frozen in a dry ice/isopropanol bath for 10 minutes. Lysates were stored at −70° C. until the LTB4 content was measured by competitive LTB4 ELISA (R&D Systems). Results are shown in Table 2.

Zymosan-Induced IL-8 Production

Zymosan A, or the heat-killed yeast *Saccharomyces cerevisiae*, binds to the adhesion molecule Mac-1 on the neutrophil surface and triggers phagocytosis, cell activation and IL-8 production. Zymosan-induced IL-8 production was measured as previously described (Au et al. 1998, *Brit. J. Pharm.* 123:1260-1266) with the following modifications. Human neutrophils were purified as described above, plated in 96-well tissue culture plates at 3×10^5 cells/well in complete medium, treated with compounds at 10, 2, 0.4, 0.08, 0.016, 0.0032, 0.00064, and 0 μM in duplicate in a final DMSO concentration of 0.1% for 1 hour at 37° C. 5% CO_2. Neutrophils were then stimulated with unopsonized, boiled Zymosan A (Sigma) at 2.5×10^5 particles/well for 18 hours. Supernatants were harvested and tested for IL-8 by ELISA (R&D Systems). Results are shown in Table 2.

fMLF-Induced CD18/CD11b Expression

CD18/CD11b (Mac-1) expression on neutrophils was measured as previously described (Derian et al. 1995, *J. Immunol.:*154:308-3 17) with the following modifications. Neutrophils were isolated as described above, then resuspended in complete medium at 1×10^6 cells/ml, pretreated with compounds at 10, 1, 0.1, 0.01, and 0 μM in duplicate at a final DMSO concentration of 0.1% for 10 minutes at 37° C. 5% CO_2. Cells were then stimulated with 30 nM fMLF for 30 minutes and then chilled to 4° C. Cells were treated with rabbit IgG (Jackson ImmunoResearch Labs, West Grove, Pa., USA) (10 μg/1×10^6 cells) to block Fc receptors, stained with CD18-FITC and CD11b-PE (Becton Dickinson), and analyzed by flow cytometry on a FACSCalibur. CD18/CD11b expression (mean fluorescence) in the absence of stimulation was subtracted from all samples to obtain inhibition curves and calculate IC_{50}s. Results are shown in Table 2.

fMLF-Induced Adhesion to HUVEC

Human umbilical vein endothelial cells (HUVEC) were used as a substrate for neutrophil adhesion as previously described (Derian et al. 1995, *J. Immunol.:* 154:308-317) with the following modifications. HUVEC cells were obtained from Anthrogenesis (Cedar Knolls, N.J., USA), and neutrophils were not treated with cytochalasin B. Cells were treated with compounds at 10, 1, 0.1, 0.01, 0.001, and 0 μM in a final DMSO concentration of 0.1% in duplicate for 10 minutes, stimulated with 500 nM fMLF for 30 minutes, and washed twice with PBS before measuring fluorescence on an FLX800 plate reader (Bio-Tek Instruments, Winooski, Vt., USA). Results are shown in Table 2.

TABLE 2

Human Neutrophil Assays (all values in nM)	Racemic Compound	Compound A
PGE_2-induced cAMP EC_{50}	12589	4570
fMLF-induced LTB4 IC_{50}	20.1	2.48
Zymosan-induced IL-8 IC_{50}	ND	94
fMLF-induced CD18 expression IC_{50}	ND	390
fMLF-induced CD11b expression IC_{50}	ND	74
fMLF-induced adhesion to HUVEC IC_{50}	ND	150

5.8. Example 8

Aqueous Solubility

Equilibrium solubilities were measured in pH 7.4 aqueous buffer. The pH 7.4 buffer was prepared by adjusting the pH of a 0.07 M NaH_2PO_4 solution to 7.4 with 10 N NaOH. The ionic strength of the solution was 0.15. At least 1 mg of powder was combined with 1 ml of buffer to make >1 mg/ml mixture. These samples were shaken for >2 hours and left to stand overnight at room temperature. The samples were then filtered through a 0.45-μm Nylon syringe filter that was first saturated with the sample. The filtrate was sampled twice, consecutively. The filtrate was assayed by HPLC against standards prepared in 50% methanol. Compound A has 3.5-fold greater aqueous solubility than the racemic mixture. Measured solubility Compound A=0.012 mg/mL; racemic mixture=0.0034 mg/mL.

5.9. Example 8

LPS-Induced Lung Neutrophilia Ferret Model

The conscious ferret model has been used to investigate anti-inflammatory, emetic and behavioral effects of PDE4

inhibitors when administered by the oral (p.o.) route. From these experiments, a therapeutic index (TI) for each PDE4 inhibitor may be determined. The TI has been calculated by dividing the threshold dose for causing emetic episodes and behavioral changes by the anti-inflammatory dose (dose that causes 50% inhibition of the LPS-induced neutrophilia).

Animal Husbandry

Male ferrets (Mustela Pulorius Euro, weighing 1-2 kg). Ferrets were supplied either by Bury Green Farm or Misay Consultancy. Following transport, the animals were allowed to acclimatize in the holding rooms for a period of not less than 7 days. The Diet comprised SDS diet C pelleted food given ad lib with Whiskers cat food given 3 times per week. Water was pasteurized animal grade drinking water and was changed daily.

Dosing with PDE4 Inhibitor

PDE4 inhibitors were administered orally (p.o.), at doses initially of 1-10 mg/kg, but subsequently up to 30 mg/kg in order to establish whether the TI was 10 or higher, and/or at lower doses to establish the minimum dose to cause 50% inhibition of neutrophilia. Ferrets were fasted overnight but allowed free access to water. The animals were orally dosed with vehicle or PDE4 inhibitor using a 15 cm dosing needle that was passed down the back of the throat into the oesophagus. After dosing, the animals were returned to holding cages fitted with Perspex doors to allow observation, and given free access to water. After dosing, the animals were constantly observed and any emesis or behavioural changes were recorded. The animals were allowed access to food 60-90 minutes after p.o. dosing

Exposure to LPS

Thirty minutes after p.o. dosing with compound or vehicle control, the ferrets were placed into sealed Perspex containers and exposed to an aerosol of LPS (100 μg/ml) for 10 minutes. Aerosols of LPS were generated by a nebulizer (DeVilbiss, USA) and this was directed into the Perspex exposure chamber. Following a 10 minute exposure period, the animals were returned to the holding cages and allowed free access to water, and at a later stage, food. Observation continued for a period of at least 2.5 hours post p.o. dosing and emetic episodes and behavioral changes were recorded.

Bronchoalveolar Lavage

Six hours after LPS exposure the animals were killed by overdose of sodium pentobarbitone administered intraperitoneally. The trachea was then cannulated with polypropylene tubing and the lungs lavaged twice with 20 ml heparinized (10 units/ml) phosphate buffered saline (PBS).

Blood Sampling/Tissue Removal

A terminal blood sample (10 ml) was removed by transthoracic cardiac puncture. The blood was spun at 2500 rpm for 15 minutes and the plasma removed and stored at −20° C. The brain also removed and frozen at −20° C. for analysis of compound content.

Cell Counts

The bronchoalveolar lavage (BAL) samples were centrifuged at 1500 rpm for 5 minutes. The supernatant was removed and the resulting cell pellet re-suspended in 1 ml PBS. A cell smear of the re-suspended fluid was prepared and stained with Leishmans stain to allow differential cell counting. A total cell count was made using the remaining re-suspended sample. From this, the total number of neutrophils in the BAL was determined.

Parameters Measured:

1. % Inhibition of LPS-induced pulmonary neutrophilia.
2. Emetic episodes—the number of vomits and retches were counted.
3. Behavioral changes—the following behavioral effects were noted: salivation, panting, mouth clawing, flattened posture, ataxia, arched back and backward walking. Any behavioral changes were semi-quantified by applying a severity rating (mild, moderate or severe).
4. The TI was calculated as the highest dose found to not cause emetic episodes divided by the lowest dose found to inhibit pulmonary neutrophilia by 50% or more.

The effect of Compound A on LPS-induced neutrophilia in the lungs of conscious ferrets is demonstrated in FIG. **1**.

Emesis and Behavioral Changes

Following p.o. dosing of the PDE4, the ferrets were observed for at least 2 hours and emetic episodes (vomits and retches) and behavioral changes were recorded.

No emetic episodes (retching or vomiting) were observed in the ferrets pre-treated p.o. with the relevant vehicle (acetone/cremophor/distilled water). In a small proportion of the control-treated animals (7/22), mild behavioral changes (lip licking and backward walking) were seen.

Compound A (0.1-3 mg/kg, p.o.), caused no emetic episodes (retching and vomiting). Some behavioral changes (flattened posture, lip licking and backward walking) were observed and classified as mild. At 10 mg/kg in 2/6 ferrets, some retching but no frank emesis was observed along with salivation and behavioral changes (scored as mild or moderate). At the highest dose tested (30 mg/kg) moderate to marked emesis was observed in 3/4 animals along with pronounced behavioral changes. These data are summarized in Table III.

TABLE III

Conscious ferret: Emetic episodes and behavioural changes following oral administration of Compound A.

Treatment/dose (mg/kg)	Vomits	Retches	Salivation	Panting	Mouth clawing	Flattened posture	Ataxia	Lip licking	Backward walking
Vehicle (acetone/cremophor/dist. H2O)	None	None	None	None	None	None	None	Mild (6/22)	Mild (7/22)
Compound A (0.1 mg/kg)	None	None	None	None	None	Mild (2/5)	None	Mild (4/5)	Mild (3/5)
Compound A (0.3 mg/kg)	None	None	None	None	None	Mild (2/6)	None	Mild (3/6)	Mild (4/6)
Compound A (1.0 mg/kg)	None	None	None	None	None	Mild (2/6)	None	Mild (6/6)	Mild (4/6)
Compound A (3.0 mg/kg)	None	None	None	None	Mild (1/8)	Marked (7/8)	None	Mild (2/8)	Moderate (5/8)
Compound A (10 mg/kg)	None	Mild (2/6)	Mild (1/6)	None	Mild (1/6)	Marked (6/6)	None	Moderate (5/6)	Marked (6/6)

TABLE III-continued

Conscious ferret: Emetic episodes and behavioural changes following oral administration of Compound A.

Treatment/dose (mg/kg)	Vomits	Retches	Salivation	Panting	Mouth clawing	Flattened posture	Ataxia	Lip licking	Backward walking
Compound A (30 mg/kg)	Moderate (3/4)	Marked (3/4)	Moderate (3/4)	Mild (1/4)	Marked (4/4)	Marked (4/4)	Mild (3/4)	Moderate (4/4)	Mild (2/4)

Animals were observed for up to 3 hours following dosing. Numbers in parentheses refer to the number of animals that responded The numbers of animals in each group range from 4-22.

Therapeutic Index Calculation

From these experiments, a therapeutic index (TI) was determined for each compound by dividing the threshold dose for inducing emetic episodes by the ED_{50} value for inhibiting the pulmonary neutrophilia. The TI calculation is summarized in Table IV. Compound A had a TI of 12, causing no emetic episodes at an anti-inflammatory dose of 1 mg/kg.

TABLE IV

Summary of the effective doses (ED_{50}) for inhibition of LPS-induced pulmonary neutrophilia and induction of emesis and the therapeutic index derived from these values.

Compound	Inhibition of LPS-induced neutrophilia (ED_{50} mg/kg)	Threshold emetic dose (mg/kg)	Therapeutic index
Compound A	0.8	10	12

5.10. Example 9

200 Mg Dosage Capsule

Table V illustrates a batch formulation and single dosage formulation for a 200 mg Compound A single dose unit, i.e., about 40 percent by weight, in a size #0 capsule.

TABLE V

Formulation for 200 mg capsule

Material	Percent By Weight	Quantity (mg/tablet)	Quantity (kg/batch)
Compound A	40.0%	200 mg	16.80 kg
Pregelatinized Corn Starch, NF5	9.5%	297.5 mg	24.99 kg
Magnesium Stearate	0.5%	2.5 mg	0.21 kg
Total	100.0%	500 mg	42.00 kg

The pregelatinized corn starch (SPRESS B-820) and Compound A components are passed through a 710 μm screen and then are loaded into a Diffusion Mixer with a baffle insert and blended for 15 minutes. The magnesium stearate is passed through a 210 μm screen and is added to the Diffusion Mixer. The blend is then encapsulated in a size #0 capsule, 500 mg per capsule (8400 capsule batch size) using a Dosator type capsule filling machine.

5.11. Example 10

100 Mg Oral Dosage Form

Table VI illustrates a batch formulation and a single dose unit formulation containing 100 mg of Compound A.

TABLE VI

Formulation for 100 mg tablet

Material	Percent by Weight	Quantity (mg/tablet)	Quantity (kg/batch)
Compound A	40%	100.00	20.00
Microcrystalline Cellulose, NF	53.5%	133.75	26.75
Pluronic F-68 Surfactant	4.0%	10.00	2.00
Croscarmellose Sodium Type A, NF	2.0%	5.00	1.00
Magnesium Stearate, NF	0.5%	1.25	0.25
Total	100.0%	250.00 mg	50.00 kg

The microcrystalline cellulose, croscarmellose sodium, and Compound A components are passed through a #30 mesh screen (about 430μ to about 655μ). The Pluronic F-68® (manufactured by JRH Biosciences, Inc. of Lenexa, Kans.) surfactant is passed through a #20 mesh screen (about 457μ to about 1041μ). The Pluronic F-68® surfactant and 0.5 kgs of croscarmellose sodium are loaded into a 16 qt. twin shell tumble blender and are mixed for about 5 minutes. The mix is then transferred to a 3 cubic foot twin shell tumble blender where the microcrystalline cellulose is added and blended for about 5 minutes. The thalidomide is added and blended for an additional 25 minutes. This pre-blend is passed through a roller compactor with a hammer mill attached at the discharge of the roller compactor and moved back to the tumble blender. The remaining croscarmellose sodium and magnesium stearate is added to the tumble blender and blended for about 3 minutes. The final mixture is compressed on a rotary tablet press with 250 mg per tablet (200,000 tablet batch size).

5.12. Example 11

Aerosol Dosage Form

A concentrate is prepared by combining Compound A, and a 12.6 kg portion of the trichloromonofluormethane in a sealed stainless steel vessel equipped with a high shear mixer. Mixing is carried out for about 20 minutes. The bulk suspension is then prepared in the sealed vessel by combining the concentrate with the balance of the propellants in a bulk product tank that is temperature controlled to 21° to 27° C. and pressure controlled to 2.8 to 4.0 BAR. 17 ml aerosol containers which have a metered valve which is designed to

provide 100 inhalations of the composition of the invention. Each container is provided with the following:

Compound A	0.0120 g
trichloromonofluoromethane	1.6939 g
dichlorodifluoromethane	3.7175 g
dichlorotetrafluoroethane	1.5766 g
total	7.0000 g

While the invention has been described with respect to the particular embodiments, it will be apparent to those skilled in the art that various changes and modifications may be made without departing from the spirit and scope of the invention as defined in the claims. Such modifications are also intended to fall within the scope of the appended claims.

The invention claimed is:

1. A method of treating depression, inflammatory skin disease, psoriasis, atopic dermatitis, contact dermatitis, rheumatoid arthritis, osteoarthritis, systemic lupus erythrematosus, inflammatory bowel disease, Crohn's Disease, Behcet's Disease or colitis, which comprises administering to a patient in need of such treatment a therapeutically effective amount of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, or a pharmaceutically acceptable salt thereof.

2. The method of claim **1** further comprising administering to a patient in need of such treatment a therapeutically effective amount of an antihistamine, anti-inflammatory drug, non-steroid anti-inflammatory drug, or steroid.

3. The method of claim **1** wherein the patient is a mammal.

4. The method of claim **1** wherein the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is administered parenterally, transdermally, mucosally, nasally, buccally, sublingually, or orally.

5. The method of claim **4** wherein the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is administered orally.

6. The method of claim **5** wherein the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is administered orally in a tablet or capsule form.

7. The method of claim **1** wherein the therapeutically effective amount is from about 1 mg to about 1000 mg per day.

8. The method of claim **7** wherein the therapeutically effective amount is from about 5 mg to about 500 mg per day.

9. The method of claim **8** wherein the therapeutically effective amount is from about 10 mg to about 200 mg per day.

10. A method of treating psoriasis which comprises administering to a patient having psoriasis a therapeutically effective amount of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

11. The method of claim **10**, wherein the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is substantially free of (−) isomer.

12. The method of claim **10**, wherein the compound is administered orally.

13. The method of claim **12**, wherein the compound is administered in the form of a tablet or capsule.

14. The method of claim **10**, wherein the compound is administered in the amount of from about 1 mg to about 1,000 mg per day.

15. The method of claim **14**, wherein the compound is administered in the amount of from about 5 mg to about 500 mg per day.

16. The method of claim **15**, wherein the compound is administered in the amount of from about 10 mg to about 200 mg per day.

17. The method of claim **16**, wherein the compound is administered in the amount of about 20 mg to about 40 mg per day.

18. The method of claim **17**, wherein the compound is administered in the amount of about 20 mg twice daily.

19. The method of claim **17**, wherein the compound is administered in the amount of about 40 mg once daily.

20. The method of claim **10**, further comprising administering to a patient in need of such treatment a therapeutically effective amount of an antihistamine, anti-inflammatory drug, non-steroid anti-inflammatory drug, or steroid.

* * * * *

EXHIBIT E

US007893101B2

(12) **United States Patent**
Muller et al.

(10) **Patent No.: US 7,893,101 B2**
(45) **Date of Patent: Feb. 22, 2011**

(54) **SOLID FORMS COMPRISING (+)-2-[1-(3-ETHOXY-4-METHOXYPHENYL)-2-METHYLSULFONYLETHYL]-4-ACETYLAMINOISOINDOLINE-1,3-DIONE, COMPOSITIONS THEREOF, AND USES THEREOF**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 265 days.

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(60) Provisional application No. 60/366,515, filed on Mar. 20, 2002, provisional application No. 60/438,450, filed on Jan. 7, 2003.

(51) **Int. Cl.**
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(52) **U.S. Cl.** **514/411**; 548/451

(58) **Field of Classification Search** 514/411; 548/451

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,031,450	A		4/1962	Fischer et al.
3,322,755	A		5/1967	Roch et al.
3,920,636	A		11/1975	Takahashi et al.
4,001,237	A		1/1977	Partyka et al.
4,001,238	A		1/1977	Partyka et al.
4,047,404	A		9/1977	Hayashi
4,060,615	A		11/1977	Matier et al.
4,101,548	A		7/1978	Crenshaw et al.
4,162,316	A		7/1979	Nishimura et al.
4,209,623	A		6/1980	Juby
4,880,810	A		11/1989	Lowe, III
4,885,301	A		12/1989	Coates
5,147,875	A		9/1992	Coates et al.
5,354,571	A		10/1994	Morikawa et al.
5,401,774	A		3/1995	Pamukcu et al.
5,439,895	A		8/1995	Lee et al.
5,488,055	A		1/1996	Kumar et al.
5,608,914	A		3/1997	Keesler
5,614,530	A		3/1997	Kumar et al.
5,614,627	A		3/1997	Takase et al.
5,658,940	A		8/1997	Muller et al.
5,698,579	A		12/1997	Muller
5,703,098	A		12/1997	Muller et al.
5,710,170	A		1/1998	Guay et al.
5,728,844	A		3/1998	Muller et al.
5,728,845	A		3/1998	Muller et al.
5,736,570	A		4/1998	Muller et al.
5,798,373	A		8/1998	Warrellow
5,801,195	A		9/1998	Muller et al.
5,849,770	A		12/1998	Head et al.
5,877,200	A		3/1999	Muller
5,891,896	A		4/1999	Warrellow et al.
6,011,050	A		1/2000	Muller et al.
6,020,339	A		2/2000	Perrier et al.
6,020,358	A	*	2/2000	Muller et al. 514/411
6,034,089	A		3/2000	Han et al.
6,046,221	A		4/2000	Muller et al.
6,069,156	A		5/2000	Oku et al.
6,162,830	A		12/2000	Connor et al.
6,166,041	A		12/2000	Cavalla et al.
6,177,471	B1		1/2001	Menander et al.
6,204,275	B1		3/2001	Friesen et al.
6,218,400	B1		4/2001	Daugan et al.
6,300,335	B1		10/2001	Campbell et al.
6,316,472	B1		11/2001	Frenette et al.
6,333,354	B1		12/2001	Schudt

(Continued)

FOREIGN PATENT DOCUMENTS

EP	0 347 146	6/1989

(Continued)

OTHER PUBLICATIONS

Brittain's, polymorphism in Pharmaceutical Solids, Drugs and the Pharmaceutical Science; 1999, V. 95, pp. 348-361.*

(Continued)

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(57) **ABSTRACT**

Solid forms comprising (+)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, compositions comprising the solid forms, methods of making the solid forms and methods of their use are disclosed. The methods include methods of treating and/or preventing disorders ameliorated by the reduction of levels of TNF-α or the inhibition of PDE4.

15 Claims, 33 Drawing Sheets

U.S. PATENT DOCUMENTS

7,427,638	B2 *	9/2008	Muller et al.	514/411
2008/0027123	A1	1/2008	Muller et al.	

FOREIGN PATENT DOCUMENTS

EP	0 349 239	A2	6/1989
EP	0 351 058		6/1989
EP	0 352 960	A2	7/1989
EP	0 395 328		4/1990
EP	0 428 268	A2	10/1990
EP	0 463 756	A1	6/1991
EP	0 526 004	A1	7/1992
EP	0 607 439	A1	9/1992
EP	0 722 937	A1	1/1996
EP	0 722 943	A1	1/1996
EP	0 722 944	A1	1/1996
GB	2 063 249	A	9/1980
WO	WO 93/07149		4/1993
WO	WO 93/12095		6/1993
WO	WO 94/01728		1/1994
WO	WO 94/05661		3/1994
WO	WO 94/29277		12/1994
WO	WO 95/19978		7/1995
WO	WO 96/32379		10/1996
WO	WO 97/03070		1/1997
WO	WO 97/03675		2/1997
WO	WO 97/03985		2/1997
WO	WO 97/24334		7/1997
WO	WO 98/06722		2/1998
WO	WO 98/08848		3/1998
WO	WO 98/14448		4/1998
WO	WO 98/16521		4/1998
WO	WO 98/17668		4/1998
WO	WO 98/23597		6/1998
WO	WO 98/38168		9/1998
WO	WO 99/06041		2/1999
WO	WO 03/080049		10/2003

OTHER PUBLICATIONS

Grant, D. J. W., "Theory and Origin of Polymorphism," *Polymorphism in Pharmaceutical Solids*, Jan. 1999:1-10.

International Search Report in corresponding application No. PCT/US2008/004021 dated Jan. 14, 2009.

U.S. Appl. No. 60/454,155, filed Mar. 12, 2003, Muller, G. et al.

U.S. Appl. No. 60/454,149, filed Mar. 12, 2003, Muller, G. et al.

U.S. Appl. No. 60/438,448, filed Jan. 7, 2003, Muller, G. et al.

U.S. Appl. No. 60/436,975, filed Dec. 30, 2002, Muller, G. et al.

U.S. Appl. No. 60/366,515, filed Mar. 20, 2002, Muller, G. et al.

Akazome, M. et al., 1997, "Asymmetric recognition of 1-arylethylamines by (R)-phenylglycyl-(R)-phenylglycine and its mechanism," Tetrahedron: Asymmetry, Elsevier Scince Publishers, Amsterdam, NL, 8(14):2331-2336.

Au et al., 1998, *Brit. J. Pharm*. 123:1260-1266.

Baehr et al., 1979, *J. Biol. Chem*. 254:11669.

Baughman et al., 1990,*J. Lab. Clin. Med*. 115:36-42.

Beavo and Reifsnyder, *Trends in Pharm.*, 11, 150-55, 1990.

Bissonnette et al., 1989, *Inflammation* 13:329-339.

Bloom and Beavo 1996, *Proc. Natl. Acad. Sci. USA* 93:14188-14192.

Brackeen, M.F. et al., 1995, "Design and synthesis of conformationally constrained analogues of 4-(3-butoxy-4-methoxybenzyl) imidazolidin-2-one (Ro 20/1724) as potent inhibitors of cAMP-specific phosphodiesterase", *J. Med. Chem*. 38:4848-54.

Carstensen, Jens T., 1995, *Drug Stability: Principles & Practice*, 2nd ed., Marcel Dekker, New York, NY pp. 379-380.

Casini et al., 1964, Farmaco Ed. Sci. 19:563.

Clouse et al, 1989, *J. Immunol*. 142:431-438.

Derian et al., 1995, *J. Immunol*. 154:308-317.

Duh et al., 1989, *Proc. Nat. Acad. Sci*. 86:5974-5978.

Featherstone, R.L., et al., 2000, "Comparison of phosphodiesterase inhibitors of differing isoenzyme selectivity added to St. Thomas' hospital cardioplegic solution used for hypothermic preservation of rat lungs", Am. J. Respir. Crit. Care Med. 162:850-6.

Gillespie et al., 1989, *Mol. Pharm*. 36:773.

Hidaka and Asano 1976, *Biochem. Biophys. Acta* 429:485.

Hinshaw et al. 1990, *Circ. Shock* 30:2797-292.

Holler et al., 1990, *Blood* 75:1011-1016.

Johnson et al., 1989, *Endocrinology* 124:1424-1427.

List et al., 1990, *J. Clin. Oncol*. 8:1424.MDS.

Luke, G.P. et al., 1999, "Synthesis of (S)-5-(1-aminoethyl)-2-(cyclohexylmethoxy) benzamide," Tetrahedron: Asymmetry, Elsevier Science Publishers, Amsterdam, NL, 10(22):4393-4403.

Merck Manual (1999) 17th ed., 953.

Monté et al., 1990, *Blood* 79:2670.

Muller, George, et al., 1999, *Bioorganic & Medicinal Chemistry Letters* 9; pp. 1625-1630.

Muller et al., 1998, *Bioorg. & Med Chem Lett*. 8:2669-2674.

Muller, et al., 1996, *J. Med. Chem*. 39:3238.

Nicholson et al., 1991, *Trends Pharmaco. Sci*. 12:19.

Shealy et al., 1965, "D- and L-thalidomide." *Chem. Indus*. 12;24:1030-1.

Tierney, et al., ed., 1998, Current Medical Diagnosis & Treatment, 37th ed., Appleton & Lange, pp. 499.

Verghese, et al., *Journal of Pharmacology and Experimental Therapeutics*, 272(3), 1313-1320, 1995.

Van Dullemen et al., 1995, *Gastroenterology*, 109:129-135.

Wilen, S.H., et al., 1977, *Tetrahedron* 33:2725.

Wilen, S.H., 1972, *Tables of Resolving Agents and Optical Resoutions* (E.L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, IN) p. 268.

Wolff, Manfred E., *ed., Burger's Medicinal Chemistry and Drug Discovery*, 5th ed. 1995 172-178, 949-982.

Dredge et al., 2003, "Angiogenesis inhibitors in cancer therapy," Curr. Opin. Investig. Drugs 4(6):667-674.

Dredge et al., 2002, "Recent developments in antiangiogenic therapy," Expert Opin. Biol. Ther. 2(8):953-966.

Gee et al., 2003, "Selective cytokine inhibitory drugs with enhanced antiangiogenic activity control tumor growth through vascular inhibition," Cancer Res. 63(23):8073-8078.

Marriott et al., 2001, "Immunotherapeutic and antitumour potential of thalidomide analogues," Expert Opin. Biol. Ther. 1(4):1-8.

Marriott et al., 2002, "Thalidomide and its analogues have distinct and opposing effects on TNF-alpha and TNFR2 during co-stimulation of both CD4(+) and CD8(+) T cells," Clin. Exp. Immunol. 130(1):75-84.

Molostvov et al., 2004, "The effects of selective cytokine inhibitory drugs (CC-10004 and CC-1088) on VEGF and IL-6 expression and apoptosis in myeloma and endothelial cell co-cultures," Br. J. Haematol.124(3):366-375.

Notice of References Cited from Office Action dated Dec. 3, 2007 in U.S. Appl. No. 11/106,142.

Craven, B.M. et al., 1969, "Crystal structures of two polymorphs of 5-ethyl-5-isoamylbarbituric acid (amobarbital)," Acta Crystallographica, Section B: Structural Crystal Chemistry, B25 (PL. 10).

Berge et al., 1977, "Pharmaceutical Salts," Journal of Pharmaceutical Sciences, 66(1):1-19.

Bundgaard, Hans, 1985, "Design of prodrugs: Bioreversible derivatives for various functional groups and chemical entities," Design of Prodrugs, Chapter 1:1-4.

U.S. Department of Health and Human Services, Food and Drug Administration, 2000, "Guidance for Industry—Impurities: Residual Solvents in New Veterinary Medicinal Products, Active Substances and Excipients," pp. 1-20.

Craven et al., 1969, CAS: 71:117431.

Berge et al., Journal of pharmaceutical Science, 1977, vol. 66, pp. 1-19.

Bundgaard et al. publication, 1985, Design of prodrug, pp. 1-4.

FDA's guidance for industry, 2000, pp. 1-20.

Jones et al., "Pharmaceutical Cocrystals: An Emerging Approach to Physical Property Enhancement," *Mater. Res. Bull.*, 31:875-79 (2006).

Andrew D. Bond & William Jones, Controlling Crystal Architecture in Molecular Solids: The Supramolecular Approach, in Supramolecular Organization and Materials Design, 391, 436 (W. Jones & C. N. R. Rao, eds., 2001).

Trask et al., "Selective polymorph transformation via solvent-drop grinding," *Chem. Commun.*, 880-882 (2005).

* cited by examiner

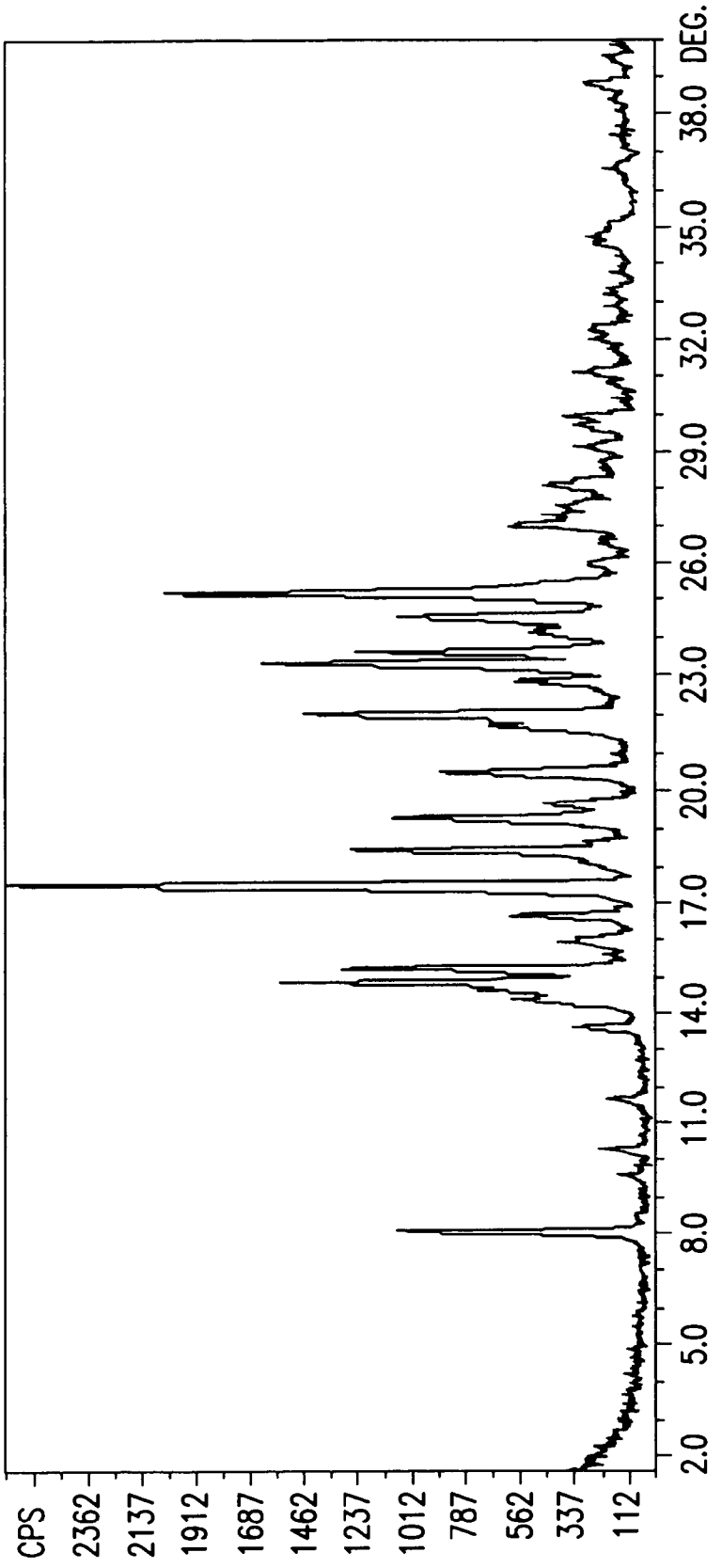

CPS
2362
2137
1912
1687
1462
1237
1012
787
562
337
112
2.0
5.0
8.0
11.0
14.0
17.0
20.0
23.0
26.0
29.0
32.0
35.0
38.0
DEG.

FIG. 1

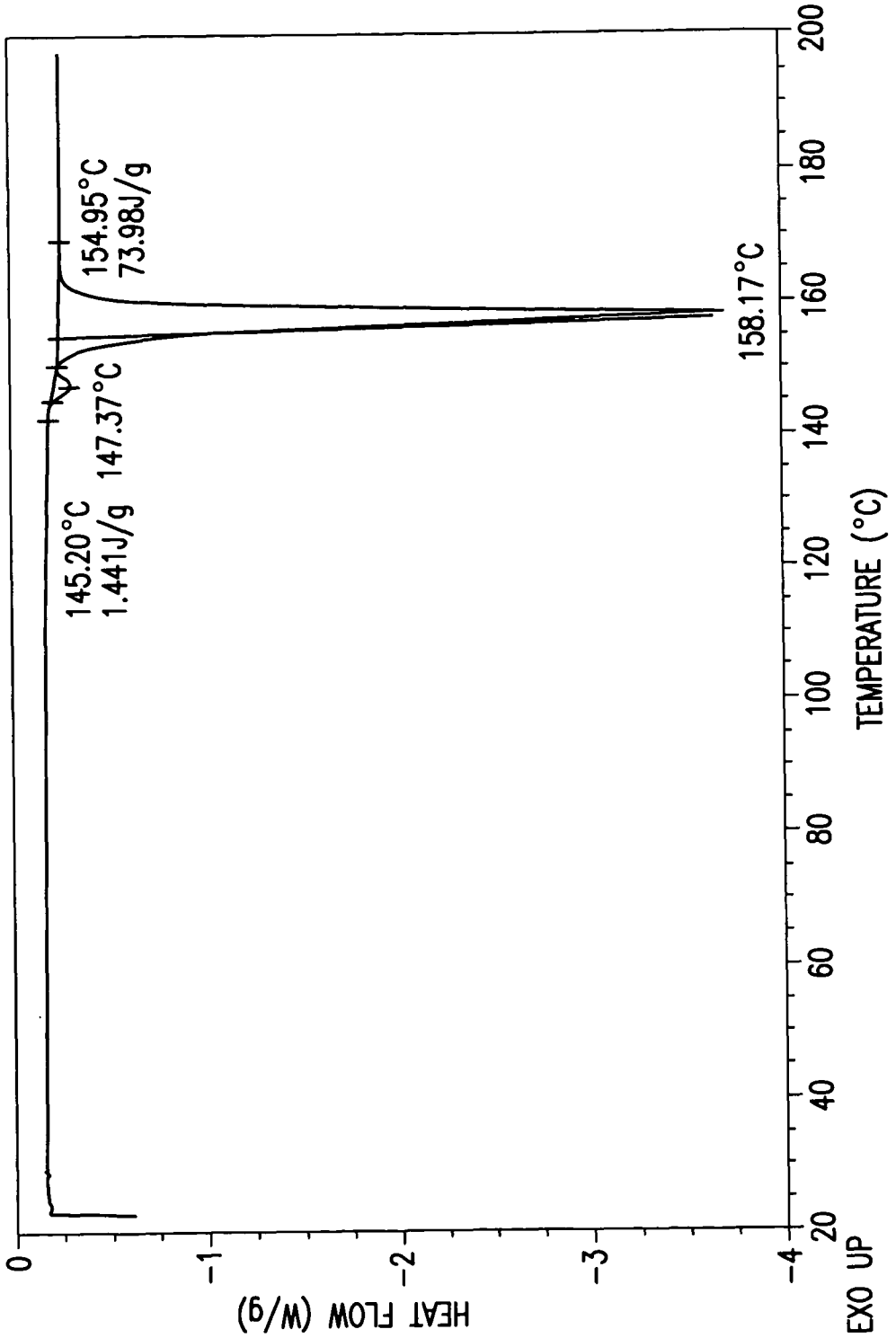

154.95°C
73.98J/g
158.17°C
147.37°C
145.20°C
1.441J/g
HEAT FLOW (W/g)
EXO UP
TEMPERATURE (°C)
0
-1
-2
-3
-4
20
40
60
80
100
120
140
160
180
200

FIG.2

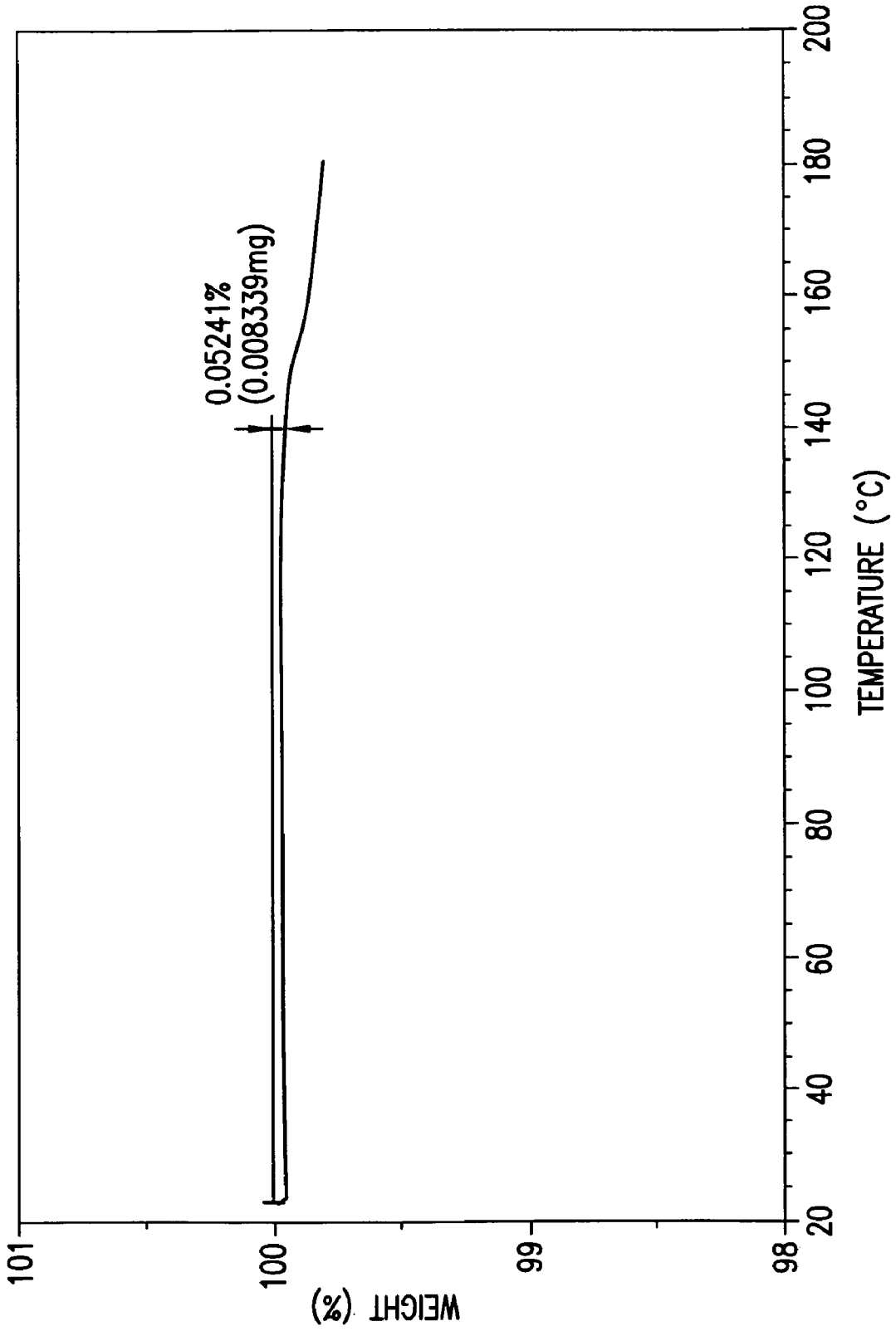

0.05241%
(0.008339mg)
WEIGHT (%)
101
100
99
98
20
40
60
80
100
120
140
160
180
200
TEMPERATURE (°C)

FIG.3

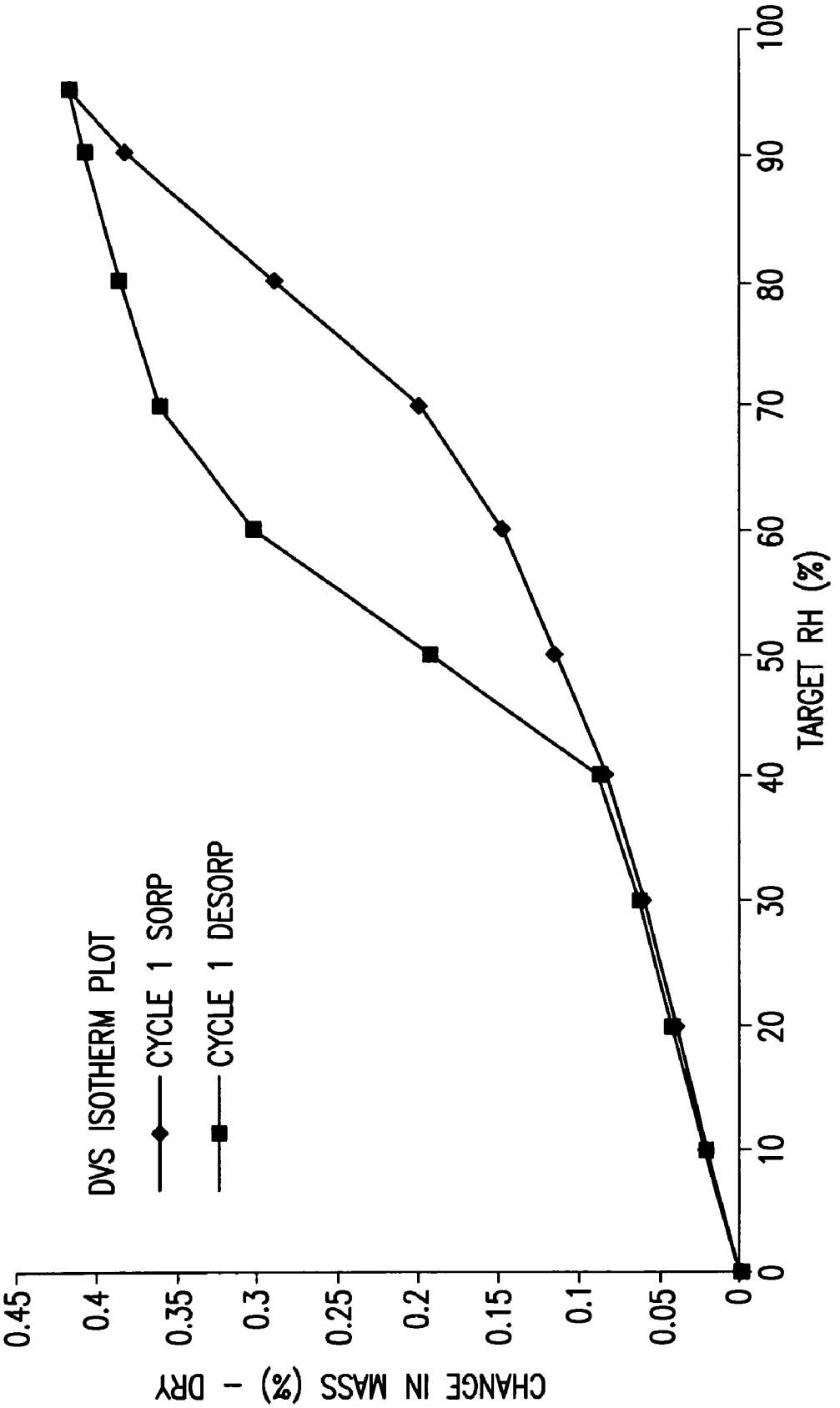

DVS ISOTHERM PLOT
CYCLE 1 SORP
CYCLE 1 DESORP
CHANGE IN MASS (%) - DRY
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
10
20
30
40
50
60
70
80
90
100
TARGET RH (%)

FIG.4

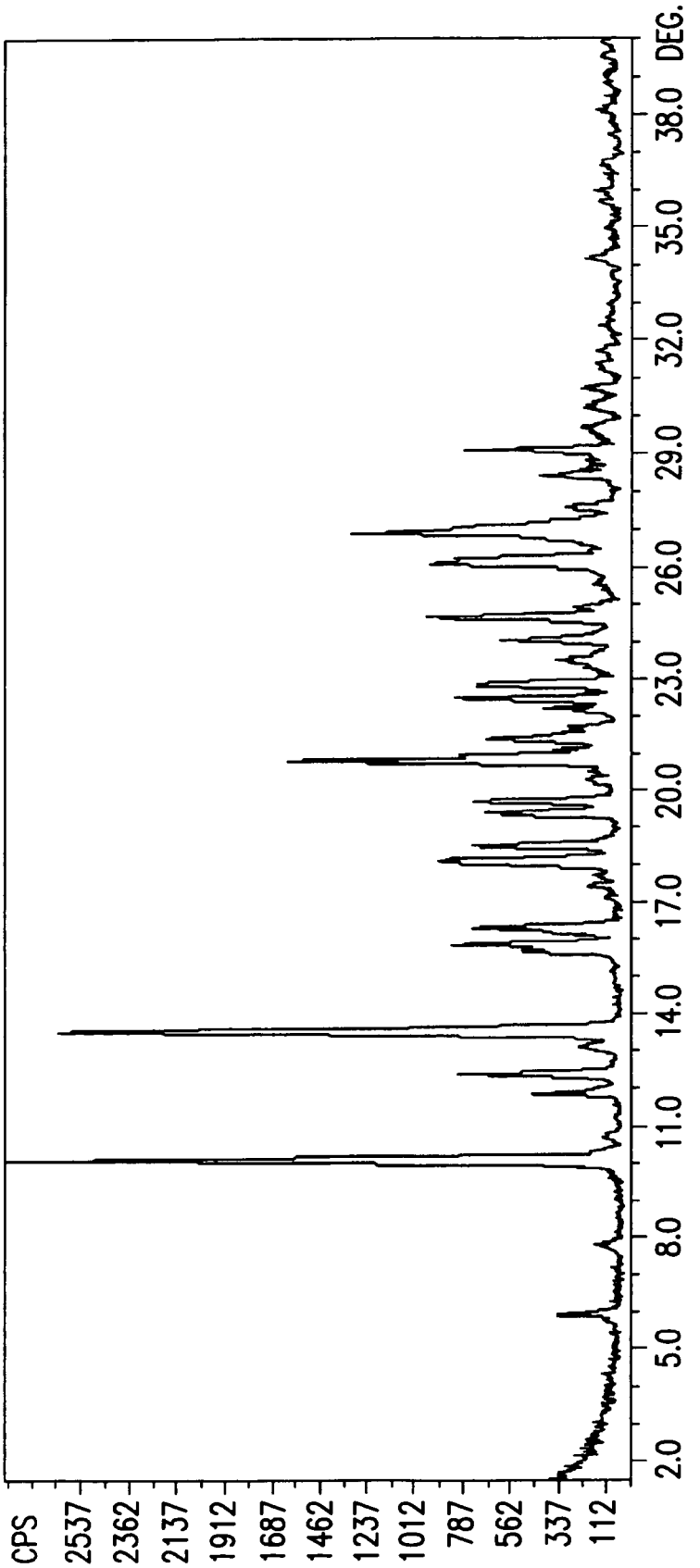

CPS
2537
2362
2137
1912
1687
1462
1237
1012
787
562
337
112
2.0
5.0
8.0
11.0
14.0
17.0
20.0
23.0
26.0
29.0
32.0
35.0
38.0
DEG.

FIG.5

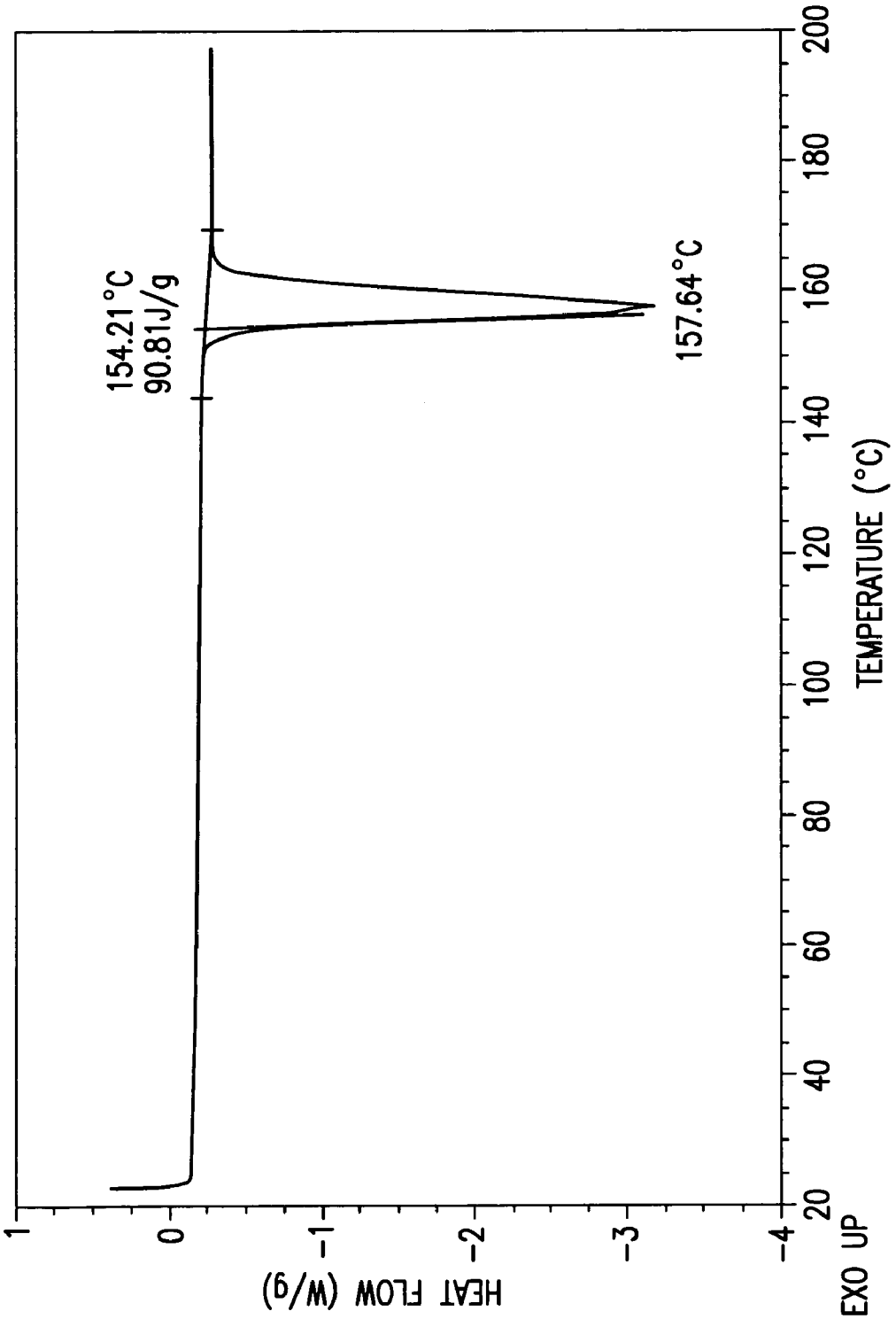

154.21°C
90.81J/g
157.64°C
HEAT FLOW (W/g)
1
0
-1
-2
-3
-4
EXO UP
20
40
60
80
100
120
140
160
180
200
TEMPERATURE (°C)

FIG.6

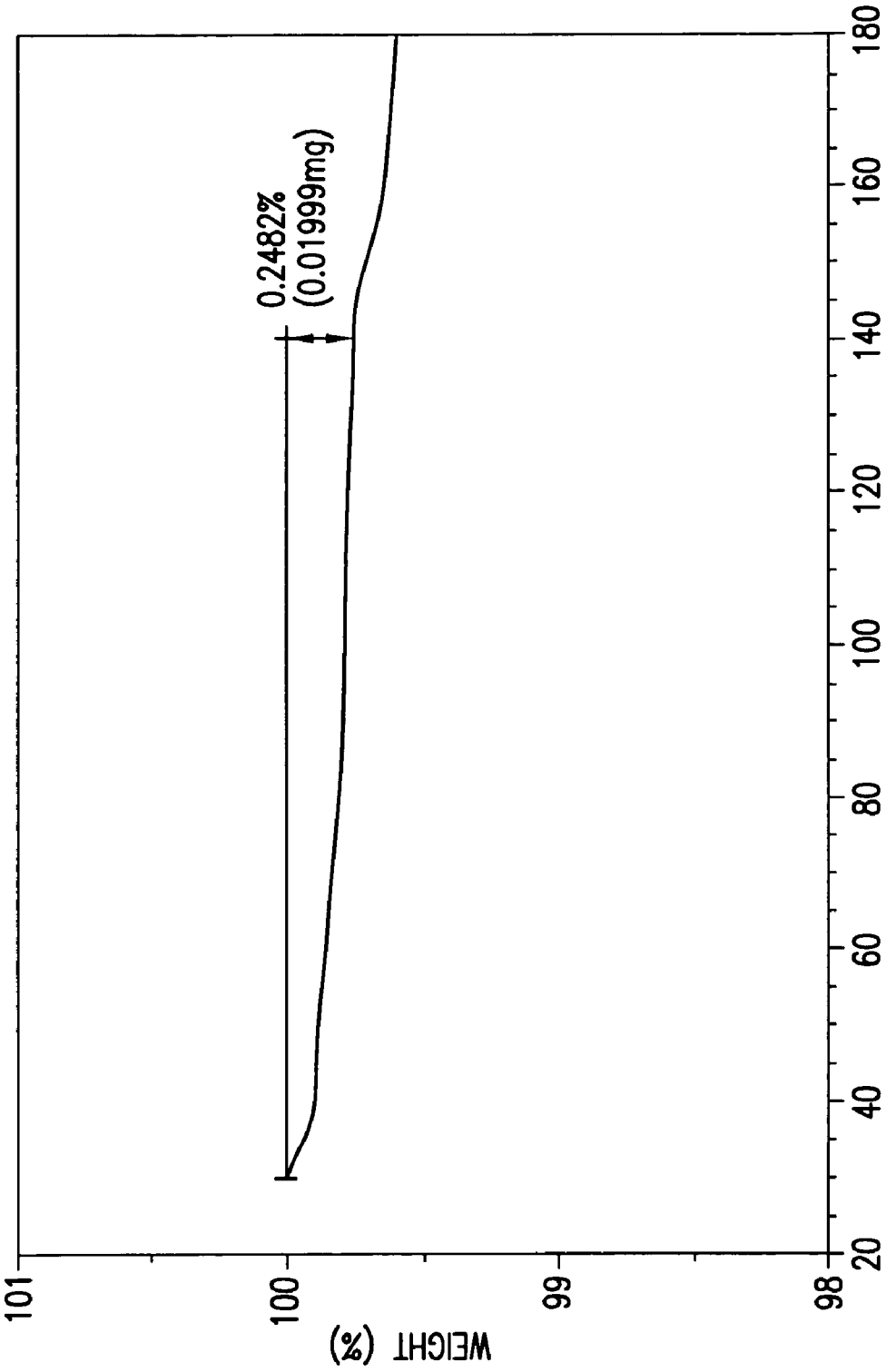

0.2482%
(0.01999mg)
WEIGHT (%)
101
100
99
98
TEMPERATURE (°C)
20
40
60
80
100
120
140
160
180

FIG. 7

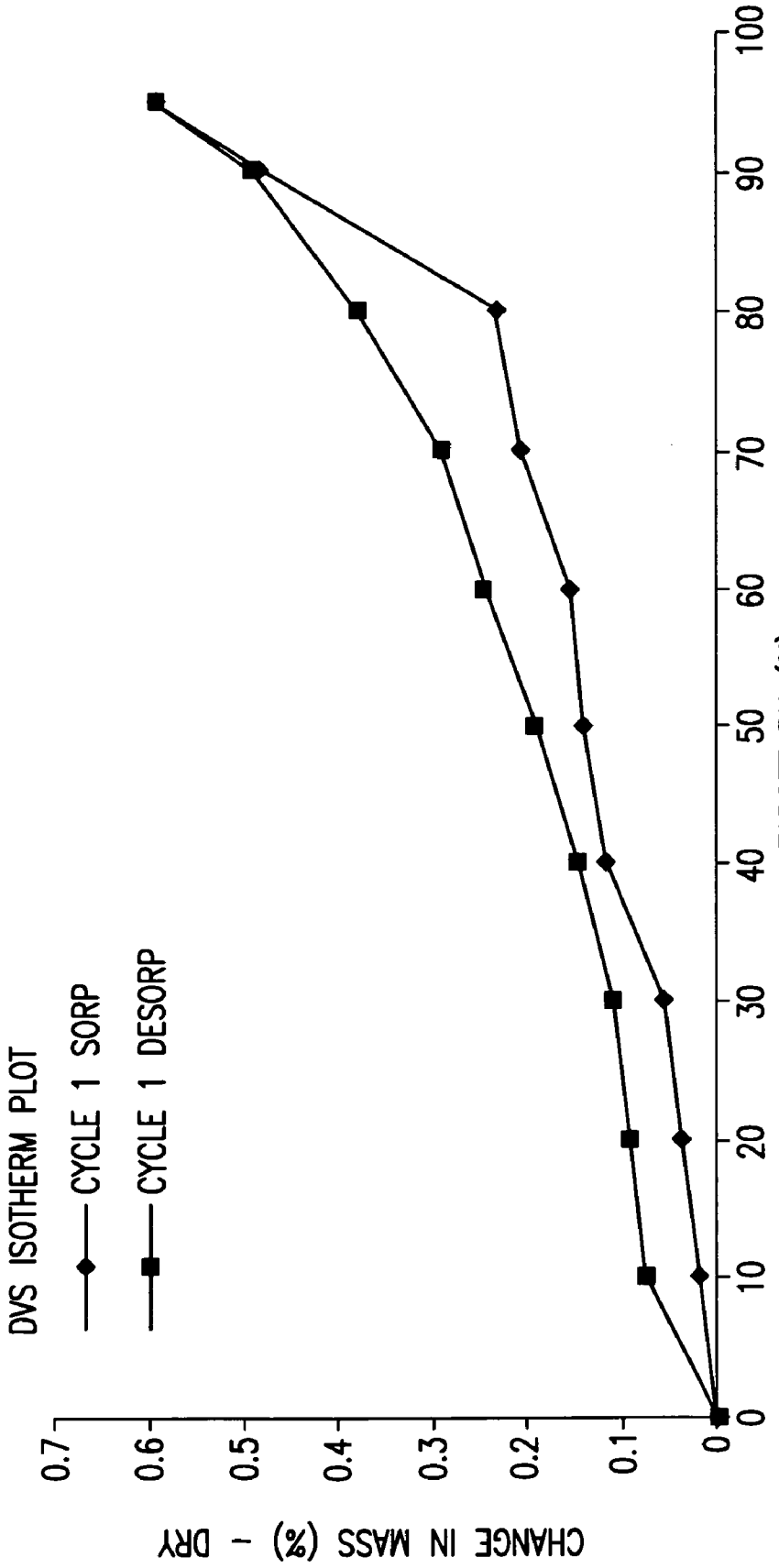

DVS ISOTHERM PLOT
CYCLE 1 SORP
CYCLE 1 DESORP
CHANGE IN MASS (%) - DRY
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
60
70
80
90
100
TARGET RH (%)

FIG.8

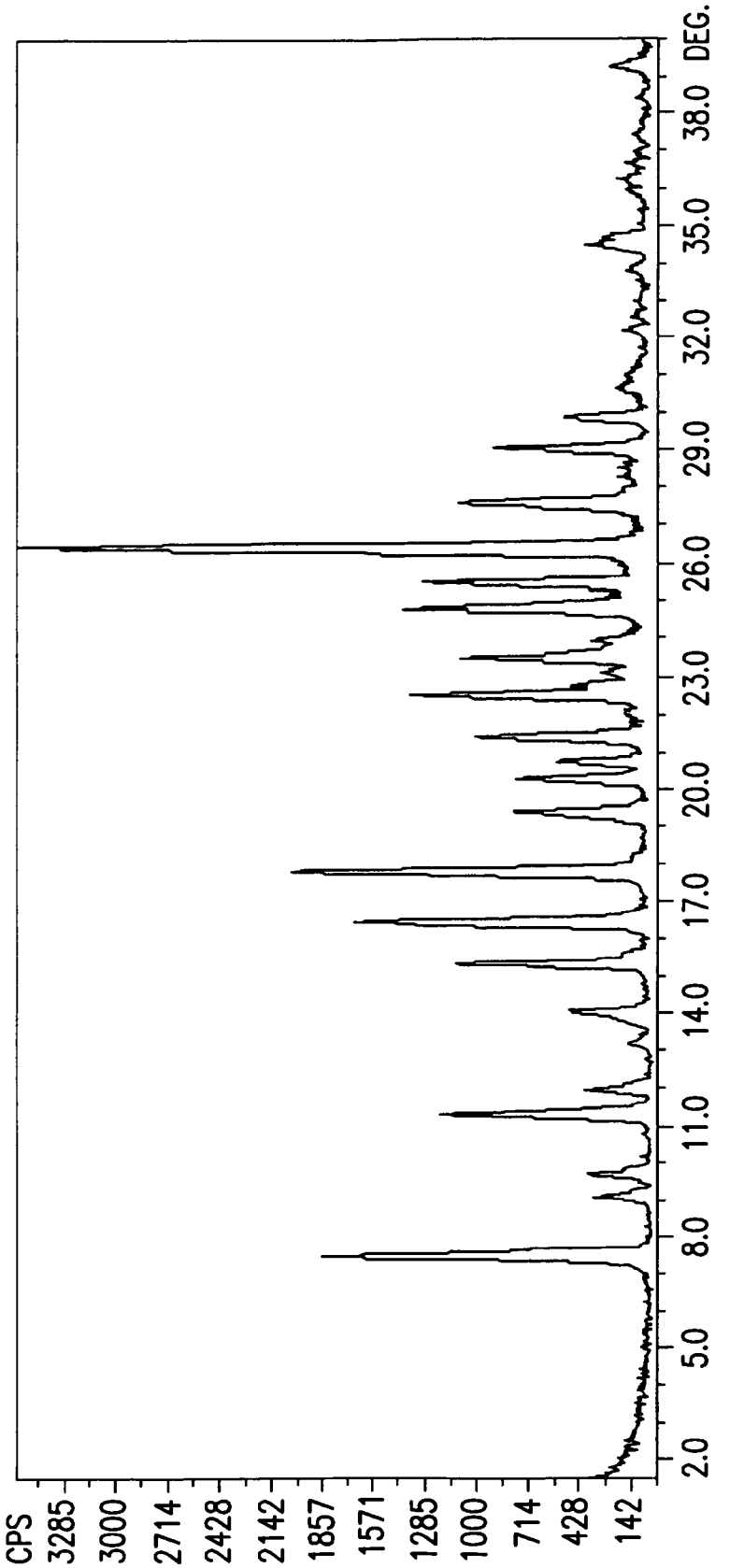

CPS
3285
3000
2714
2428
2142
1857
1571
1285
1000
714
428
142
2.0
5.0
8.0
11.0
14.0
17.0
20.0
23.0
26.0
29.0
32.0
35.0
38.0
DEG.

FIG.9

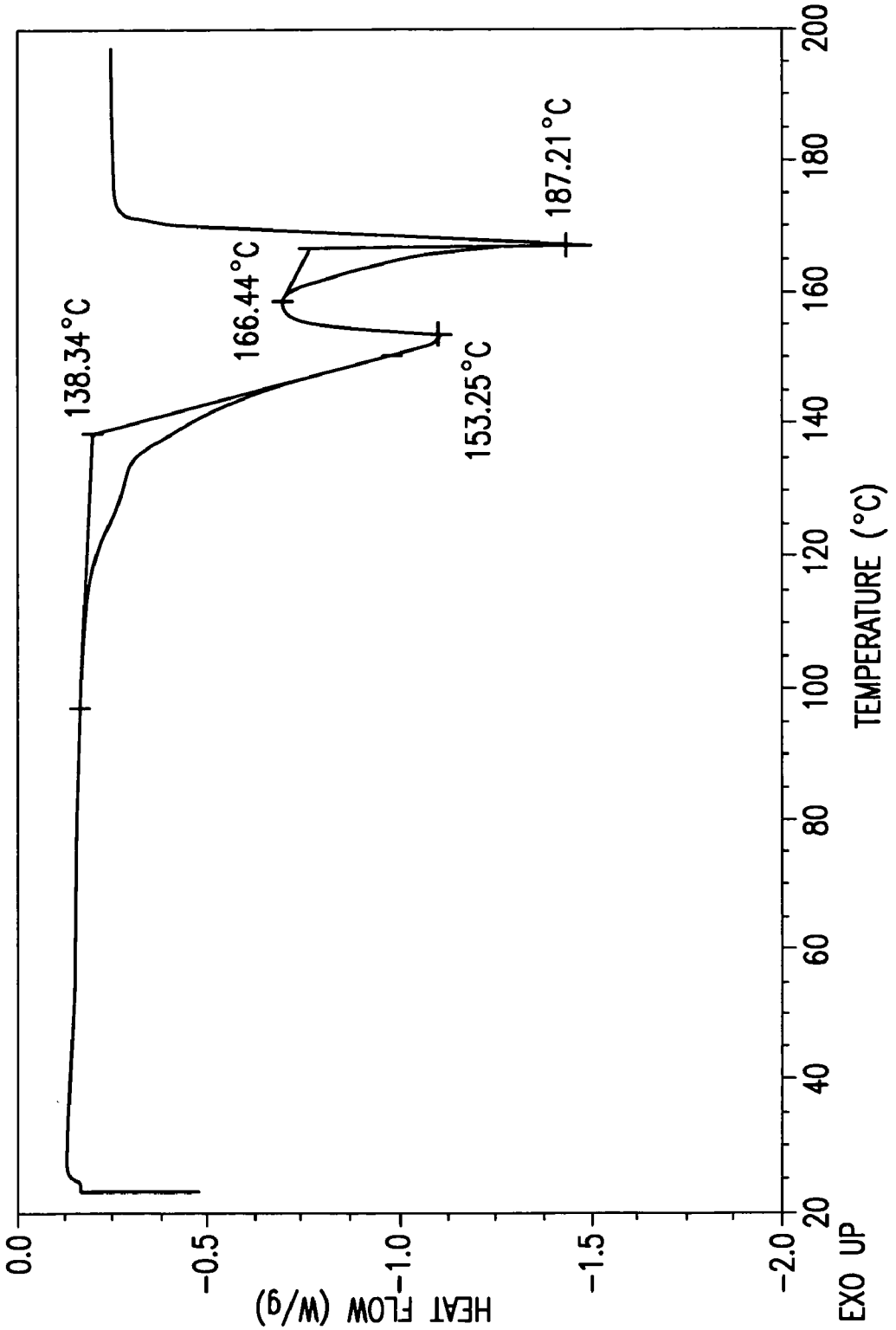

138.34°C
166.44°C
187.21°C
153.25°C
0.0
-0.5
-1.0
-1.5
-2.0
HEAT FLOW (W/g)
EXO UP
20
40
60
80
100
120
140
160
180
200
TEMPERATURE (°C)

FIG. 10

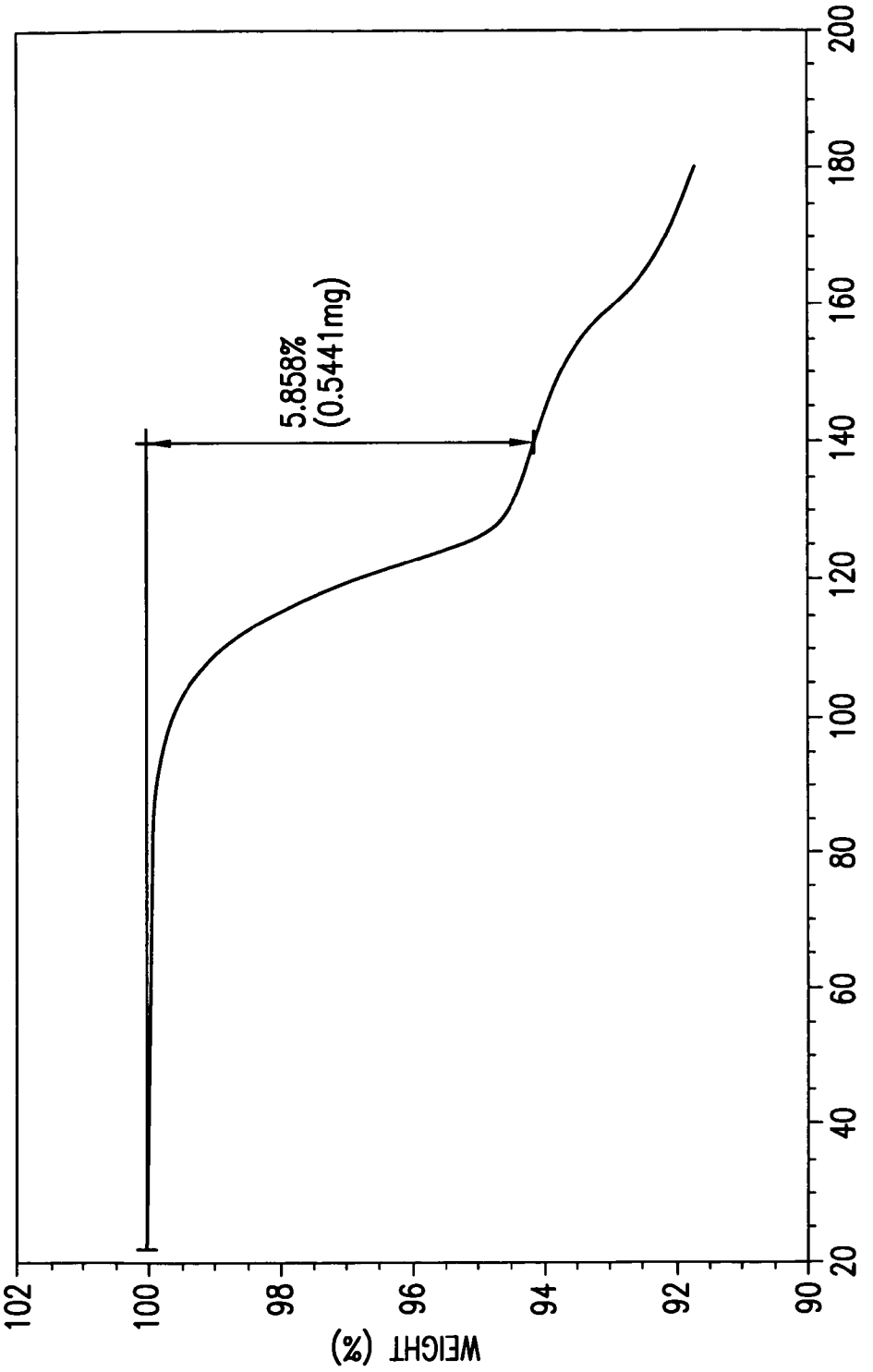

5.858%
(0.5441mg)
WEIGHT (%)
102
100
98
96
94
92
90
TEMPERATURE (°C)
20
40
60
80
100
120
140
160
180
200

FIG. 11

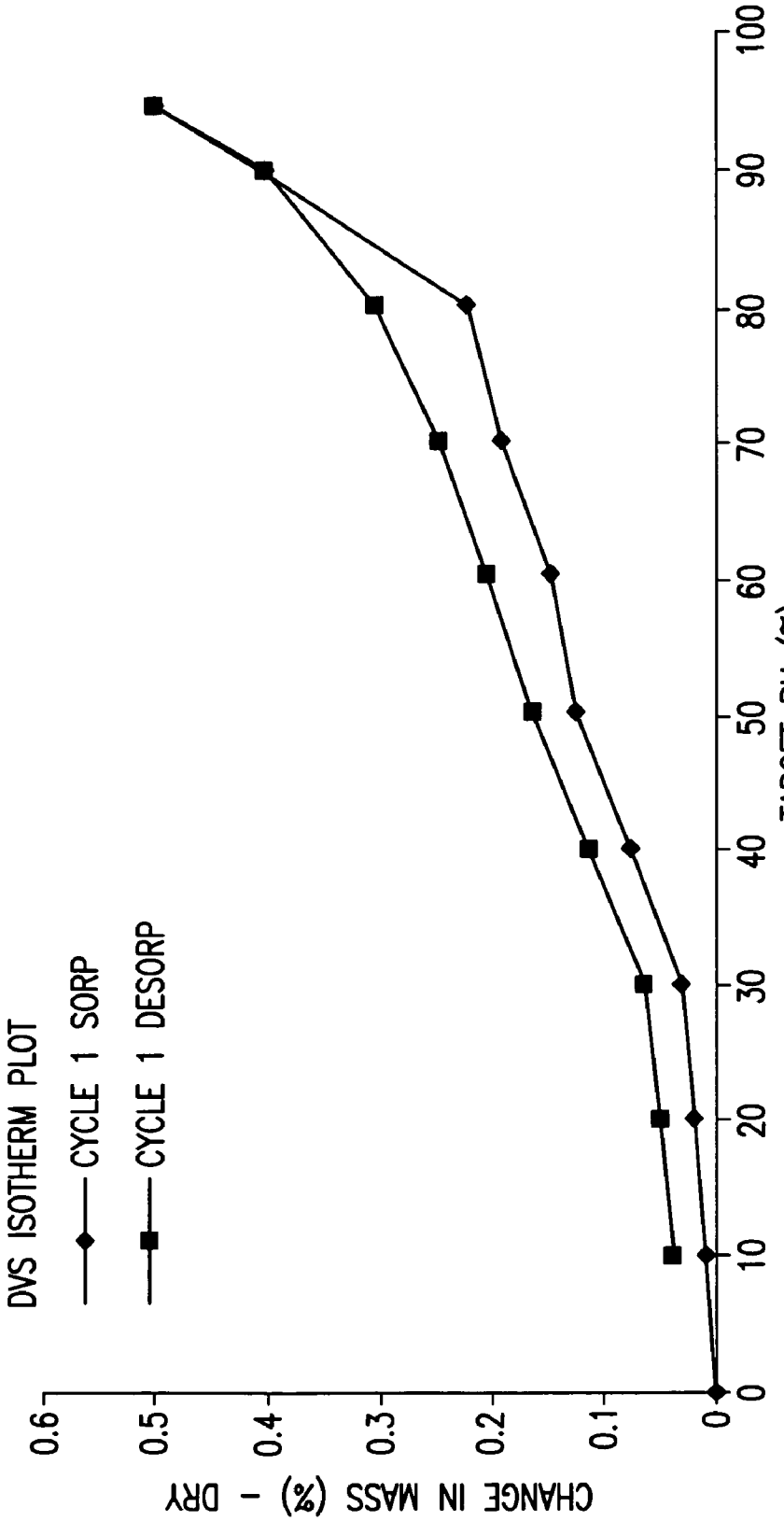

DVS ISOTHERM PLOT
CYCLE 1 SORP
CYCLE 1 DESORP
CHANGE IN MASS (%) - DRY
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
60
70
80
90
100
TARGET RH (%)

FIG.12

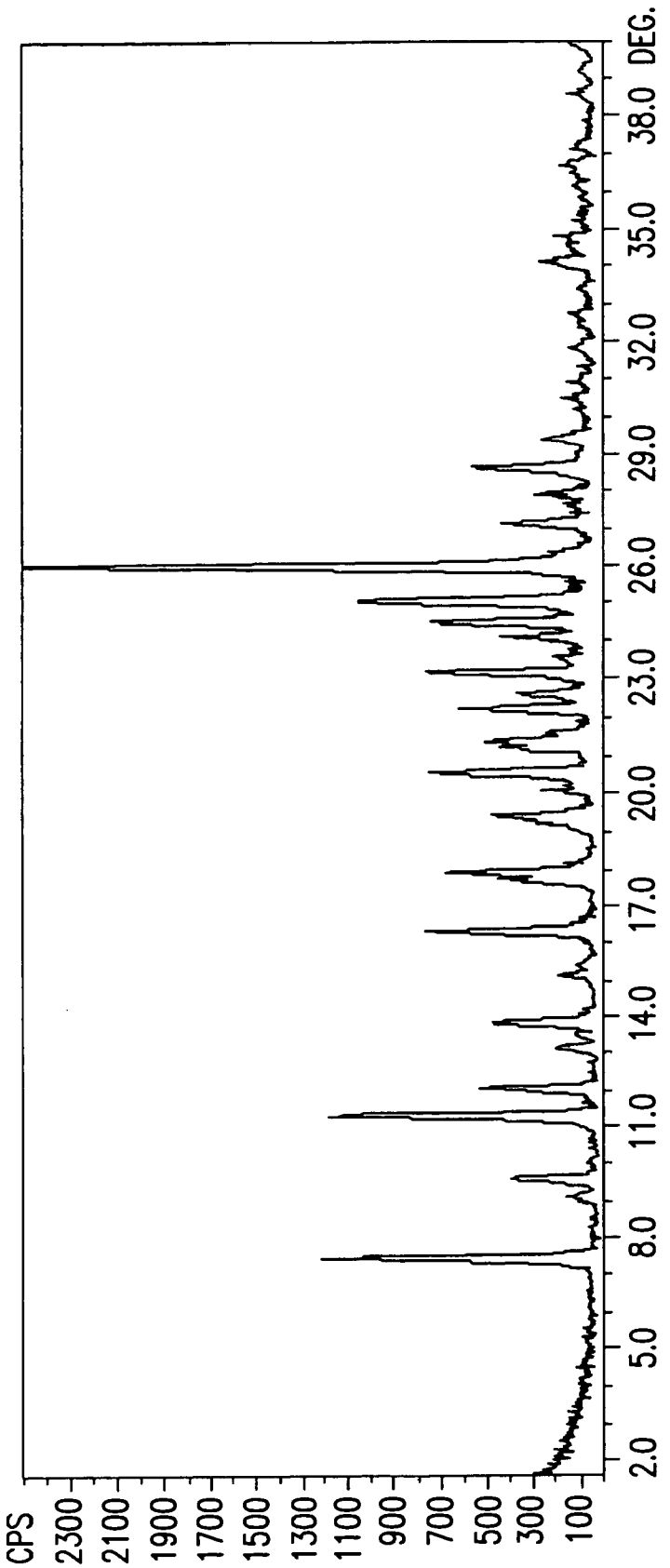

CPS
2300
2100
1900
1700
1500
1300
1100
900
700
500
300
100
2.0
5.0
8.0
11.0
14.0
17.0
20.0
23.0
26.0
29.0
32.0
35.0
38.0
DEG.

FIG. 13

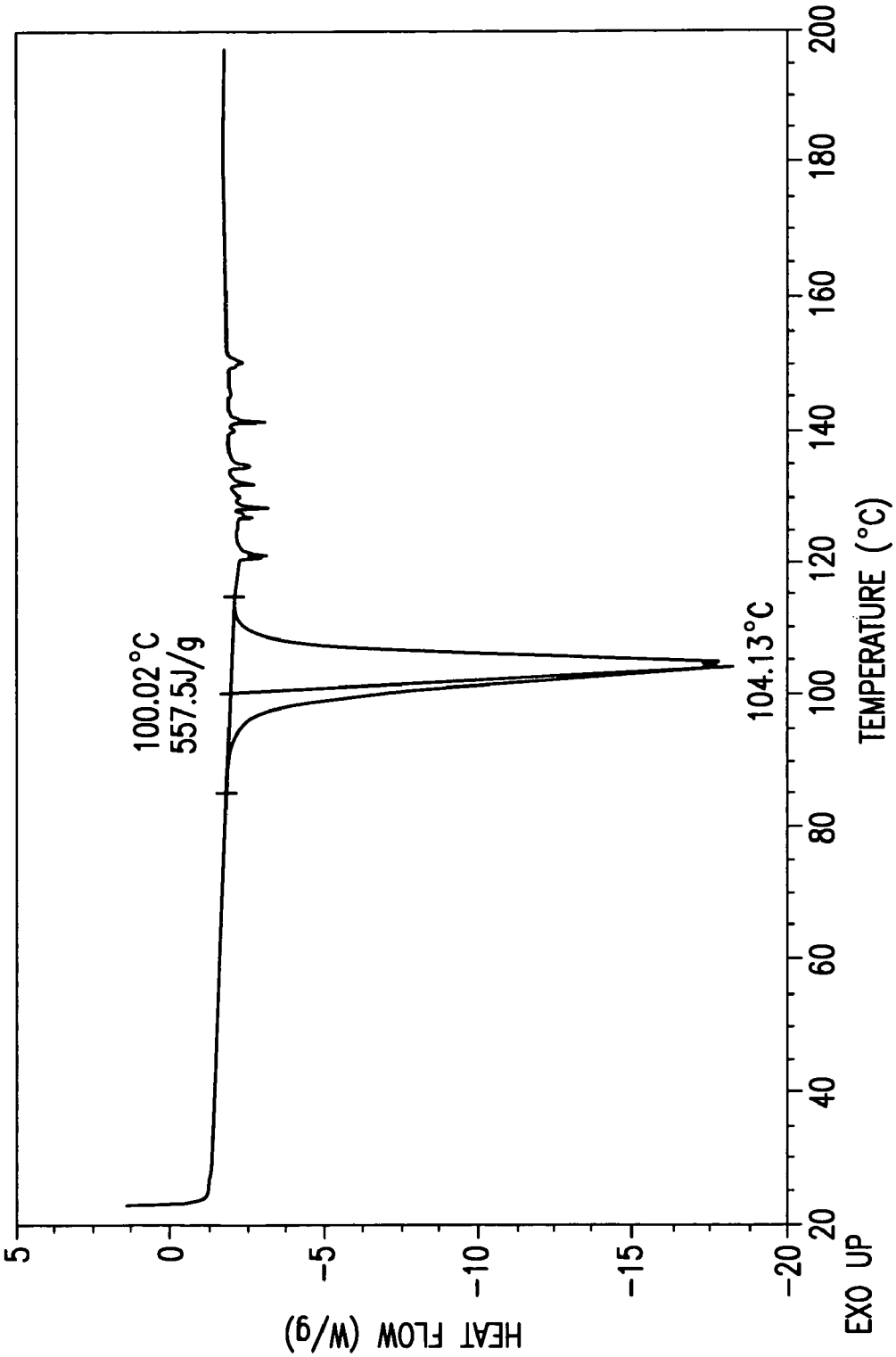

100.02°C
557.5J/g
104.13°C
HEAT FLOW (W/g)
EXO UP
TEMPERATURE (°C)
5
0
-5
-10
-15
-20
20
40
60
80
100
120
140
160
180
200

FIG. 14

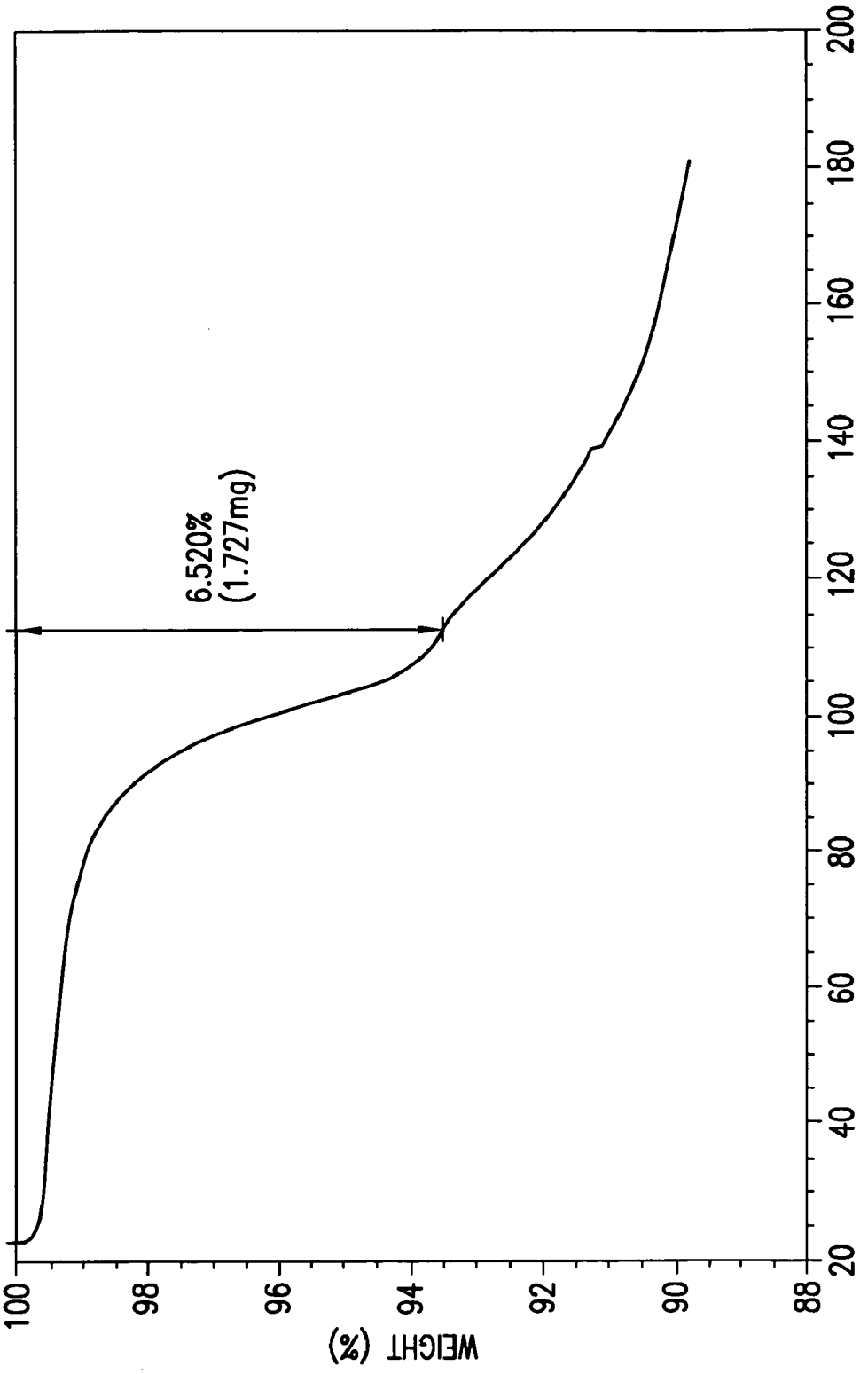

6.520%
(1.727mg)
WEIGHT (%)
100
98
96
94
92
90
88
TEMPERATURE (°C)
20
40
60
80
100
120
140
160
180
200

FIG. 15

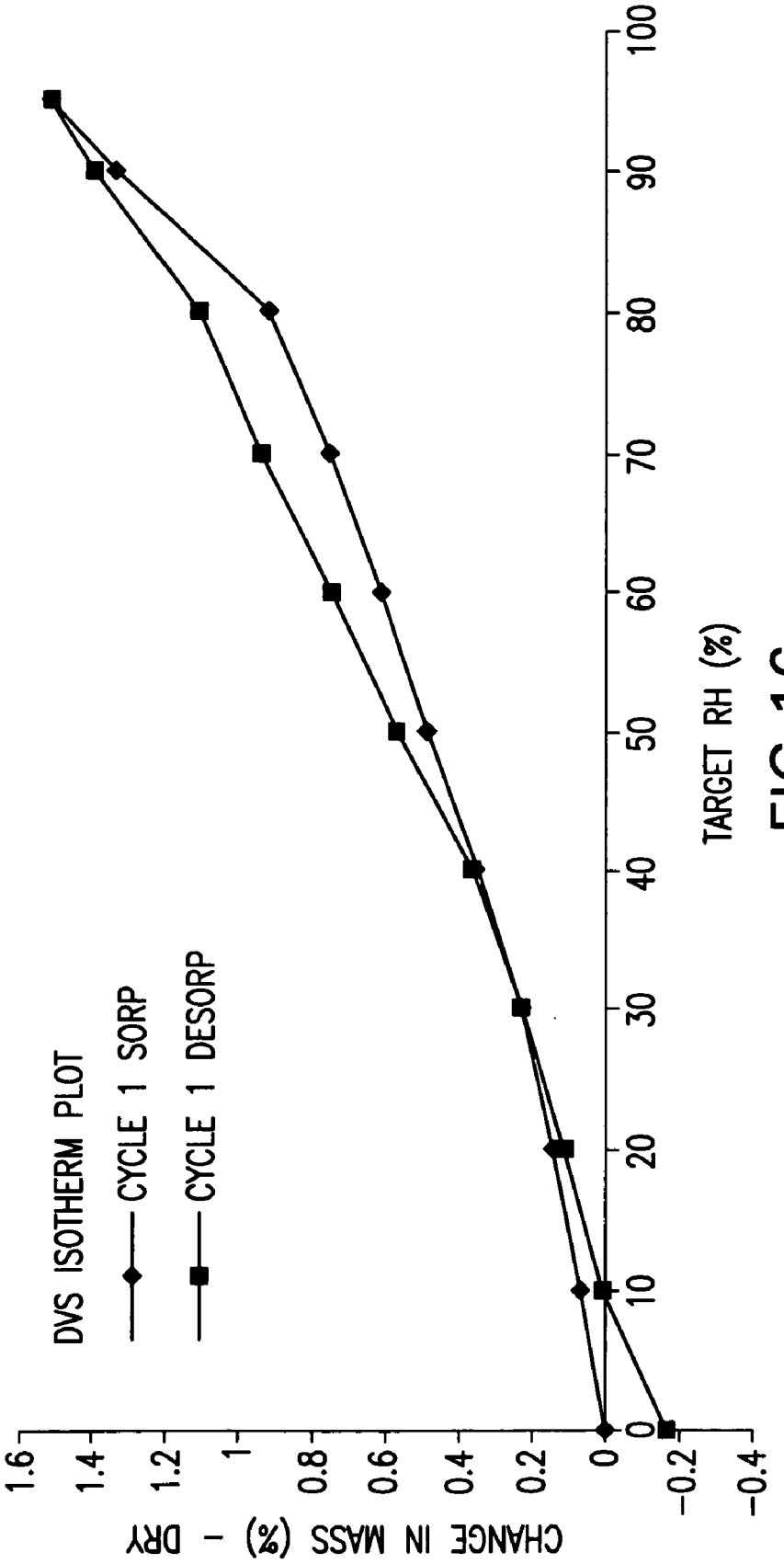

DVS ISOTHERM PLOT
CYCLE 1 SORP
CYCLE 1 DESORP
CHANGE IN MASS (%) - DRY
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
0
10
20
30
40
50
60
70
80
90
100
TARGET RH (%)

FIG.16

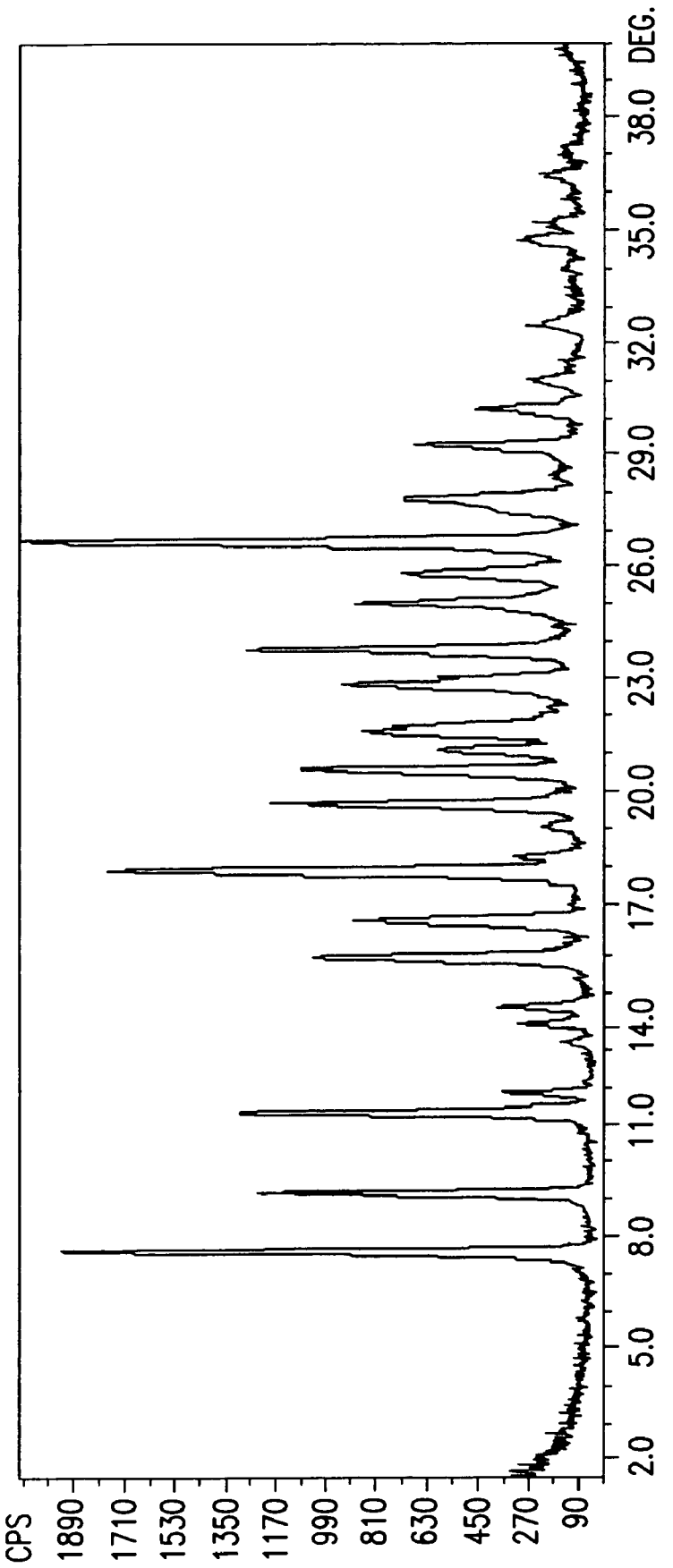

CPS
1890
1710
1530
1350
1170
990
810
630
450
270
90
2.0
5.0
8.0
11.0
14.0
17.0
20.0
23.0
26.0
29.0
32.0
35.0
38.0
DEG.

FIG. 17

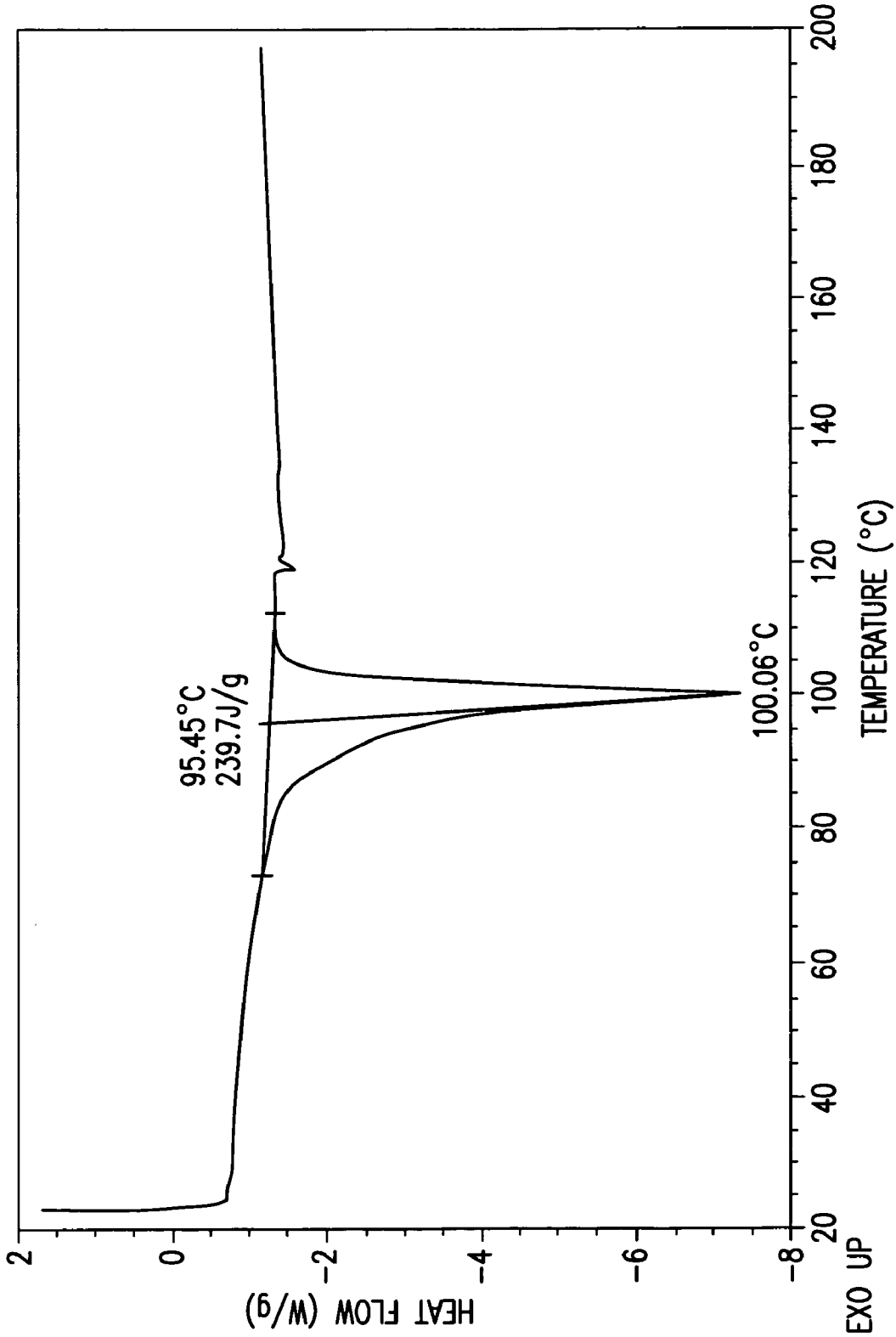

95.45°C
239.7J/g
100.06°C
HEAT FLOW (W/g)
TEMPERATURE (°C)
EXO UP
2
0
-2
-4
-6
-8
20
40
60
80
100
120
140
160
180
200

FIG.18

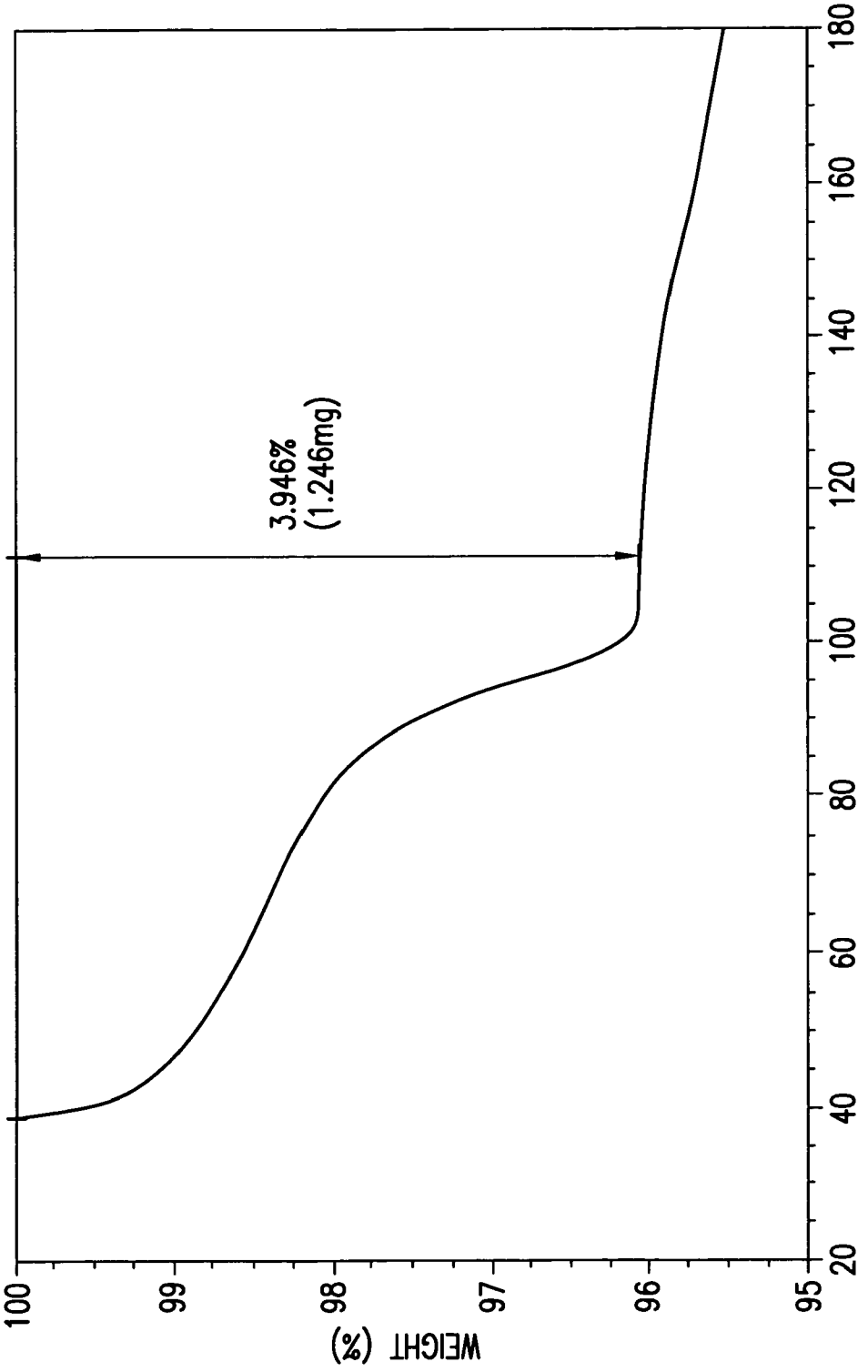

3.946%
(1.246mg)
WEIGHT (%)
100
99
98
97
96
95
TEMPERATURE (°C)
20
40
60
80
100
120
140
160
180

FIG.19

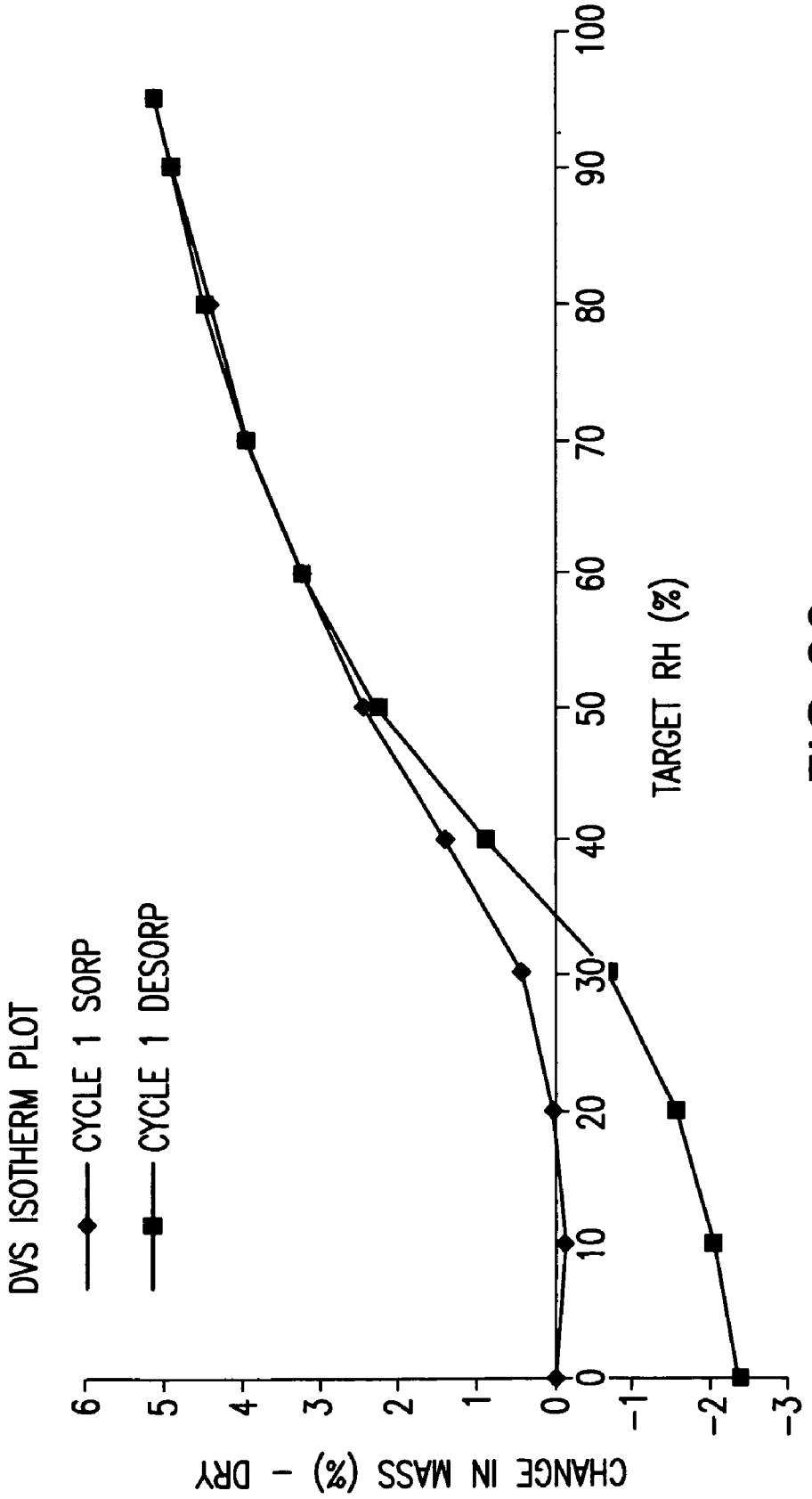

DVS ISOTHERM PLOT
CYCLE 1 SORP
CYCLE 1 DESORP
TARGET RH (%)
CHANGE IN MASS (%) - DRY
100
90
80
70
60
50
40
30
20
10
0
-1
-2
-3
6
5
4
3
2
1
0

FIG.20

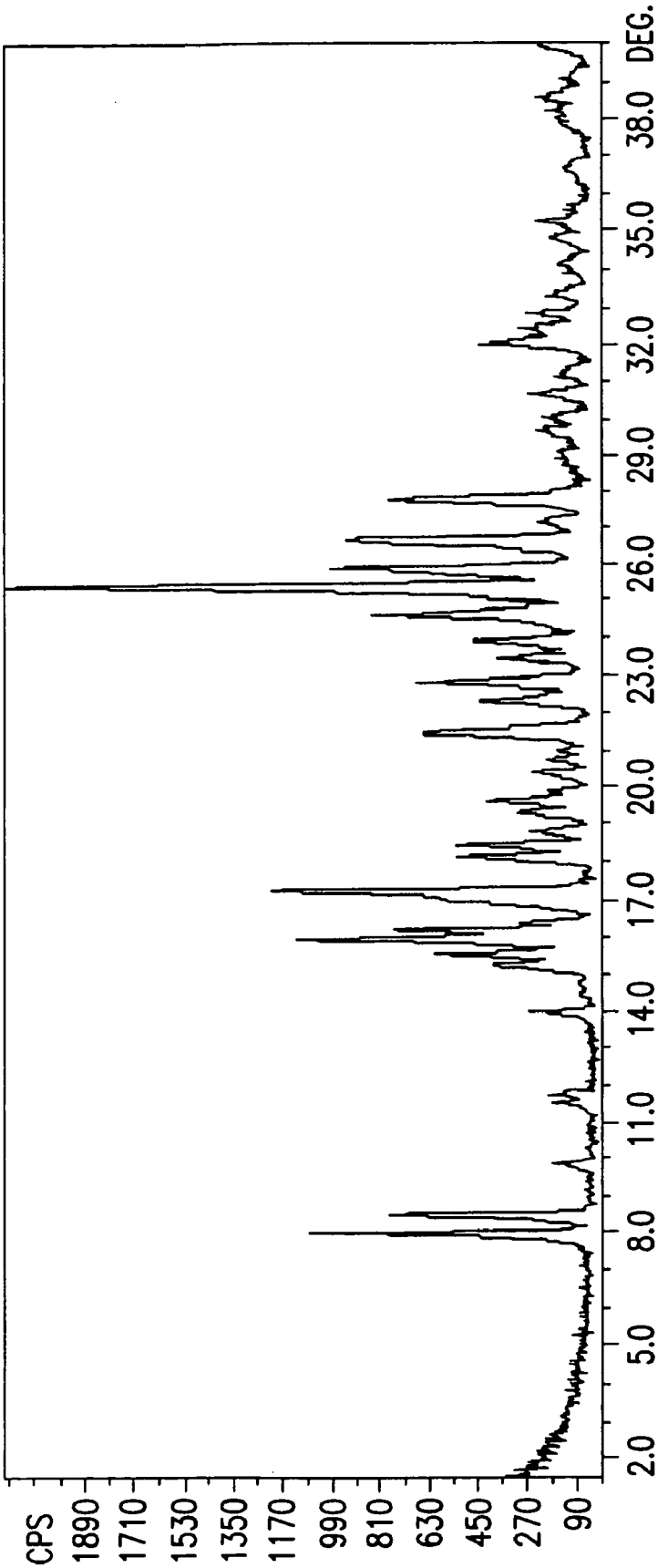

CPS
1890
1710
1530
1350
1170
990
810
630
450
270
90
2.0
5.0
8.0
11.0
14.0
17.0
20.0
23.0
26.0
29.0
32.0
35.0
38.0
DEG.

FIG.21

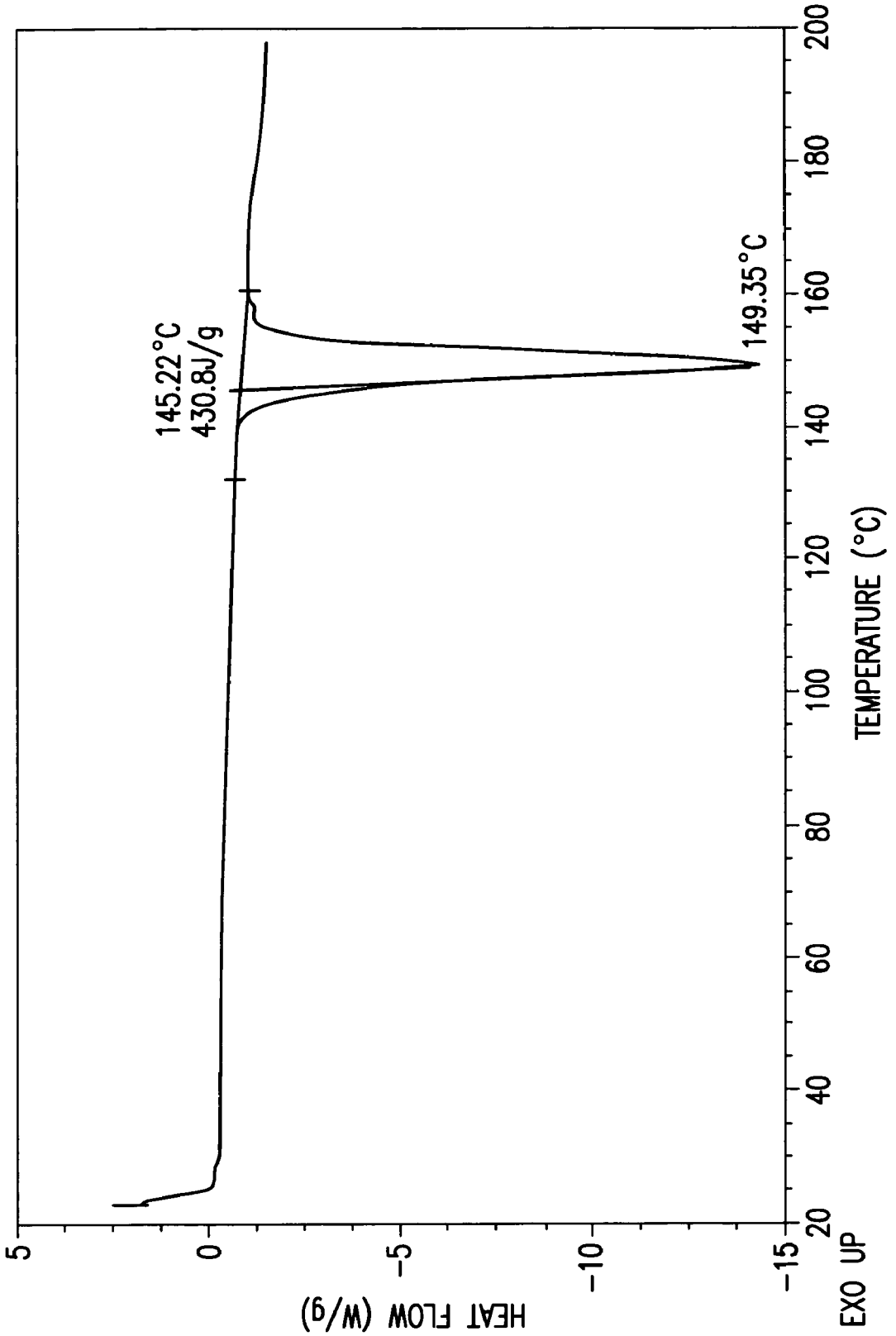

145.22°C
430.8J/g
149.35°C
HEAT FLOW (W/g)
EXO UP
TEMPERATURE (°C)
5
0
-5
-10
-15
20
40
60
80
100
120
140
160
180
200

FIG.22

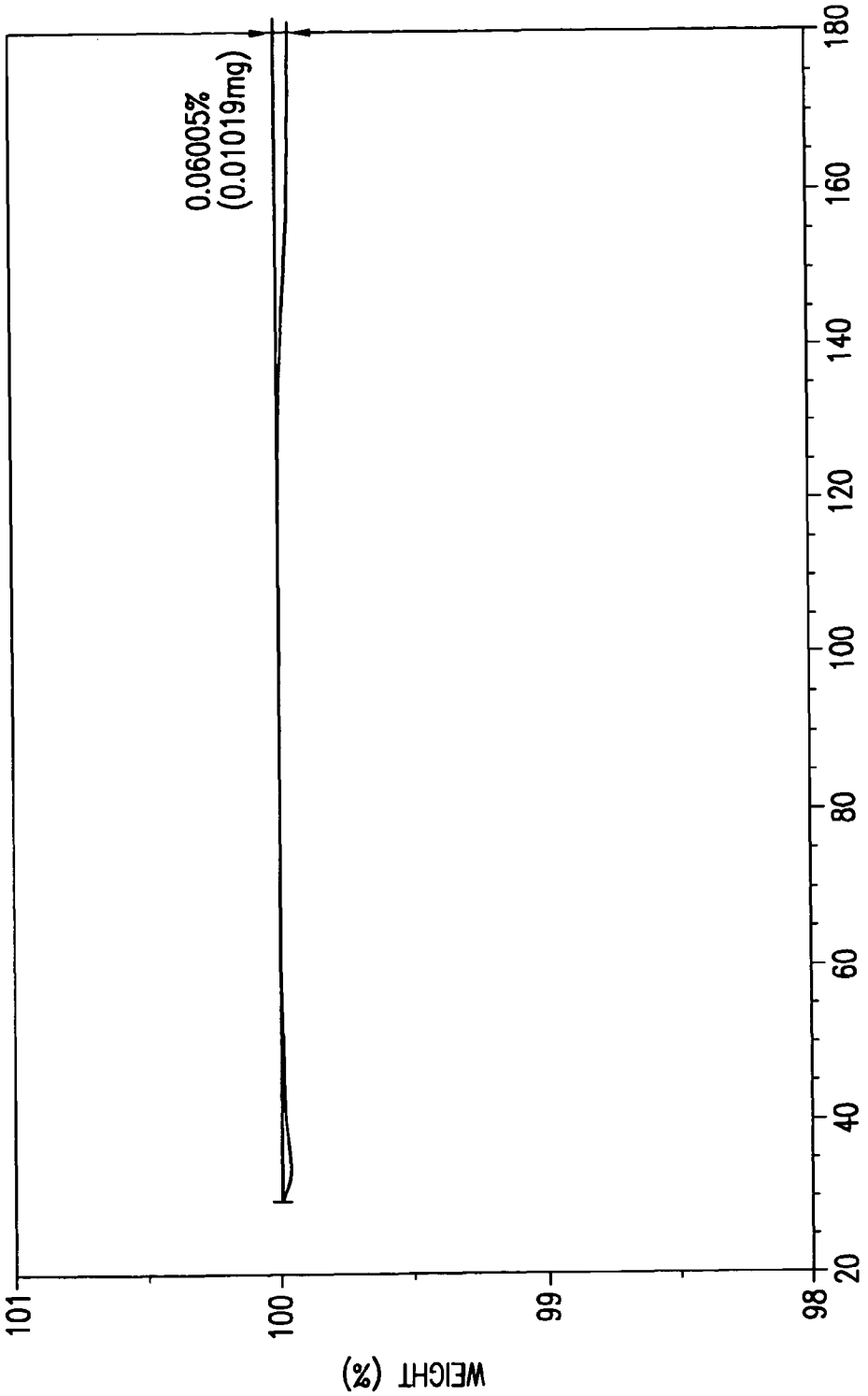

0.06005%
(0.01019mg)
WEIGHT (%)
101
100
99
98
20
40
60
80
100
120
140
160
180
TEMPERATURE (°C)

FIG.23

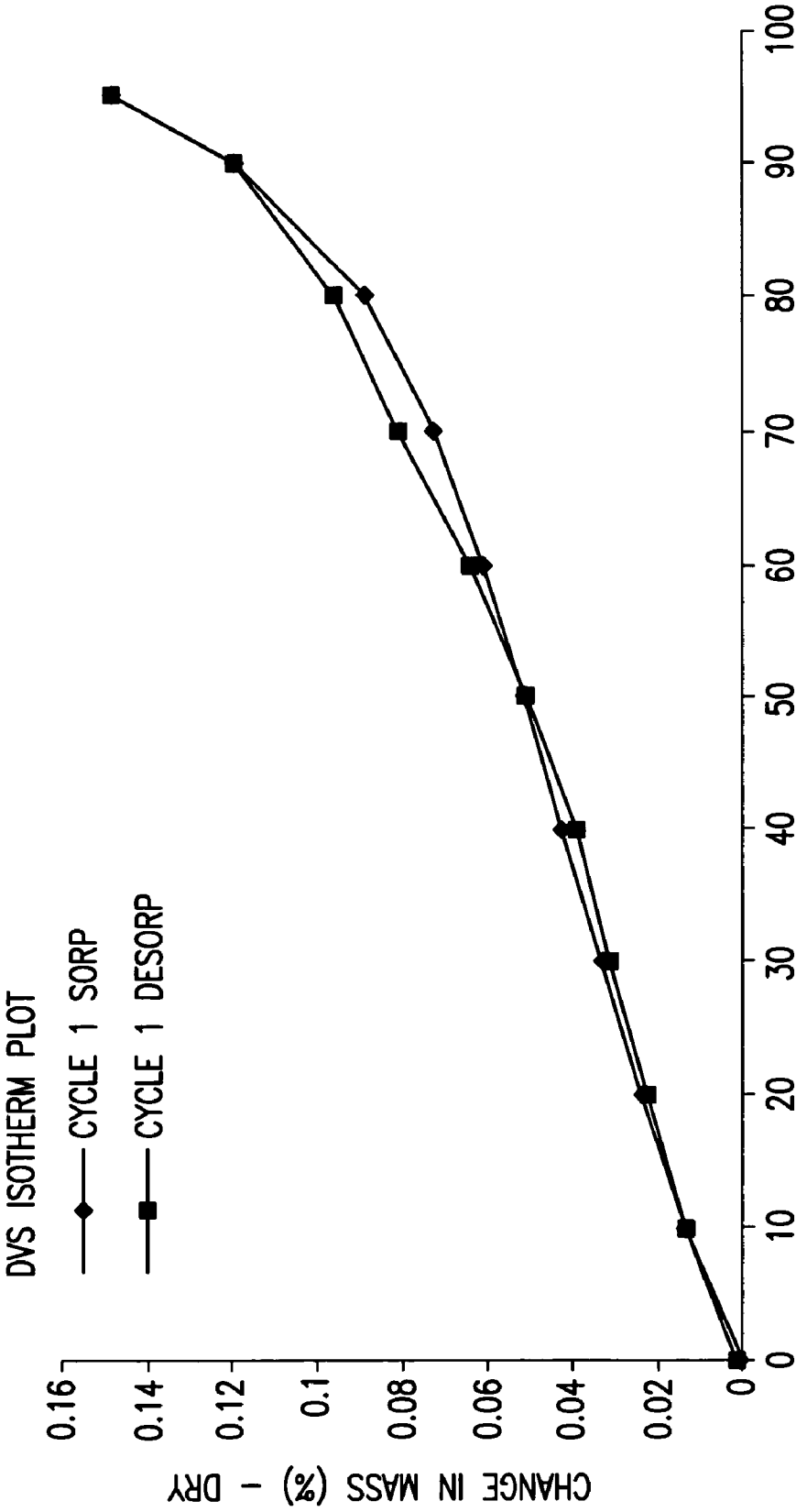

DVS ISOTHERM PLOT
CYCLE 1 SORP
CYCLE 1 DESORP
CHANGE IN MASS (%) - DRY
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
10
20
30
40
50
60
70
80
90
100
TARGET RH (%)

FIG.24

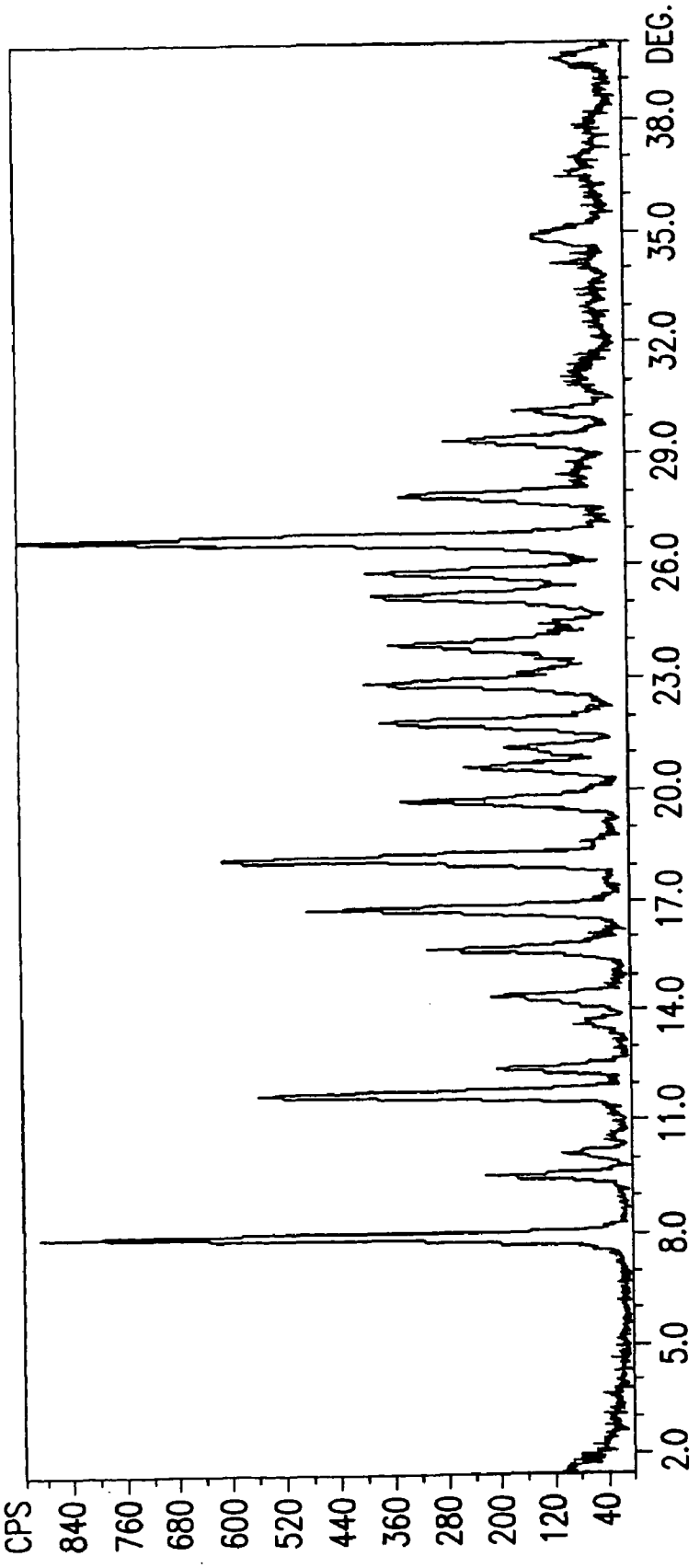

CPS
840
760
680
600
520
440
360
280
200
120
40
2.0
5.0
8.0
11.0
14.0
17.0
20.0
23.0
26.0
29.0
32.0
35.0
38.0
DEG.

FIG.25

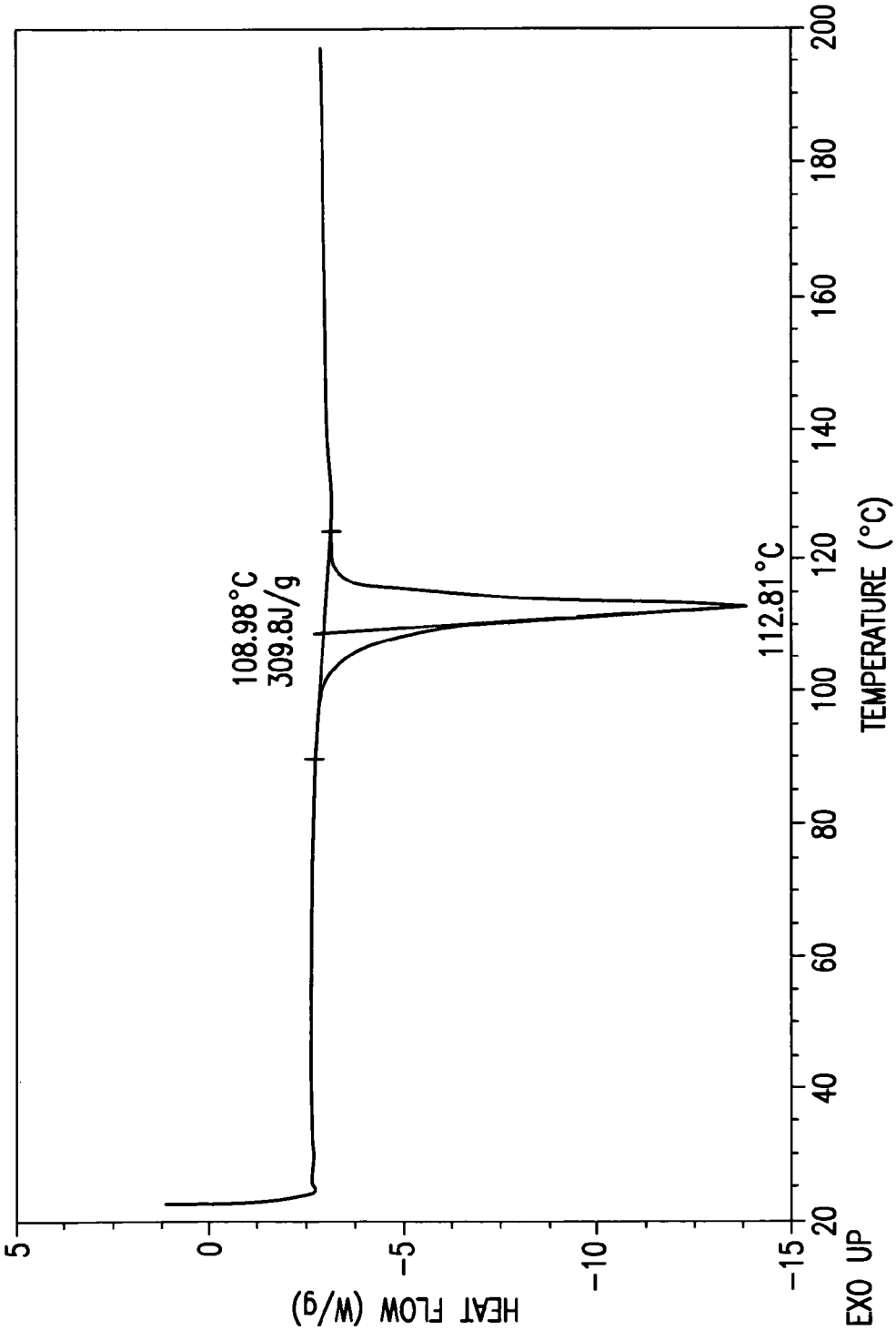

108.98°C
309.8J/g
112.81°C
HEAT FLOW (W/g)
EXO UP
TEMPERATURE (°C)
5
0
-5
-10
-15
20
40
60
80
100
120
140
160
180
200

FIG.26

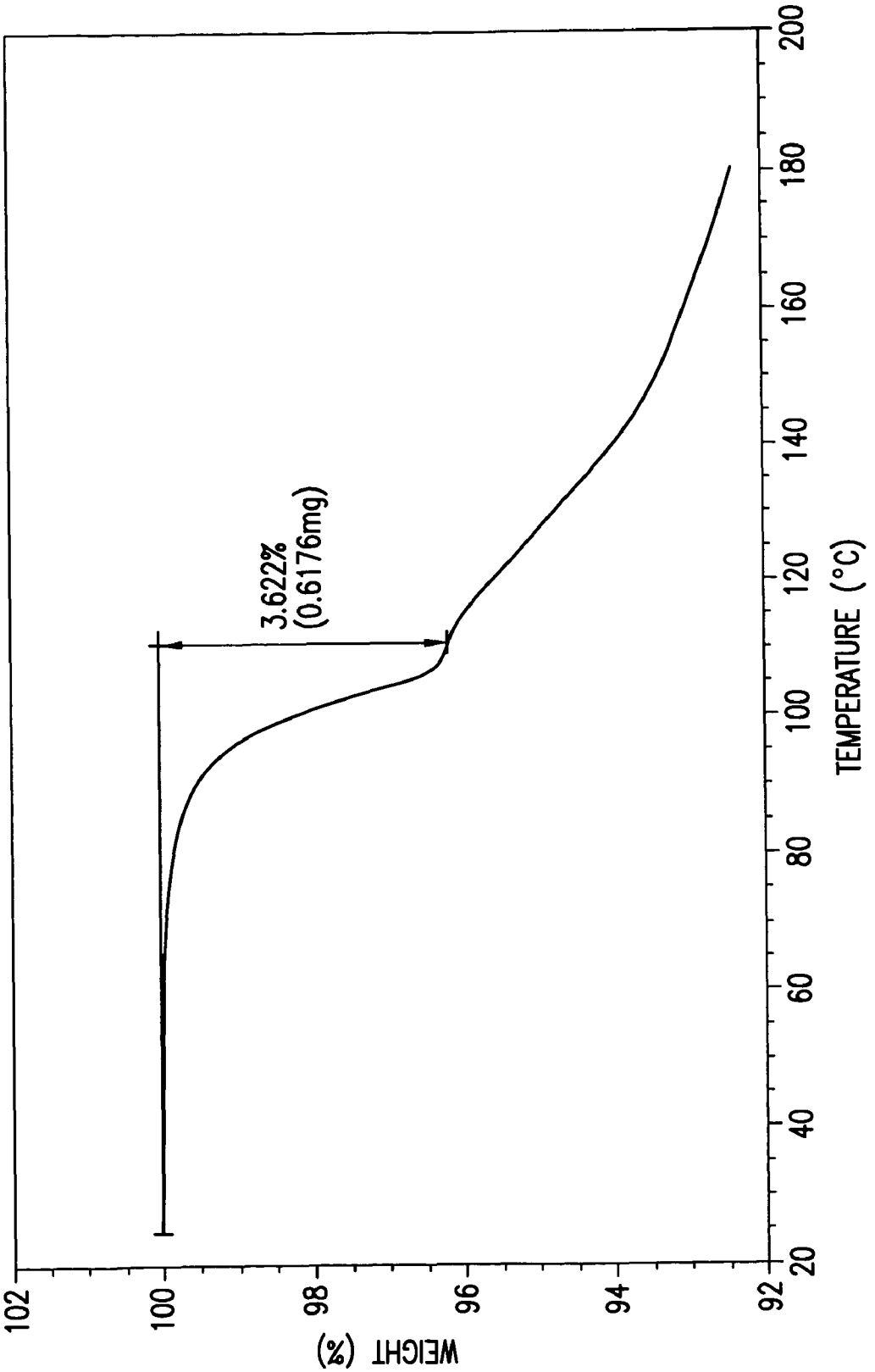

3.622%
(0.6176mg)
WEIGHT (%)
102
100
98
96
94
92
TEMPERATURE (°C)
20
40
60
80
100
120
140
160
180
200

FIG.27

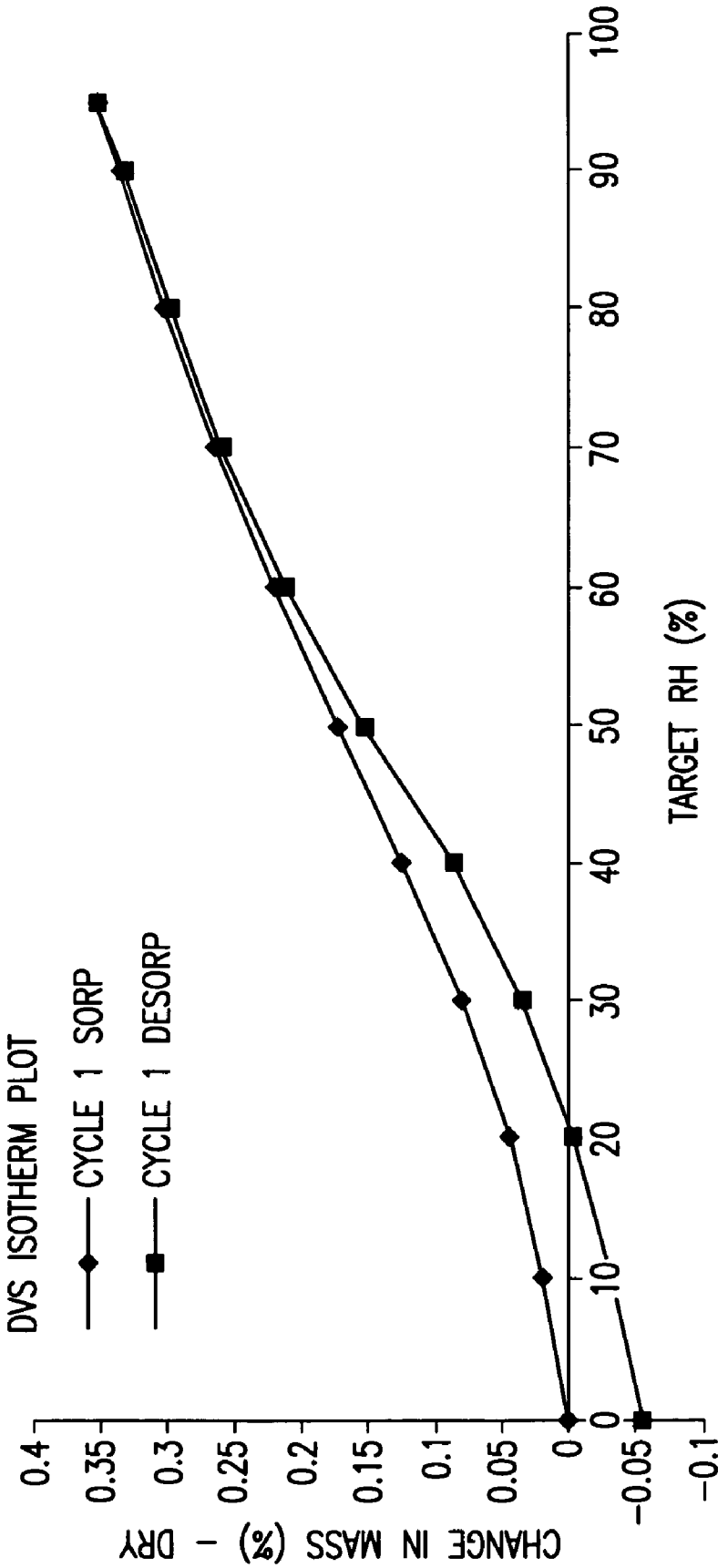

DVS ISOTHERM PLOT
CYCLE 1 SORP
CYCLE 1 DESORP
CHANGE IN MASS (%) - DRY
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
0
10
20
30
40
50
60
70
80
90
100
TARGET RH (%)

FIG.28

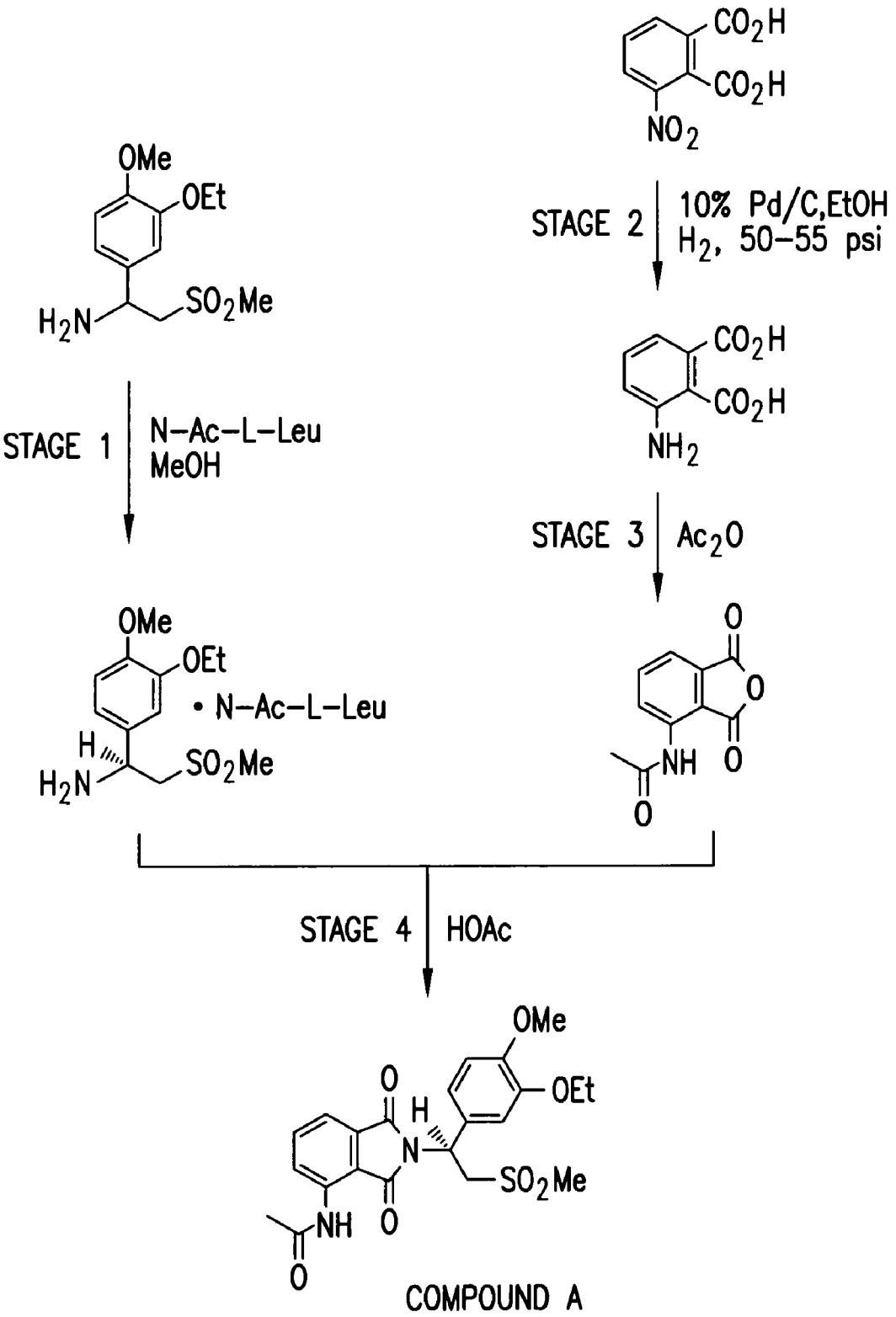

OMe
OEt
H_2N
SO_2Me
STAGE 1
N-Ac-L-Leu
MeOH
CO_2H
CO_2H
NO_2
STAGE 2
10% Pd/C,EtOH
H_2, 50-55 psi
CO_2H
CO_2H
NH_2
STAGE 3
Ac_2O
• N-Ac-L-Leu
NH
STAGE 4
HOAc
COMPOUND A

FIG.29

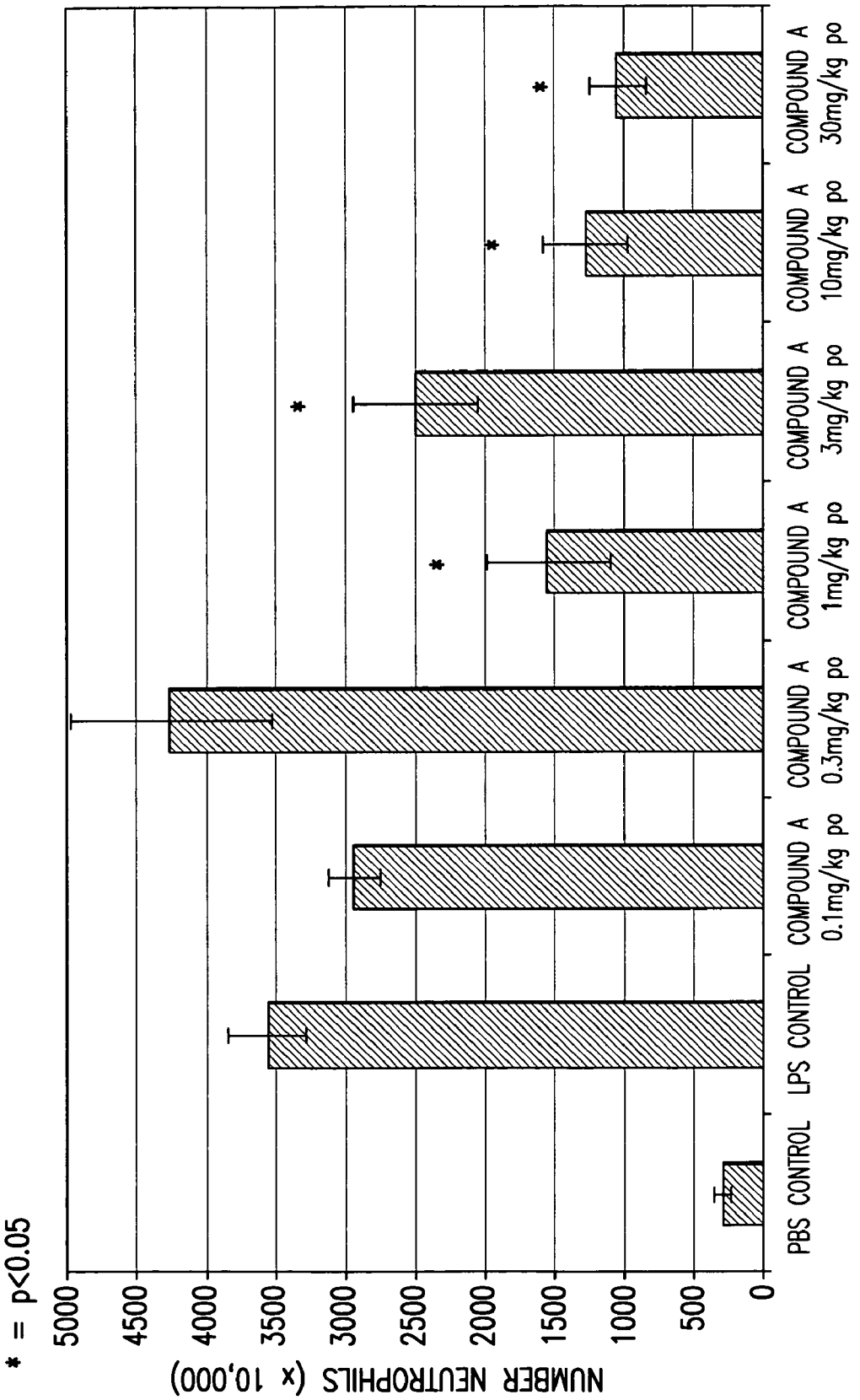

NUMBER NEUTROPHILS (x 10,000)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
* = p<0.05
PBS CONTROL
LPS CONTROL
COMPOUND A 0.1mg/kg po
COMPOUND A 0.3mg/kg po
COMPOUND A 1mg/kg po
COMPOUND A 3mg/kg po
COMPOUND A 10mg/kg po
COMPOUND A 30mg/kg po

FIG.30

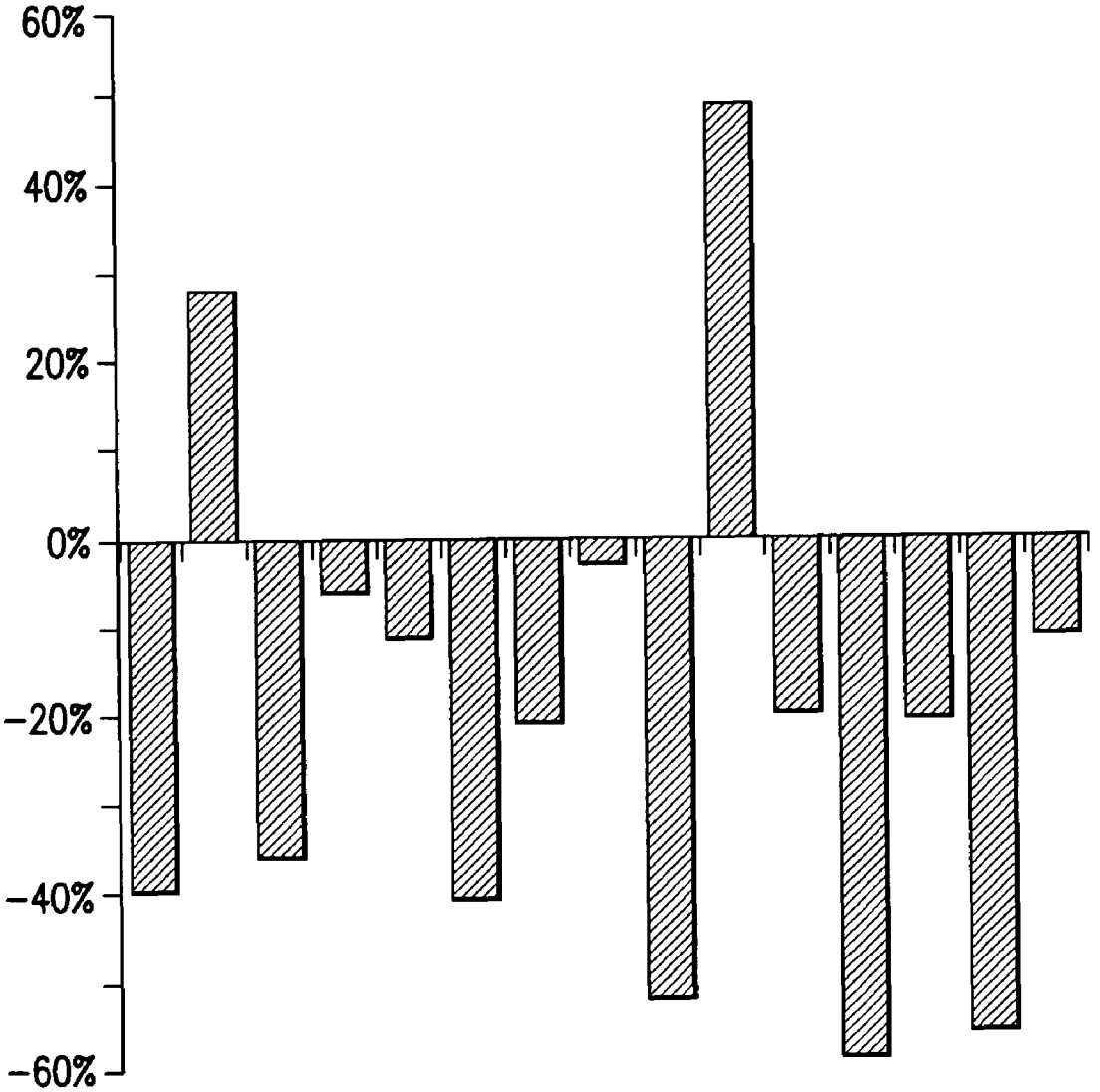


FIG.31

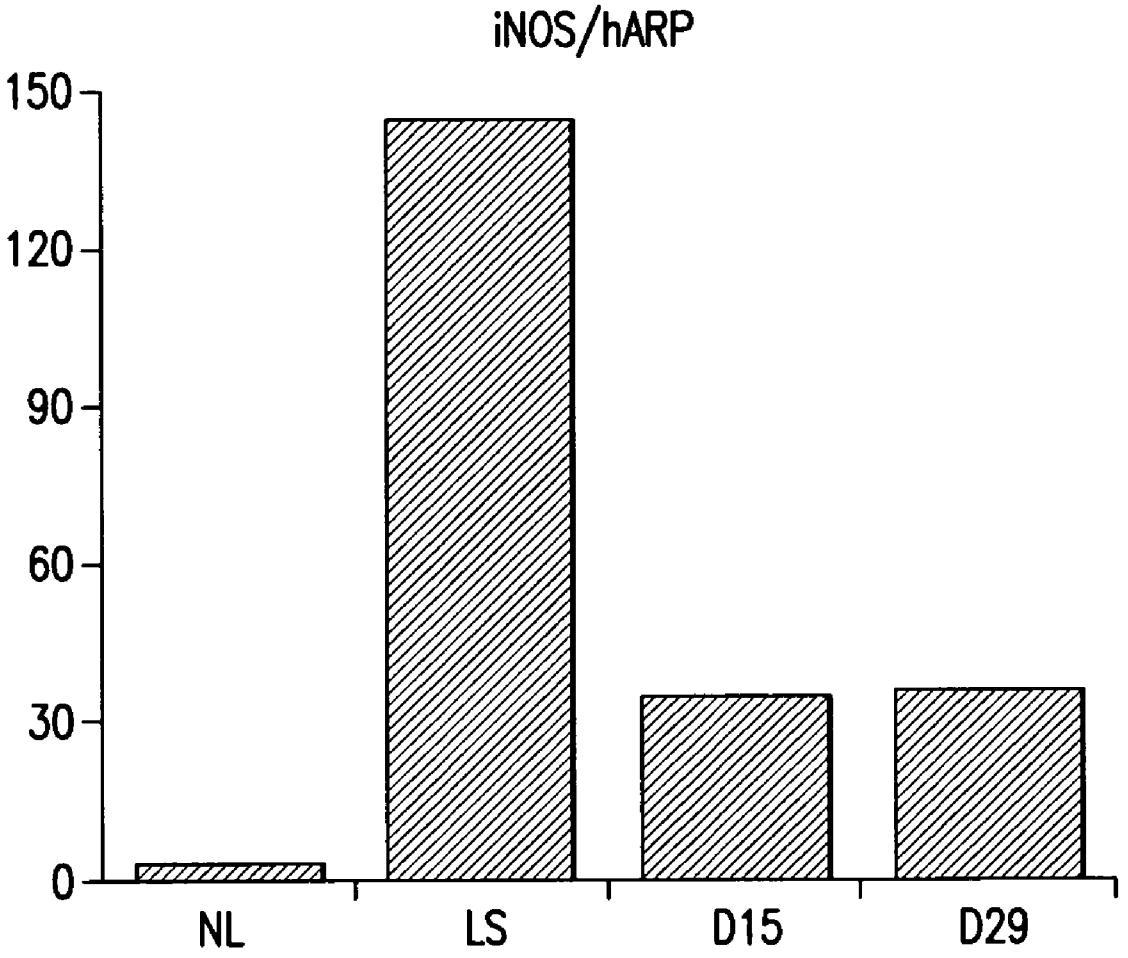


FIG.32

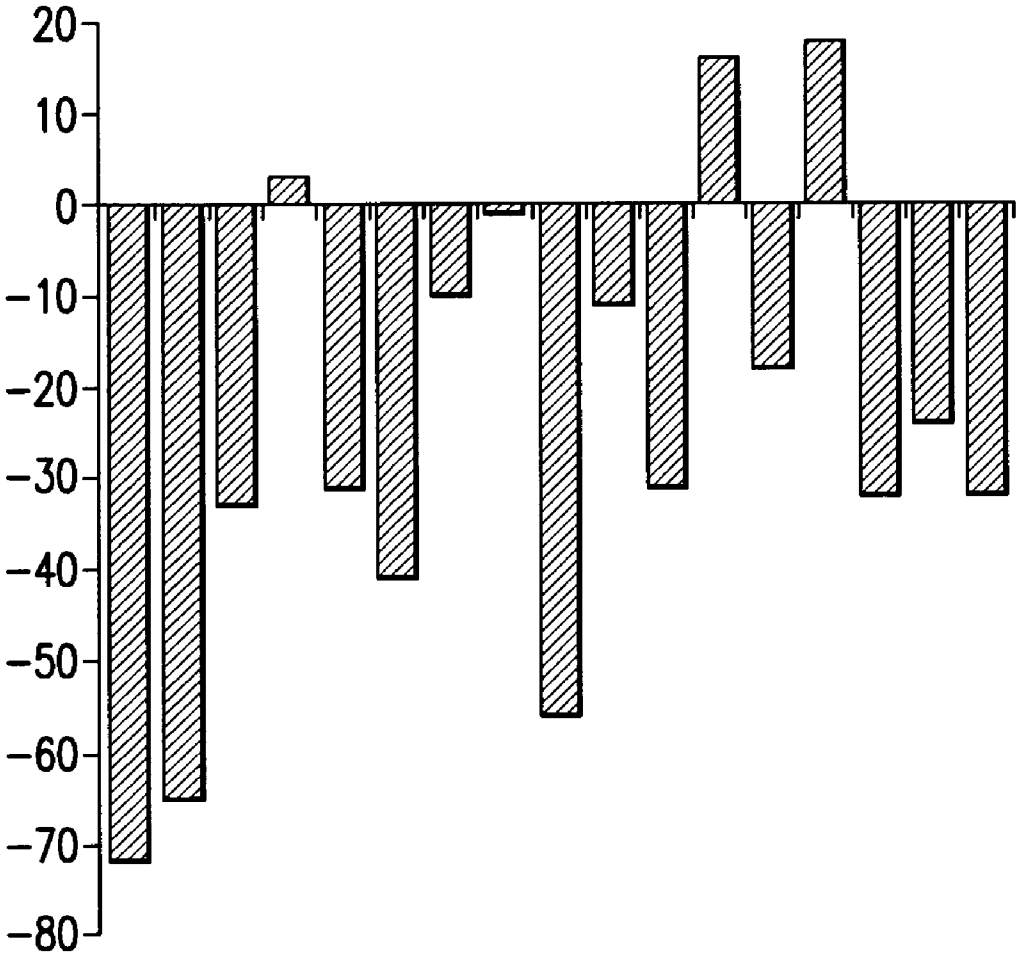


FIG.33

SOLID FORMS COMPRISING (+)-2-[1-(3-ETHOXY-4-METHOXYPHENYL)-2-METHYLSULFONYLETHYL]-4-ACETYLAMINOISOINDOLINE-1,3-DIONE, COMPOSITIONS THEREOF, AND USES THEREOF

This application is a continuation-in-part of U.S. patent application Ser. No. 11/106,142, filed Apr. 13, 2005 now U.S. Pat. No. 7,427,638, which is a divisional of U.S. patent application Ser. No. 10/392,195, filed on Mar. 19, 2003, issued as U.S. Pat. No. 6,962,940, which claims the benefit of U.S. Provisional Patent Application No. 60/366,515, filed on Mar. 20, 2002, and U.S. Provisional Patent Application No. 60/438,450, filed on Jan. 7, 2003, the entireties of which are incorporated herein by reference.

1. FIELD OF INVENTION

Provided herein are solid forms comprising (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, compositions comprising the solid forms, methods of making the solid forms and methods of their use for the treatment of various diseases and/or disorders.

2. BACKGROUND OF THE INVENTION

Tumor necrosis factor alpha (TNF-α) is a cytokine that is released primarily by mononuclear phagocytes in response to immunostimulators. TNF-α is capable of enhancing most cellular processes, such as differentiation, recruitment, proliferation, and proteolytic degradation. At low levels, TNF-α confers protection against infective agents, tumors, and tissue damage. However, TNF-α also has a role in many diseases. When administered to a patient, TNF-α causes or aggravates inflammation, fever, cardiovascular effects, hemorrhage, coagulation, and acute phase responses similar to those seen during acute infections and shock states. Enhanced or unregulated TNF-α production has been implicated in a number of diseases and medical conditions, for example, cancers, such as solid tumors and blood-borne tumors; heart disease, such as congestive heart failure; and viral, genetic, inflammatory, allergic, and autoimmune diseases.

Adenosine 3',5'-cyclic monophosphate (cAMP) also plays a role in many diseases and conditions, such as, but not limited to, asthma and inflammation, and other conditions (Lowe and Cheng, *Drugs of the Future,* 17(9), 799-807, 1992). It has been shown that the elevation of cAMP in inflammatory leukocytes inhibits their activation and the subsequent release of inflammatory mediators, including TNF-α and NF-κB. Increased levels of cAMP also leads to the relaxation of airway smooth muscle.

It is believed that the primary cellular mechanism for the inactivation of cAMP is the breakdown of cAMP by a family of isoenzymes referred to as cyclic nucleotide phosphodiesterases (PDE) (Beavo and Reitsnyder, *Trends in Pharm.,* 11, 150-155, 1990). There are eleven known PDE families. It is recognized, for example, that the inhibition of PDE type IV is particularly effective in both the inhibition of inflammatory mediator release and the relaxation of airway smooth muscle (Verghese, et al., *J. Pharm. Exper. Therapeut.,* 272(3), 1313-1320, 1995). Thus, compounds that inhibit PDE4 (PDE IV) specifically, may inhibit inflammation and aid the relaxation of airway smooth muscle with a minimum of unwanted side effects, such as cardiovascular or anti-platelet effects. Currently used PDE4 inhibitors lack the selective action at acceptable therapeutic doses.

Cancer is a particularly devastating disease, and increases in blood TNF-α levels are implicated in the risk of and the spreading of cancer. Normally, in healthy subjects, cancer cells fail to survive in the circulatory system, one of the reasons being that the lining of blood vessels acts as a barrier to tumor-cell extravasation. However, increased levels of cytokines have been shown to substantially increase the adhesion of cancer cells to endothelium in vitro. One explanation is that cytokines, such as TNF-α, stimulate the biosynthesis and expression of a cell surface receptors called ELAM-1 (endothelial leukocyte adhesion molecule). ELAM-1 is a member of a family of calcium-dependent cell adhesion receptors, known as LEC-CAMs, which includes LECAM-1 and GMP-140. During an inflammatory response, ELAM-1 on endothelial cells functions as a "homing receptor" for leukocytes. Recently, ELAM-1 on endothelial cells was shown to mediate the increased adhesion of colon cancer cells to endothelium treated with cytokines (Rice et al., 1989, *Science* 246:1303-1306).

Inflammatory diseases such as arthritis, related arthritic conditions (e.g., osteoarthritis and rheumatoid arthritis), inflammatory bowel disease (e.g., Crohn's disease and ulcerative colitis), sepsis, psoriasis, atopic dermatitis, contact dermatitis, chronic obstructive pulmonary disease, and chronic inflammatory pulmonary diseases are also prevalent and problematic ailments. TNF-α plays a central role in the inflammatory response and the administration of their antagonists block chronic and acute responses in animal models of inflammatory disease.

Enhanced or unregulated TNF-α production has been implicated in viral, genetic, inflammatory, allergic, and autoimmune diseases. Examples of such diseases include but are not limited to: HIV; hepatitis; adult respiratory distress syndrome; bone-resorption diseases; chronic obstructive pulmonary diseases; chronic pulmonary inflammatory diseases; asthma; dermatitis; cystic fibrosis; septic shock; sepsis; endotoxic shock; hemodynamic shock; sepsis syndrome; post ischemic reperfusion injury; meningitis; psoriasis; fibrotic disease; cachexia; graft rejection; auto-immune disease; rheumatoid spondylitis; arthritic conditions, such as rheumatoid arthritis and osteoarthritis; osteoporosis; Crohn's disease; ulcerative colitis; inflammatory-bowel disease; multiple sclerosis; systemic lupus erythrematosus; ENL in leprosy; radiation damage; asthma; and hyperoxic alveolar injury. Tracey et al., 1987, *Nature* 330:662-664 and Hinshaw et al., 1990, *Circ. Shock* 30:279-292 (endotoxic shock); Dezube et al., 1990, *Lancet,* 335:662 (cachexia); Millar et al., 1989, *Lancet* 2:712-714 and Ferrai-Baliviera et al., 1989, *Arch. Surg.* 124:1400-1405 (adult respiratory distress syndrome); Bertolini et al., 1986, *Nature* 319:516-518, Johnson et al., 1989, *Endocrinology* 124:1424-1427, Holler et al., 1990, *Blood* 75:1011-1016, and Grau et al., 1989, *N. Engl. J. Med.* 320:1586-1591 (bone resorption diseases); Pignet et al., 1990, *Nature,* 344:245-247, Bissonnette et al., 1989, *Inflammation* 13:329-339 and Baughman et al., 1990, *J. Lab. Clin. Med.* 115:36-42 (chronic pulmonary inflammatory diseases); Elliot et al., 1995, *Int. J. Pharmac.* 17:141-145 (rheumatoid arthritis); von Dullemen et al., 1995, *Gastroenterology,* 109: 129-135 (Crohn's disease); Duh et al., 1989, *Proc. Nat. Acad. Sci.* 86:5974-5978, Poll et al., 1990, *Proc. Nat. Acad. Sci.* 87:782-785, Monto et al., 1990, *Blood* 79:2670, Clouse et al., 1989, *J. Immunol.* 142, 431-438, Poll et al., 1992, *AIDS Res. Hum. Retrovirus,* 191-197, Poli et al. 1990, *Proc. Natl. Acad. Sci.* 87:782-784, Folks et al., 1989, PNAS 86:2365-2368 (HIV and opportunistic infections resulting from HIV).

Pharmaceutical compounds that can block the activity or inhibit the production of certain cytokines, including TNF-α,

may be beneficial therapeutics. Many small-molecule inhibitors have demonstrated an ability to treat or prevent inflammatory diseases implicated by TNF-α (for a review, see Lowe, 1998 *Exp. Opin. Ther. Patents* 8:1309-1332). One such class of molecules are the substituted phenethylsulfones described in U.S. Pat. No. 6,020,358.

The preparation and selection of a solid form of a pharmaceutical compound is complex, given that a change in solid form may affect a variety of physical and chemical properties, which may provide benefits or drawbacks in processing, formulation, stability and bioavailability, among other important pharmaceutical characteristics. Potential pharmaceutical solids include crystalline solids and amorphous solids. Amorphous solids are characterized by a lack of long-range structural order, whereas crystalline solids are characterized by structural periodicity. The desired class of pharmaceutical solid depends upon the specific application; amorphous solids are sometimes selected on the basis of, e.g., an enhanced dissolution profile, while crystalline solids may be desirable for properties such as, e.g., physical or chemical stability (see, e.g., S. R. Vippagunta et al., *Adv. Drug. Deliv. Rev.*, (2001) 48:3-26; L. Yu, *Adv. Drug. Deliv. Rev.*, (2001) 48:27-42).

Whether crystalline or amorphous, potential solid forms of a pharmaceutical compound include single-component and multiple-component solids. Single-component solids consist essentially of the pharmaceutical compound in the absence of other compounds. Variety among single-component crystalline materials may potentially arise, e.g., from the phenomenon of polymorphism, wherein multiple three-dimensional arrangements exist for a particular pharmaceutical compound (see, e.g., S. R. Byrn et al., *Solid State Chemistry of Drugs*, (1999) SSCI, West Lafayette). The importance of studying polymorphs was underscored by the case of Ritonavir, an HIV protease inhibitor that was formulated as soft gelatin capsules. About two years after the product was launched, the unanticipated precipitation of a new, less soluble polymorph in the formulation necessitated the withdrawal of the product from the market until a more consistent formulation could be developed (see S. R. Chemburkar et al., *Org. Process Res. Dev.*, (2000) 4:413-417).

Additional diversity among the potential solid forms of a pharmaceutical compound may arise, e.g., from the possibility of multiple-component solids. Crystalline solids comprising two or more ionic species may be termed salts (see, e.g., *Handbook of Pharmaceutical Salts Properties, Selection and Use*, P. H. Stahl and C. G. Wermuth, Eds., (2002), Wiley, Weinheim). Additional types of multiple-component solids that may potentially offer other property improvements for a pharmaceutical compound or salt thereof include, e.g., hydrates, solvates, co-crystals and clathrates, among others (see, e.g., S. R. Byrn et al., *Solid State Chemistry of Drugs*, (1999) SSCI, West Lafayette). Moreover, multiple-component crystal forms may potentially be susceptible to polymorphism, wherein a given multiple-component composition may exist in more than one three-dimensional crystalline arrangement. The preparation of solid forms is of great importance in the development of a safe, effective, stable and marketable pharmaceutical compound.

Provided herein are embodiments addressing a need for solid forms of the compound chemically named (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione ("Compound A"), which was disclosed in U.S. application Ser. No. 10/392,195, filed Mar. 19, 2003 (issued as U.S. Pat. No. 6,962,940), as well as U.S. Provisional Application Ser. Nos. 60/366,515, filed Mar. 20, 2002 and 60/438,450, filed Jan. 7, 2003.

3. SUMMARY OF THE INVENTION

This invention relates to methods of treating diseases and disorders utilizing an enantiomer of a substituted phenethylsulfone compound and pharmaceutically acceptable solvates, hydrates, co-crystals, clathrates, prodrugs and polymorphs thereof and methods for reducing the level of cytokines and their precursors in mammals. The invention also relates to pharmaceutical compositions comprising the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione and a pharmaceutically acceptable carrier. The invention further relates to the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione substantially free of its (−) enantiomer.

This invention particularly relates to the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione. This compound is believed to have increased potency and other benefits as compared to its racemate, 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

The invention encompasses the use of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione for treating or preventing diseases or disorders ameliorated by the inhibition of TNF-αproduction in mammals. In certain embodiments, this treatment includes the reduction or avoidance of adverse effects. Such disorders include, but are not limited to, cancers, including, but not limited to cancer of the head, thyroid, neck, eye, skin, mouth, throat, esophagus, chest, bone, blood, bone marrow, lung, colon, sigmoid, rectum, stomach, prostate, breast, ovaries, kidney, liver, pancreas, brain, intestine, heart, adrenal, subcutaneous tissue, lymph nodes, heart, and combinations thereof. Specific cancers that can be treated by this method are multiple myeloma, malignant melanoma, malignant glioma, leukemia and solid tumors.

The invention also encompasses the use of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione in the treatment or prevention of heart disease, including, but not limited to congestive heart failure, cardiomyopathy, pulmonary edema, endotoxin-mediated septic shock, acute viral myocarditis, cardiac allograft rejection, and myocardial infarction.

The invention also encompasses the use of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione to treat diseases or disorders ameliorated by the inhibition of PDE4. For example, the compounds and compositions of the invention may be useful to treat or prevent viral, genetic, inflammatory, allergic, and autoimmune diseases. Examples of such diseases include, but are not limited to: HIV; hepatitis; adult respiratory distress syndrome; bone-resorption diseases; chronic obstructive pulmonary diseases; chronic pulmonary inflammatory diseases; dermatitis; inflammatory skin disease, atopic dermatitis, cystic fibrosis; septic shock; sepsis; endotoxic shock; hemodynamic shock; sepsis syndrome; post ischemic reperfusion injury; meningitis; psoriasis; fibrotic disease; cachexia; graft rejection including graft versus host disease; auto-immune disease; rheumatoid spondylitis; arthritic conditions, such as rheumatoid arthritis and osteoarthritis; osteoporosis; Crohn's disease; ulcerative colitis; inflammatory-bowel disease; multiple sclerosis; systemic

lupus erythrematosus; erythema nodosum leprosum (ENL) in leprosy; radiation damage; asthma; and hyperoxic alveolar injury.

In yet another embodiment, the stereomerically pure (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is also useful in the treatment or prevention of microbial infections or the symptoms of microbial infections including, but not limited to, bacterial infections, fungal infections, malaria, mycobacterial infection, and opportunistic infections resulting from HIV.

The invention further encompasses pharmaceutical compositions and single unit dosage forms comprising the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione and pharmaceutically acceptable polymorphs, prodrugs, hydrates, clathrates, and solvates thereof.

In a separate embodiment, the invention encompasses the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

In a further embodiment, the invention encompasses a method of producing the stereomerically pure (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione which comprises contacting 1-(3-Ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethylamine with a chiral amino acid and contacting the product of the first step with N-(1,3-Dioxo-1,3-dihydroisobenzofuran-4-yl)-acetamide. In a related embodiment the invention encompasses a chiral salt of 1-(3-Ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethylamine.

Embodiments herein provide solid forms comprising the compound chemically named (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione ("Compound A"). Compound A can be synthesized or obtained according to any method apparent to those of skill in the art based upon the teachings herein, including the methods described in the Examples below. Compound A can also be prepared according to the methods described in U.S. Pat. No. 6,962,940, issued Nov. 8, 2005, the entirety of which is incorporated by reference herein.

In certain embodiments, the solid forms are single-component crystal forms of Compound A. In certain embodiments, the solid forms are multiple-component crystal forms, including, but not limited to, co-crystals and/or solvates (including hydrates) comprising Compound A. In other embodiments, the solid forms are single-component amorphous forms of Compound A. In other embodiments, the solid forms are multiple-component amorphous forms. Without intending to be limited by any particular theory, certain novel solid forms provided herein have particular advantageous physical and/or chemical properties making them useful, e.g., for manufacturing, processing, formulation and/or storage, while also possessing particularly advantageous biological properties, such as, e.g., bioavailability and/or biological activity.

In particular embodiments, solid forms provided herein include solid forms comprising Compound A, including, but not limited to, single-component and multiple-component solid forms comprising Compound A. In certain embodiments, solid forms provided herein include polymorphs, solvates (including hydrates) and co-crystals comprising Compound A. Certain embodiments herein provide methods of making, isolating and/or characterizing the solid forms provided herein.

The solid forms provided herein are useful as active pharmaceutical ingredients for the preparation of formulations for use in patients. Thus, embodiments herein encompass the use of these solid forms as a final drug product. Certain embodiments provide solid forms useful in making final dosage forms with improved properties, e.g., powder flow properties, compaction properties, tableting properties, stability properties, and excipient compatibility properties, among others, that are needed for manufacturing, processing, formulation and/or storage of final drug products. Certain embodiments herein provide pharmaceutical compositions comprising a single-component crystal form, a multiple-component crystal form, a single-component amorphous form and/or a multiple-component amorphous form comprising Compound A and a pharmaceutically acceptable diluent, excipient or carrier. The solid forms and the final drug products provided herein are useful, for example, for the treatment, prevention or management of diseases and disorders provided herein.

Certain embodiments herein provide methods using the solid forms provided herein for treating, preventing or managing diseases or disorders ameliorated by the inhibition of TNF-α production in mammals, such as HIV; hepatitis; adult respiratory distress syndrome; bone resorption diseases; chronic obstructive pulmonary diseases; chronic pulmonary inflammatory diseases; asthma; dermatitis; cystic fibrosis; septic shock; sepsis; endotoxic shock; hemodynamic shock; sepsis syndrome; post ischemic reperfusion injury; meningitis; psoriasis; fibrotic disease; cachexia; graft rejection; auto immune disease; rheumatoid spondylitis; arthritic conditions, such as psoriatic arthritis, rheumatoid arthritis and osteoarthritis; osteoporosis; Crohn's disease; ulcerative colitis; inflammatory bowel disease; multiple sclerosis; systemic lupus erythematosus; cutaneous lupus erythematosus; pulmonary sarcoidosis; ENL in leprosy; radiation damage; asthma; and hyperoxic alveolar injury. Such disorders further include, but are not limited to, cancers, including, but not limited to cancer of the head, thyroid, neck, eye, skin, mouth, throat, esophagus, chest, bone, blood, bone marrow, lung, colon, sigmoid, rectum, stomach, prostate, breast, ovaries, kidney, liver, pancreas, brain, intestine, heart, adrenal, subcutaneous tissue, lymph nodes, heart, and combinations thereof. Specific cancers that can be treated by this method are multiple myeloma, malignant melanoma, malignant glioma, leukemia and solid tumors. In certain embodiments, methods using the solid forms provided herein include the reduction or avoidance of certain adverse effects.

Certain embodiments herein provide methods of using the solid forms provided herein in the treatment or prevention of heart disease, including, but not limited to congestive heart failure, cardiomyopathy, pulmonary edema, endotoxin-mediated septic shock, acute viral myocarditis, cardiac allograft rejection, and myocardial infarction.

Certain embodiments herein provide methods of using the solid forms provided herein to treat diseases or disorders ameliorated by the inhibition of PDE4. For example, the solid forms provided herein may be useful to treat or prevent viral, genetic, inflammatory, allergic, and autoimmune diseases. Examples of such diseases include, but are not limited to: HIV; hepatitis; adult respiratory distress syndrome; bone-resorption diseases; chronic obstructive pulmonary diseases; chronic pulmonary inflammatory diseases; dermatitis; inflammatory skin disease; atopic dermatitis; cystic fibrosis; septic shock; sepsis; endotoxic shock; hemodynamic shock; sepsis syndrome; post ischemic reperfusion injury; meningitis; psoriasis; fibrotic disease; cachexia; graft rejection including graft versus host disease; auto-immune disease; rheumatoid spondylitis; arthritic conditions, such as rheumatoid arthritis and osteoarthritis; osteoporosis; Crohn's disease; ulcerative colitis; inflammatory-bowel disease; multiple sclerosis; systemic lupus erythrematosus; erythema

nodosum leprosum (ENL) in leprosy; radiation damage; asthma; and hyperoxic alveolar injury.

Certain embodiments herein provide methods of using the solid forms provided herein in the treatment or prevention of microbial infections or the symptoms of microbial infections including, but not limited to, bacterial infections, fungal infections, malaria, mycobacterial infection, and opportunistic infections resulting from HIV.

Particular embodiments herein provide methods of using the solid forms provided herein in the treatment or prevention of diseases including: psoriasis; psoriatic arthritis; rheumatoid arthritis; chronic cutaneous sarcoid; giant cell arteritis; Parkinson's; prurigo nodularis; lichen planus; complex apthosis; Behcet's disease; lupus; hepatitis; uveitis; Sjogren's disease; depression (including major depression); interstitial cystitis; vulvodynia; prostatitis; osteoarthritis; diffuse large B cell lymphoma; polymysoitis; dermatomyositis; inclusion body myositis; erosive osteoarthritis; interstitial cystitis; hepatitis; endometriosis; radiculopathy; and pyoderma gangrenosum.

Certain embodiments herein provide pharmaceutical compositions and single unit dosage forms comprising one or more solid forms provided herein.

3.1. BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** provides a representative X-ray Powder Diffraction ("XRPD") pattern of Form A of Compound A.

FIG. **2** provides a representative Differential Scanning Calorimetry ("DSC") plot of Form A of Compound A.

FIG. **3** provides a representative Thermal Gravimetric Analysis ("TGA") plot of Form A of Compound A.

FIG. **4** provides a representative Dynamic Vapor Sorption ("DVS") plot of Form A of Compound A.

FIG. **5** provides a representative XRPD pattern of Form B of Compound A.

FIG. **6** provides a representative DSC plot of Form B of Compound A.

FIG. **7** provides a representative TGA plot of Form B of Compound A.

FIG. **8** provides a representative DVS plot of Form B of Compound A.

FIG. **9** provides a representative XRPD pattern of Form C of Compound A.

FIG. **10** provides a representative DSC plot of Form C of Compound A.

FIG. **11** provides a representative TGA plot of Form C of Compound A.

FIG. **12** provides a representative DVS plot of Form C of Compound A.

FIG. **13** provides a representative XRPD pattern of Form D of Compound A.

FIG. **14** provides a representative DSC plot of Form D of Compound A.

FIG. **15** provides a representative TGA plot of Form D of Compound A.

FIG. **16** provides a representative DVS plot of Form D of Compound A.

FIG. **17** provides a representative XRPD pattern of Form E of Compound A.

FIG. **18** provides a representative DSC plot of Form E of Compound A.

FIG. **19** provides a representative TGA plot of Form E of Compound A.

FIG. **20** provides a representative DVS plot of Form E of Compound A.

FIG. **21** provides a representative XRPD pattern of Form F of Compound A.

FIG. **22** provides a representative DSC plot of Form F of Compound A.

FIG. **23** provides a representative TGA plot of Form F of Compound A.

FIG. **24** provides a representative DVS plot of Form F of Compound A.

FIG. **25** provides a representative XRPD of Form G of Compound A.

FIG. **26** provides a representative DSC plot of Form G of Compound A.

FIG. **27** provides a representative TGA plot of Form G of Compound A.

FIG. **28** provides a representative DVS plot of Form G of Compound A.

FIG. **29** illustrates a preparation of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

FIG. **30** illustrates the effect of Compound A on LPS-induced neutrophilia in the lungs of conscious ferrets.

FIG. **31** illustrates the percent change in epidermal thickness among all 15 subjects at Day 29 in a clinical study evaluating Compound A in patients with severe plaque-type psoriasis.

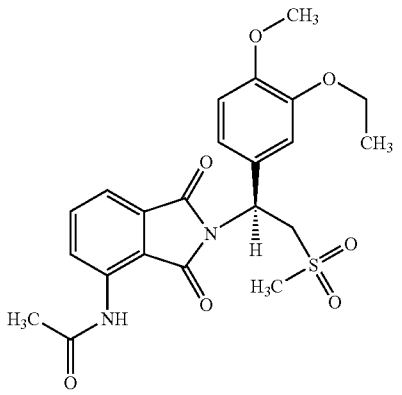
FIG. **32** illustrates the change in mean iNOS (normalized to hARP) in biopsy specimens of lesional skin at Day 29 in a clinical study evaluating Compound A in patients with severe plaque-type psoriasis.

FIG. **33** illustrates the percentage change in total Psoriasis Area and Severity Index (PASI) score among evaluable patients from baseline at Day 29 in a clinical study evaluating Compound A in patients with severe plaque-type psoriasis.

3.2. DEFINITIONS

As used herein, term "Compound A" refers to enantiomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione which comes off of an HPLC column at about 25.4 minutes when that column is a 150 mm×4.6 mm Ultron Chiral ES-OVS chiral HPLC column (Agilent Technology), the eluent is 15:85 ethanol: 20 mM KH_2PO_4 at pH 3.5, and the observation wavelength is 240 nm. The ^1H NMR spectrum of Compound A is substantially as follows: δ($CDCl_3$); 1.47 (t, 3H); 2.26 (s, 3H); 2.87 (s, 3H); 3.68-3.75 (dd, 1H); 3.85 (s, 3H); 4.07-4.15 (q, 2H); 4.51-4.61 (dd, 1H); 5.84-5.90 (dd, 1H); 6.82-8.77 (m, 6H); 9.46 (s, 1H). The ^{13}C NMR spectrum of Compound A is substantially as follows: δ(DMSO-d_6); 14.66; 24.92; 41.61; 48.53; 54.46; 55.91; 64.51; 111.44; 112.40; 115.10; 118.20; 120.28; 124.94; 129.22; 131.02; 136.09; 137.60; 148.62; 149.74; 167.46; 169.14; 169.48. Compound A dissolved in methanol rotates plane polarized light in the (+) direction.

Without being limited by theory, Compound A is believed to be S-{2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione}, which has the following structure:



As used herein, the term "patient" refers to a mammal, particularly a human.

As used herein, the term "pharmaceutically acceptable salts" refer to salts prepared from pharmaceutically acceptable non-toxic acids or bases including inorganic acids and bases and organic acids and bases.

As used herein and unless otherwise indicated, the term "prodrug" means a derivative of a compound that can hydrolyze, oxidize, or otherwise react under biological conditions (in vitro or in vivo) to provide the compound. Examples of prodrugs include, but are not limited to, derivatives and metabolites of Compound A that include biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Prodrugs can typically be prepared using well-known methods, such as those described by 1 *Burger's Medicinal Chemistry and Drug Discovery,* 172-178, 949-982 (Manfred E. Wolff ed., 5th ed. 1995).

As used herein and unless otherwise indicated, the terms "biohydrolyzable amide," "biohydrolyzable ester," "biohydrolyzable carbamate," "biohydrolyzable carbonate," "biohydrolyzable ureide," "biohydrolyzable phosphate" mean an amide, ester, carbamate, carbonate, ureide, or phosphate, respectively, of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties in vivo, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted in vivo to the biologically active compound. Examples of biohydrolyzable esters include, but are not limited to, lower alkyl esters, alkoxyacyloxy esters, alkyl acylamino alkyl esters, and choline esters. Examples of biohydrolyzable amides include, but are not limited to, lower alkyl amides, α-amino acid amides, alkoxyacyl amides, and alkylaminoalkylcarbonyl amides. Examples of biohydrolyzable carbamates include, but are not limited to, lower alkylamines, substituted ethylenediamines, aminoacids, hydroxyalkylamines, heterocyclic and heteroaromatic amines, and polyether amines.

As used herein and unless otherwise indicated, the term "stereomerically pure" means a composition that comprises one stereoisomer of a compound and is substantially free of other stereoisomers of that compound. For example, a stereomerically pure composition of a compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically pure composition of a compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, more preferably greater than about 90% by weight of one stereoisomer of the compound and less than about 10% by weight of the other stereoisomers of the compound, even more preferably greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, and most preferably greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound.

As used herein and unless otherwise indicated, the term "enantiomerically pure" means a stereomerically pure composition of a compound having one chiral center.

As used herein, term "adverse effects" includes, but is not limited to gastrointestinal, renal and hepatic toxicities, leukopenia, increases in bleeding times due to, e.g., thrombocytopenia, and prolongation of gestation, nausea, vomiting, somnolence, asthenia, dizziness, teratogenicity, extra-pyramidal symptoms, akathisia, cardiotoxicity including cardiovascular disturbances, inflammation, male sexual dysfunction, and elevated serum liver enzyme levels. The term "gastrointestinal toxicities" includes but is not limited to gastric and intestinal ulcerations and erosions. The term "renal toxicities" includes but is not limited to such conditions as papillary necrosis and chronic interstitial nephritis.

As used herein and unless otherwise indicated, the phrases "reduce or avoid adverse effects" and "reducing or avoiding adverse effects" mean the reduction of the severity of one or more adverse effects as defined herein.

It should be noted that if there is a discrepancy between a depicted structure and a name given that structure, the depicted structure is to be accorded more weight. In addition, if the stereochemistry of a structure or a portion of a structure is not indicated with, for example, bold or dashed lines, the structure or portion of the structure is to be interpreted as encompassing all stereoisomers of it.

As used herein and unless otherwise specified, the terms "solid form" and related terms refer to a physical form which is not predominantly in a liquid or a gaseous state. As used herein and unless otherwise specified, the term "solid form" and related terms, when used herein to refer to Compound A, refer to a physical form comprising Compound A which is not predominantly in a liquid or a gaseous state. Solid forms may be crystalline, amorphous or mixtures thereof. In particular embodiments, solid forms may be liquid crystals. A "single-component" solid form comprising Compound A consists essentially of Compound A. A "multiple-component" solid form comprising Compound A comprises a significant quantity of one or more additional species, such as ions and/or molecules, within the solid form. For example, in particular embodiments, a crystalline multiple-component solid form comprising Compound A further comprises one or more species non-covalently bonded at regular positions in the crystal lattice. Multiple-component solid forms comprising Compound A include co-crystals, solvates (e.g., hydrates), and clathrates of Compound A. In particular embodiments, the term "solid form comprising Compound A" and related terms include single-component and multiple-component solid forms comprising Compound A. In particular embodiments, "solid forms comprising Compound A" and related terms include crystal forms comprising Compound A, amorphous forms comprising Compound A, and mixtures thereof.

As used herein and unless otherwise specified, the term "crystalline" and related terms used herein, when used to describe a compound, substance, modification, material,

component or product, unless otherwise specified, mean that the compound, substance, modification, material, component or product is substantially crystalline as determined by X-ray diffraction. See, e.g., *Remington: The Science and Practice of Pharmacy,* 21st edition, Lippincott, Williams and Wilkins, Baltimore, Md. (2005); *The United States Pharmacopeia,* 23rd ed., 1843-1844 (1995).

As used herein and unless otherwise specified, the term "crystal forms," "crystalline forms" and related terms herein refer to solid forms that are crystalline. Crystal forms include single-component crystal forms and multiple-component crystal forms, and include, but are not limited to, polymorphs, solvates, hydrates, and/or other molecular complexes. In certain embodiments, a crystal form of a substance may be substantially free of amorphous forms and/or other crystal forms. In certain embodiments, a crystal form of a substance may contain less than about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45% or 50% of one or more amorphous forms and/or other crystal forms on a weight basis. In certain embodiments, a crystal form of a substance may be physically and/or chemically pure. In certain embodiments, a crystal form of a substance may be about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91% or 90% physically and/or chemically pure.

As used herein and unless otherwise specified, the terms "polymorphs," "polymorphic forms" and related terms herein, refer to two or more crystal forms that consist essentially of the same molecule, molecules, and/or ions. Like different crystal forms, different polymorphs may have different physical properties such as, e.g., melting temperature, heat of fusion, solubility, dissolution properties and/or vibrational spectra, as a result of the arrangement or conformation of the molecules and/or ions in the crystal lattice. The differences in physical properties may affect pharmaceutical parameters such as storage stability, compressibility and density (important in formulation and product manufacturing), and dissolution rate (an important factor in bioavailability). Differences in stability can result from changes in chemical reactivity (e.g., differential oxidation, such that a dosage form discolors more rapidly when comprised of one polymorph than when comprised of another polymorph) or mechanical changes (e.g., tablets crumble on storage as a kinetically favored polymorph converts to thermodynamically more stable polymorph) or both (e.g., tablets of one polymorph are more susceptible to breakdown at high humidity). As a result of solubility/dissolution differences, in the extreme case, some solid-state transitions may result in lack of potency or, at the other extreme, toxicity. In addition, the physical properties may be important in processing (e.g., one polymorph might be more likely to form solvates or might be difficult to filter and wash free of impurities, and particle shape and size distribution might be different between polymorphs).

As used herein and unless otherwise specified, the terms "solvate" and "solvated," refer to a crystal form of a substance which contains solvent. The terms "hydrate" and "hydrated" refer to a solvate wherein the solvent comprises water. "Polymorphs of solvates" refers to the existence of more than one crystal form for a particular solvate composition. Similarly, "polymorphs of hydrates" refers to the existence of more than one crystal form for a particular hydrate composition. The term "desolvated solvate," as used herein, refers to a crystal form of a substance which may be prepared by removing the solvent from a solvate.

As used herein and unless otherwise specified, the term "amorphous," "amorphous form," and related terms used herein, mean that the substance, component or product in question is not substantially crystalline as determined by X-ray diffraction. In particular, the term "amorphous form" describes a disordered solid form, i.e., a solid form lacking long range crystalline order. In certain embodiments, an amorphous form of a substance may be substantially free of other amorphous forms and/or crystal forms. In other embodiments, an amorphous form of a substance may contain less than about 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45% or 50% of one or more other amorphous forms and/or crystal forms on a weight basis. In certain embodiments, an amorphous form of a substance may be physically and/or chemically pure. In certain embodiments, an amorphous form of a substance be about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91% or 90% physically and/or chemically pure.

Techniques for characterizing crystal forms and amorphous forms include, but are not limited to, thermal gravimetric analysis (TGA), differential scanning calorimetry (DSC), X-ray powder diffractometry (XRPD), single-crystal X-ray diffractometry, vibrational spectroscopy, e.g., infrared (IR) and Raman spectroscopy, solid-state and solution nuclear magnetic resonance (NMR) spectroscopy, optical microscopy, hot stage optical microscopy, scanning electron microscopy (SEM), electron crystallography and quantitative analysis, particle size analysis (PSA), surface area analysis, solubility measurements, dissolution measurements, elemental analysis and Karl Fischer analysis. Characteristic unit cell parameters may be determined using one or more techniques such as, but not limited to, X-ray diffraction and neutron diffraction, including single-crystal diffraction and powder diffraction. Techniques useful for analyzing powder diffraction data include profile refinement, such as Rietveld refinement, which may be used, e.g., to analyze diffraction peaks associated with a single phase in a sample comprising more than one solid phase. Other methods useful for analyzing powder diffraction data include unit cell indexing, which allows one of skill in the art to determine unit cell parameters from a sample comprising crystalline powder.

As used herein and unless otherwise specified, the terms "about" and "approximately," when used in connection with a numeric value or a range of values which is provided to characterize a particular solid form, e.g., a specific temperature or temperature range, such as, e.g., that describing a DSC or TGA thermal event, including, e.g., melting, dehydration, desolvation or glass transition events; a mass change, such as, e.g., a mass change as a function of temperature or humidity; a solvent or water content, in terms of, e.g., mass or a percentage; or a peak position, such as, e.g., in analysis by IR or Raman spectroscopy or XRPD; indicate that the value or range of values may deviate to an extent deemed reasonable to one of ordinary skill in the art while still describing the particular solid form. For example, in particular embodiments, the terms "about" and "approximately," when used in this context and unless otherwise specified, indicate that the numeric value or range of values may vary within 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1.5%, 1%, 0.5%, or 0.25% of the recited value or range of values.

As used herein and unless otherwise specified, a sample comprising a particular crystal form or amorphous form that is "substantially pure," e.g., substantially free of other solid forms and/or of other chemical compounds, contains, in particular embodiments, less than about 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.75%, 0.5%, 0.25% or 0.1% percent by weight of one or more other solid forms and/or of other chemical compounds.

As used herein and unless otherwise specified, a sample or composition that is "substantially free" of one or more other solid forms and/or other chemical compounds means that the

composition contains, in particular embodiments, less than about 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.75%, 0.5%, 0.25% or 0.1% percent by weight of one or more other solid forms and/or other chemical compounds.

As used herein, and unless otherwise specified, the terms "treat," "treating" and "treatment" refer to the eradication or amelioration of a disease or disorder, or of one or more symptoms associated with the disease or disorder. In certain embodiments, the terms refer to minimizing the spread or worsening of the disease or disorder resulting from the administration of one or more prophylactic or therapeutic agents to a patient with such a disease or disorder. In some embodiments, the terms refer to the administration of a compound provided herein, with or without other additional active agent, after the onset of symptoms of the particular disease.

As used herein, and unless otherwise specified, the terms "prevent," "preventing" and "prevention" refer to the prevention of the onset, recurrence or spread of a disease or disorder, or of one or more symptoms thereof. In certain embodiments, the terms refer to the treatment with or administration of a compound provided herein, with or without other additional active compound, prior to the onset of symptoms, particularly to patients at risk of diseases or disorders provided herein. The terms encompass the inhibition or reduction of a symptom of the particular disease. Patients with familial history of a disease in particular are candidates for preventive regimens in certain embodiments. In addition, patients who have a history of recurring symptoms are also potential candidates for the prevention. In this regard, the term "prevention" may be interchangeably used with the term "prophylactic treatment."

As used herein, and unless otherwise specified, the terms "manage," "managing" and "management" refer to preventing or slowing the progression, spread or worsening of a disease or disorder, or of one or more symptoms thereof. Often, the beneficial effects that a patient derives from a prophylactic and/or therapeutic agent do not result in a cure of the disease or disorder. In this regard, the term "managing" encompasses treating a patient who had suffered from the particular disease in an attempt to prevent or minimize the recurrence of the disease.

As used herein, and unless otherwise specified, a "therapeutically effective amount" of a compound is an amount sufficient to provide a therapeutic benefit in the treatment or management of a disease or disorder, or to delay or minimize one or more symptoms associated with the disease or disorder. A therapeutically effective amount of a compound means an amount of therapeutic agent, alone or in combination with other therapies, which provides a therapeutic benefit in the treatment or management of the disease or disorder. The term "therapeutically effective amount" can encompass an amount that improves overall therapy, reduces or avoids symptoms or causes of disease or disorder, or enhances the therapeutic efficacy of another therapeutic agent.

As used herein, and unless otherwise specified, a "prophylactically effective amount" of a compound is an amount sufficient to prevent a disease or disorder, or prevent its recurrence. A prophylactically effective amount of a compound means an amount of therapeutic agent, alone or in combination with other agents, which provides a prophylactic benefit in the prevention of the disease. The term "prophylactically effective amount" can encompass an amount that improves overall prophylaxis or enhances the prophylactic efficacy of another prophylactic agent.

The term "composition" as used herein is intended to encompass a product comprising the specified ingredients (and in the specified amounts, if indicated), as well as any product which results, directly or indirectly, from combination of the specified ingredients in the specified amounts. By "pharmaceutically acceptable" it is meant that the diluent, excipient or carrier must be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

4. DETAILED DESCRIPTION OF THE INVENTION

This invention relates to stereomerically pure Compound A, which is the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, substantially free of its (−) enantiomer, as well as novel methods of using, and compositions comprising, stereomerically pure Compound A and/or solid forms comprising Compound A. For example, the present invention encompasses the in vitro and in vivo use of Compound A, and the incorporation of Compound A into pharmaceutical compositions and single unit dosage forms useful in the treatment and prevention of a variety of diseases and disorders. Diseases and disorders which are ameliorated by the reduction of levels of TNF-α or inhibition of PDE4 are well known in the art and are described herein. Specific methods of the invention reduce or avoid the adverse effects associated with compounds used as TNF-αinhibitor. Other specific methods of the invention reduce or avoid the adverse effects associated with use of racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

Specific methods of the invention include methods of treating or preventing diseases and disorders including, but not limited to, solid tumors, blood-borne tumors and inflammatory diseases.

Pharmaceutical and dosage forms of the invention, which comprise Compound A or a pharmaceutically acceptable polymorph, prodrug, clathrate, solvate or hydrate thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein) can be used in the methods of the invention.

Without being limited by theory, it is believed that Compound A, including solid forms comprising Compound A, can inhibit TNF-α production. Consequently, a first embodiment of the invention relates to a method of inhibiting TNF-α production which comprises contacting a cell exhibiting abnormal TNF-α production with an effective amount of stereomerically pure Compound A or a pharmaceutically acceptable prodrug, metabolite, polymorph, solvate, hydrate, or clathrate thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein). In a particular embodiment, the invention relates to a method of inhibiting TNF-α production which comprises contacting a mammalian cell exhibiting abnormal TNF-α production with an effective amount of stereomerically pure Compound A or a pharmaceutically acceptable prodrug, metabolite, polymorph, solvate, hydrate, or clathrate thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein).

The invention also relates to a method of treating, preventing or managing disorders ameliorated by the reduction of levels of TNF-α in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A or a pharmaceutically acceptable prodrug, metabolite, polymorph, solvate, hydrate, or clathrate

thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein). In particular embodiments, diseases or disorders ameliorated by the inhibition of TNF-α production in mammals include, but are not limited to: HIV; hepatitis; adult respiratory distress syndrome; bone resorption diseases; chronic obstructive pulmonary diseases; chronic pulmonary inflammatory diseases; asthma; dermatitis; cystic fibrosis; septic shock; sepsis; endotoxic shock; hemodynamic shock; sepsis syndrome; post ischemic reperfusion injury; meningitis; psoriasis; fibrotic disease; cachexia; graft rejection; auto immune disease; rheumatoid spondylitis; arthritic conditions, such as psoriatic arthritis, rheumatoid arthritis and osteoarthritis; osteoporosis; Crohn's disease; ulcerative colitis; inflammatory bowel disease; multiple sclerosis; systemic lupus erythematosus; cutaneous lupus erythematosus; pulmonary sarcoidosis; erythema nodosum leprosum (ENL) in leprosy; radiation damage; asthma; and hyperoxic alveolar injury. Such disorders further include, but are not limited to, cancers, including, but not limited to cancer of the head, thyroid, neck, eye, skin, mouth, throat, esophagus, chest, bone, blood, bone marrow, lung, colon, sigmoid, rectum, stomach, prostate, breast, ovaries, kidney, liver, pancreas, brain, intestine, heart, adrenal, subcutaneous tissue, lymph nodes, heart, and combinations thereof. Specific cancers that can be treated by this method are multiple myeloma, malignant melanoma, malignant glioma, leukemia and solid tumors.

A further embodiment of the invention relates to a method of treating or preventing cancer, including but not limited to, solid tumor, blood-borne tumor, leukemias, and in particular, multiple myeloma in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically effective amount of stereomerically pure Compound A or a pharmaceutically acceptable prodrug, metabolite, polymorph, solvate, hydrate, or clathrate thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein); in particular wherein the patient is a mammal.

In another embodiment, the invention relates to a method of inhibiting PDE4 which comprises contacting PDE4 in a cell (e.g. a mammalian cell) with an effective amount of stereomerically pure Compound A or a pharmaceutically acceptable prodrug, metabolite, polymorph, solvate, hydrate, or clathrate thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein).

A further embodiment of the invention relates to a method of treating or preventing diseases or disorders ameliorated by the inhibition of PDE4 in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A or a pharmaceutically acceptable prodrug, metabolite, polymorph, solvate, hydrate, or clathrate thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein). Disorders ameliorated by the inhibition of PDE4 include, but are not limited to, asthma, inflammation (e.g., inflammation due to reperfusion), chronic or acute obstructive pulmonary diseases, chronic or acute pulmonary inflammatory diseases, inflammatory bowel disease, Crohn's Disease, Behcet's Disease, or colitis.

In another embodiment, the invention relates to a method of controlling cAMP levels in a cell which comprises contacting a cell with an effective amount of stereomerically pure Compound A or a pharmaceutically acceptable prodrug, metabolite, polymorph, solvate, hydrate, or clathrate thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein). As used herein the term "controlling cAMP levels" includes preventing or reducing the rate of the breakdown of Adenosine 3',5'-cyclic monophosphate (cAMP) in a cell or increasing the amount of Adenosine 3',5'-cyclic monophosphate present in a cell, preferably a mammalian cell, more preferably a human cell. In a particular method, the rate of cAMP breakdown is reduced by about 10, 25, 50, 100, 200, or 500 percent as compared to the rate in comparable cells which have not been contacted with a compound of the invention.

A further embodiment of the invention relates to a method of treating or preventing depression, asthma, inflammation (e.g., contact dermatitis, atopic dermatitis, psoriasis, rheumatoid arthritis, osteoarthritis, inflammatory skin disease, inflammation due to reperfusion), chronic or acute obstructive pulmonary diseases, chronic or pulmonary inflammatory diseases, inflammatory bowel disease, Crohn's Disease, Behcet's Disease or colitis in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A or a pharmaceutically acceptable prodrug, metabolite, polymorph, solvate, hydrate, or clathrate thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein); in particular wherein the patient is a mammal.

A separate embodiment of the invention encompasses methods of treating or preventing myelodysplastic syndrome (MDS) which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A or a pharmaceutically acceptable solvate, hydrate, stereoisomer, clathrate, or prodrug thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein). MDS refers to a diverse group of hematopoietic stem cell disorders. MDS is characterized by a cellular marrow with impaired morphology and maturation (dysmyelopoiesis), peripheral blood cytopenias, and a variable risk of progression to acute leukemia, resulting from ineffective blood cell production. See *The Merck Manual* 953 (17th ed. 1999) and List et al., 1990, *J. Clin. Oncol.* 8:1424.

A separate embodiment of the invention encompasses methods of treating or preventing myeloproliferative disease (MPD) which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A or a pharmaceutically acceptable solvate, hydrate, stereoisomer, clathrate, or prodrug thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein). Myeloproliferative disease (MPD) refers to a group of disorders characterized by clonal abnormalities of the hematopoietic stem cell. See e.g., *Current Medical Diagnosis & Treatment*, pp. 499 (37th ed., Tierney et al., ed., Appleton & Lange, 1998).

The invention also encompasses a method of treating, preventing or managing pain, including, but not limited to, complex regional pain syndrome, which comprises administering to a patient in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of a stereomerically pure Compound A or a pharmaceutically acceptable solvate, hydrate, stereoisomer, clathrate, or prodrug thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein). In a specific embodiment, the administration is before, during or after surgery or physical therapy directed at reducing or avoiding a symptom of complex regional pain syndrome in the patient.

In particular methods of the invention, stereomerically pure Compound A or a pharmaceutically acceptable polymorph, prodrug, solvate, hydrate, or clathrate thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein), is adjunctively administered with at least one additional therapeutic agent. Examples of additional therapeutic agents include, but are not limited to, anti-cancer drugs, anti-inflammatories, antihistamines and decongestants.

4.1. SOLID FORMS COMPRISING COMPOUND A

Certain embodiments herein provide solid forms comprising Compound A, which has the chemical structure shown above. Racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is readily prepared using the methods in U.S. Pat. No. 6,020,358, which is incorporated herein by reference. Compound A, which is the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, can be prepared according to any method apparent to those of skill in the art, including the methods described in U.S. Pat. No. 6,962,940, which is incorporated herein by reference.

Solid forms comprising Compound A include single-component and multiple-component forms, including crystal forms and amorphous forms, and including, but not limited to, polymorphs, solvates, hydrates, co-crystals and clathrates. Particular embodiments herein provide single-component amorphous solid forms of Compound A. Particular embodiments herein provide single-component crystalline solid forms of Compound A. Particular embodiments herein provide multiple-component amorphous forms comprising Compound A. Particular embodiments herein provide multiple-component crystalline solid forms comprising Compound A. Multiple-component solid forms provided herein include solid forms which may be described by the terms salt, co-crystal, hydrate, solvate, clathrate and/or polymorph, and include solid forms which may be described by one or more of these terms.

Solid forms comprising Compound A can be prepared by the methods described herein, including the methods described in the Examples below, or by techniques known in the art, including heating, cooling, freeze drying, lyophilization, quench cooling the melt, rapid solvent evaporation, slow solvent evaporation, solvent recrystallization, antisolvent addition, slurry recrystallization, crystallization from the melt, desolvation, recrystallization in confined spaces such as, e.g., in nanopores or capillaries, recrystallization on surfaces or templates such as, e.g., on polymers, recrystallization in the presence of additives, such as, e.g., co-crystal counter-molecules, desolvation, dehydration, rapid cooling, slow cooling, exposure to solvent and/or water, drying, including, e.g., vacuum drying, vapor diffusion, sublimation, grinding (including, e.g., cryo-grinding, solvent-drop grinding or liquid assisted grinding), microwave-induced precipitation, sonication-induced precipitation, laser-induced precipitation and precipitation from a supercritical fluid. The particle size of the resulting solid forms, which can vary, (e.g., from nanometer dimensions to millimeter dimensions), can be controlled, e.g., by varying crystallization conditions, such as, e.g., the rate of crystallization and/or the crystallization solvent system, or by particle-size reduction techniques, e.g., grinding, milling, micronizing or sonication.

While not intending to be bound by any particular theory, certain solid forms are characterized by physical properties, e.g., stability, solubility and dissolution rate, appropriate for pharmaceutical and therapeutic dosage forms. Moreover, while not wishing to be bound by any particular theory, certain solid forms are characterized by physical properties (e.g., density, compressibility, hardness, morphology, cleavage, stickiness, solubility, water uptake, electrical properties, thermal behavior, solid-state reactivity, physical stability, and chemical stability) affecting particular processes (e.g., yield, filtration, washing, drying, milling, mixing, tableting, flowability, dissolution, formulation, and lyophilization) which make certain solid forms suitable for the manufacture of a solid dosage form. Such properties can be determined using particular analytical chemical techniques, including solid-state analytical techniques (e.g., X-ray diffraction, microscopy, spectroscopy and thermal analysis), as described herein and known in the art.

Certain embodiments herein provide compositions comprising one or more of the solid forms. Certain embodiments provide compositions of one or more solid forms in combination with other active ingredients. Certain embodiments provide methods of using these compositions in the treatment, prevention or management of diseases and disorders including, but not limited to, the diseases and disorders provided herein.

In addition to solid forms comprising Compound A, provided herein are solid forms comprising prodrugs of Compound A.

Solid forms provided herein may also comprise unnatural proportions of atomic isotopes at one or more of the atoms in Compound A. For example, the compound may be radiolabeled with radioactive isotopes, such as for example tritium (^{3}H), iodine-125 (^{125}I) sulfur-35 (^{35}S), or carbon-14 (^{14}C). Radiolabeled compounds are useful as therapeutic agents, e.g., cancer therapeutic agents, research reagents, e.g., binding assay reagents, and diagnostic agents, e.g., in vivo imaging agents. All isotopic variations of Compound A, whether radioactive or not, are intended to be encompassed within the scope of the embodiments provided herein.

4.1.1. Form A of Compound A

Certain embodiments herein provide the Form A crystal form of Compound A. In certain embodiments, Form A of Compound A can be obtained from various solvents, including, but not limited to, solvent systems comprising acetone, ethanol, and mixtures thereof. In certain embodiments, Form A can be obtained using a fast cooling crystallization process.

In certain embodiments, Form A of Compound A may be characterized by X-ray powder diffraction analysis. A representative XRPD pattern of Form A of Compound A is provided in FIG. **1**. In certain embodiments, Form A of Compound A is characterized by XRPD peaks located at one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve of the following approximate positions: 8.1, 14.4, 15.2, 17.4, 18.4, 19.2, 20.5, 22.8, 23.2 23.6, 24.5, 25.1 degrees 2θ. In certain embodiments, Form A of Compound A is characterized by an XRPD pattern which matches the pattern exhibited in FIG. **1**. In certain embodiments, Form A of Compound A is characterized by an XRPD pattern having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 peaks matching peaks in the representative Form A pattern provided herein.

In certain embodiments, Form A of Compound A may be characterized by thermal analysis. A representative DSC plot for Form A of Compound A is shown in FIG. **2**. In certain embodiments, Form A is characterized by a DSC plot comprising an endothermic event with an onset temperature of about 145° C. In certain embodiments, Form A is characterized by a DSC plot further comprising an endothermic event

with an onset temperature of about 155° C. A representative TGA plot for Form A of Compound A is shown in FIG. **3**. In certain embodiments, Form A is characterized by a TGA plot comprising a mass loss of less than about 1%, e.g., about 0.05%, of the total mass of the sample upon heating from about 25° C. to about 140° C. In certain embodiments, Form A of Compound A does not contain substantial amounts of either water or other solvent in the crystal lattice. In certain embodiments, Form A is unsolvated. In certain embodiments, Form A is anhydrous.

In certain embodiments, Form A of Compound A may be characterized by moisture sorption analysis. A representative moisture sorption isotherm plot is shown in FIG. **4**. In certain embodiments, when the relative humidity ("RH") is increased from about 0% to about 95% RH, Form A exhibits a mass change of less than about 1%, e.g., about 0.4%, of the starting mass of the sample. In certain embodiments, mass gained upon adsorption is lost when the RH is decreased back to about 0% RH. Accordingly, in certain embodiments, Form A is substantially nonhygroscopic. In certain embodiments, the XRPD pattern of the Form A material is substantially unchanged following the adsorption/desorption analysis. In certain embodiments, Form A is stable with respect to humidity.

In certain embodiments, Form A of Compound A may be characterized by its stability profile. In certain embodiments, Form A material is stable, e.g., its XRPD pattern remains substantially unchanged, upon exposure to elevated temperature, upon exposure to elevated humidity, upon exposure to one or more solvents, and/or upon compression. In certain embodiments, for example, Form A is stable following exposure to an environment of about 40° C. and about 75% RH environment for about four weeks. In certain embodiments, Form A is stable following exposure to one or more solvent systems comprising, e.g., ethanol, water and/or heptane, at about 40° C. for at least about four weeks. In certain embodiments, Form A converts to Form C of Compound A upon exposure to a solvent including, but not limited to, toluene for four weeks. In certain embodiments, Form A is stable upon compression at about 2000 psi pressure for about one minute.

In certain embodiments, Form A of Compound A may be characterized by particle analysis. In certain embodiments, Form A is characterized as a white powder. In certain embodiments, a sample of Form A comprises particles having a plate-like morphology. In certain embodiments, a sample of Form A comprises particles with a D_{90} of less than about 18 μm. (As used herein, the D_{90} value represents the 90th percentile of the particle size distribution as measured by length; i.e., 90% of the particles have a length of this value or less).

Certain embodiments herein provide Form A of Compound A which is substantially pure. Certain embodiments herein provide Form A of Compound A which is substantially free of other solid forms comprising Compound A including, e.g., Forms B, C, D, E, F, G and/or an amorphous solid form comprising Compound A as provided herein. Certain embodiments herein provide Form A as a mixture of solid forms comprising Compound A, including, e.g., a mixture comprising one or more of the following: Forms B, C, D, E, F, G and an amorphous solid form comprising Compound A as provided herein.

4.1.2. Form B of Compound A

Certain embodiments herein provide the Form B crystal form of Compound A. In certain embodiments, Form B of Compound A can be obtained from various solvents, including, but not limited to, solvent systems comprising 2-propanol, acetone, acetonitrile, ethanol, ethyl acetate, heptane, methanol, methyl ethyl ketone, methyl t-butyl ether, methylene chloride, n-butanol, n-butyl acetate, tetrahydrofuran, toluene, water and mixtures comprising two or more thereof. For example, in certain embodiments, Form B can be obtained by crystallization from a solvent system comprising 1:1 ethanol:water, e.g., by a process comprising evaporation of the 1:1 ethanol:water solvent system at about 25° C., followed by isolation of Form B. For example, in certain embodiments, Form B can be obtained by crystallization from a solvent system comprising 1:1 acetone:ethanol, e.g., by a process comprising slurrying a solid form comprising Compound A in 1:1 acetone:ethanol at about 25° C. for about 2 days, followed by isolation of Form B.

In certain embodiments, Form B of Compound A may be characterized by X-ray powder diffraction analysis. A representative XRPD pattern of Form B of Compound A is provided in FIG. **5**. In certain embodiments, Form B of Compound A is characterized by XRPD peaks located at one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve of the following approximate positions: 10.1, 12.4, 13.5, 15.7, 16.3, 18.1, 20.7, 22.5, 24.7, 26.2, 26.9, 29.1 degrees 2θ. In certain embodiments, Form B of Compound A is characterized by an XRPD pattern which matches the pattern exhibited in FIG. **5**. In certain embodiments, Form B of Compound A is characterized by an XRPD pattern having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 peaks matching peaks in the representative Form B pattern provided herein.

In certain embodiments, Form B of Compound A may be characterized by thermal analysis. A representative DSC plot for Form B of Compound A is shown in FIG. **6**. In certain embodiments, Form B is characterized by a DSC plot comprising an endothermic event with an onset temperature of about 154° C. A representative TGA plot for Form B of Compound A is shown in FIG. **7**. In certain embodiments, Form B is characterized by a TGA plot comprising a mass loss of less than about 1%, e.g., about 0.25%, of the total mass of the sample upon heating from about 25° C. to about 140° C. In certain embodiments, Form B of Compound A does not contain substantial amounts of either water or other solvent in the crystal lattice. In certain embodiments, Form B is anhydrous. In certain embodiments, Form B is unsolvated.

In certain embodiments, Form B of Compound A may be characterized by moisture sorption analysis. A representative moisture sorption isotherm plot is shown in FIG. **8**. In certain embodiments, when the RH is increased from about 0% to about 95% RH, Form B exhibits a mass change of less than about 1%, e.g., about 0.6%, of the starting mass of the sample. In certain embodiments, mass gained upon adsorption is lost when the RH is decreased back to about 0% RH. In certain embodiments, Form B is substantially nonhygroscopic. In certain embodiments, the XRPD pattern of Form B material is substantially unchanged following the adsorption/desorption analysis. In certain embodiments, Form B is stable with respect to humidity.

In certain embodiments, Form B of Compound A may be characterized by its stability profile. In certain embodiments, Form B material is stable, e.g., its XRPD pattern remains substantially unchanged, upon exposure to elevated temperature, upon exposure to elevated humidity, upon exposure to one or more solvents, and/or upon compression. In certain embodiments, for example, Form B is stable following exposure to an environment of about 40° C. and about 75% RH environment for about four weeks. In certain embodiments, Form B is stable following exposure to a solvent system comprising, e.g., ethanol, water or heptane, at about 40° C. for at least about four weeks. In certain embodiments, Form B converts to Form C of Compound A upon exposure to a

solvent system comprising, e.g., toluene for about four weeks. In certain embodiments, Form B is stable following compression at about 2000 psi pressure for about one minute.

In certain embodiments, Form B of Compound A may be characterized by particle analysis. In certain embodiments, Form B is characterized as a white powder. In certain embodiments, a sample of Form B comprises particles having a flake-like morphology. In certain embodiments, a sample of Form B comprises particles with a D_{90} of less than about 12 μm.

Certain embodiments herein provide Form B of Compound A which is substantially pure. Certain embodiments herein provide Form B of Compound A which is substantially free of other solid forms comprising Compound A including, e.g., Forms A, C, D, E, F, G and/or an amorphous solid form comprising Compound A as provided herein. Certain embodiments herein provide Form B as a mixture of solid forms comprising Compound A, including, e.g., a mixture comprising one or more of the following: Forms A, C, D, E, F, G and an amorphous solid form comprising Compound A as provided herein.

4.1.3. Form C of Compound A

Certain embodiments herein provide the Form C crystal form of Compound A. In certain embodiments, Form C of Compound A can be obtained from various solvent systems, including, but not limited to, solvent systems comprising acetone, acetonitrile, ethanol, heptane, methanol, methyl ethyl ketone, tetrahydrofuran, toluene, water, and mixtures comprising two or more thereof. For example, in certain embodiments, Form C can be obtained by crystallization from a solvent system comprising toluene, e.g., by a process comprising the use of toluene as an anti-solvent, followed by isolation of Form C.

In certain embodiments, Form C of Compound A may be characterized by X-ray powder diffraction analysis. A representative XRPD pattern of Form C of Compound A is provided in FIG. **9**. In certain embodiments, Form C of Compound A is characterized by XRPD peaks located at one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve of the following approximate positions: 7.5, 11.3, 15.3, 16.4, 17.8, 21.4, 22.6, 23.5, 24.8, 25.5, 26.4, 27.6 degrees 2θ. In certain embodiments, Form C of Compound A is characterized by an XRPD pattern which matches the pattern exhibited in FIG. **9**. In certain embodiments, Form C of Compound A is characterized by an XRPD pattern having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 peaks matching peaks in the representative Form C pattern provided herein.

In certain embodiments, Form C of Compound A may be characterized by thermal analysis. A representative DSC plot for Form C of Compound A is shown in FIG. **10**. In certain embodiments, Form C is characterized by a DSC plot comprising an endothermic event with an onset temperature of about 138° C. In certain embodiments, a characteristic Form C DSC plot further comprises one or more additional events, such as, e.g., an endothermic event with an onset temperature of about 166° C. A representative TGA plot for Form C of Compound A is shown in FIG. **11**. In certain embodiments, Form C is characterized by a TGA plot comprising a mass loss of less than about 10%, e.g., about 5.9%, of the total mass of the sample upon heating from about 25° C. to about 140° C. In certain embodiments, the TGA mass loss event comprises the loss of the solvent toluene, as indicated, e.g., by TG-IR analysis. In certain embodiments, Form C of Compound A is solvated. In certain embodiments, Form C is a toluene solvate. In certain embodiments, the crystal lattice of Form C comprises about three molar equivalents of toluene per mole of Compound A.

In certain embodiments, Form C of Compound A may be characterized by moisture sorption analysis. A representative moisture sorption isotherm plot is shown in FIG. **12**. In certain embodiments, when the RH is increased from about 0% to about 95% RH, Form C exhibits a mass change of less than about 1%, e.g., about 0.5%, of the starting mass of the sample. In certain embodiments, mass gained upon adsorption is lost when the RH is decreased back to about 0% RH. In certain embodiments, Form C is substantially nonhygroscopic. In certain embodiments, the XRPD pattern of Form C material is substantially unchanged following the adsorption/desorption analysis. In certain embodiments, Form C is stable with respect to humidity.

In certain embodiments, Form C of Compound A may be characterized by its stability profile. In certain embodiments, Form C material is stable, e.g., its XRPD pattern remains substantially unchanged, upon exposure to elevated temperature, upon exposure to elevated humidity, upon exposure to one or more solvents, and/or upon compression. In certain embodiments, for example, Form C is stable following exposure to an environment of about 40° C. and about 75% RH environment for about four weeks. In certain embodiments, Form C is stable following exposure to a solvent system comprising, e.g., ethanol, water, heptane or toluene, at about 40° C. for at least about four weeks. In certain embodiments, Form C is stable following compression at about 2000 psi pressure for about one minute.

In certain embodiments, Form C of Compound A may be characterized by particle analysis. In certain embodiments, Form C is characterized as a white powder. In certain embodiments, a sample of Form C comprises particles having a plate-like morphology. In certain embodiments, a sample of Form C comprises particles with a D_{90} of less than about 12 μm.

Certain embodiments herein provide Form C of Compound A which is substantially pure. Certain embodiments herein provide Form C of Compound A which is substantially free of other solid forms comprising Compound A including, e.g., Forms A, B, D, E, F, G and/or an amorphous solid form comprising Compound A as provided herein. Certain embodiments herein provide Form C as a mixture of solid forms comprising Compound A, including, e.g., a mixture comprising one or more of the following: Forms A, B, D, E, F, G and an amorphous solid form comprising Compound A as provided herein.

4.1.4. Form D of Compound A

Certain embodiments herein provide the Form D crystal form of Compound A. In certain embodiments, Form D of Compound A can be obtained from various solvents, including, but not limited to, solvent systems comprising methylene chloride. For example, in certain embodiments, Form D can be obtained by crystallization from a solvent system comprising methylene chloride, e.g., by a process comprising the evaporation of methylene chloride, followed by isolation of Form D.

In certain embodiments, Form D of Compound A may be characterized by X-ray powder diffraction analysis. A representative XRPD pattern of Form D of Compound A is provided in FIG. **13**. In certain embodiments, Form D of Compound A is characterized by XRPD peaks located at one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve of the following approximate positions: 7.5, 9.6, 11.3, 13.9, 16.3, 17.7, 20.5, 23.2, 24.6, 25.2, 26.0, 28.8 degrees 2θ. In certain embodiments, Form D of Compound A is character-

ized by an XRPD pattern which matches the pattern exhibited in FIG. **13**. In certain embodiments, Form D of Compound A is characterized by an XRPD pattern having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 peaks matching peaks in the representative Form D pattern provided herein.

In certain embodiments, Form D of Compound A may be characterized by thermal analysis. A representative DSC plot for Form D of Compound A is shown in FIG. **14**. In certain embodiments, Form D is characterized by a DSC plot comprising an endothermic event with an onset temperature of about 100° C. A representative TGA plot for Form D of Compound A is shown in FIG. **15**. In certain embodiments, Form D is characterized by a TGA plot comprising a mass loss of less than about 10%, e.g., about 6.5%, of the total mass of the sample upon heating from about 25° C. to about 110° C. In certain embodiments, the TGA mass loss event comprises the loss of the solvent methylene chloride (i.e. dichloromethane), as indicated, e.g., by TG-IR analysis. In certain embodiments, Form D of Compound A is solvated. In certain embodiments, Form D is a methylene chloride solvate. In certain embodiments, the crystal lattice of Form D comprises about 2.5 molar equivalents of methylene chloride per mole of Compound A.

In certain embodiments, Form D of Compound A may be characterized by moisture sorption analysis. A representative moisture sorption isotherm plot is shown in FIG. **16**. In certain embodiments, when the RH is increased from about 0% to about 95% RH, Form D exhibits a mass change of less than about 3%, e.g., about 1.5%, of the starting mass of the sample. In certain embodiments, mass gained upon adsorption is lost when the RH is decreased back to about 0% RH. Accordingly, in certain embodiments, Form D is slightly hygroscopic. In certain embodiments, the XRPD pattern of Form D material is substantially unchanged following the adsorption/desorption analysis. In certain embodiments, Form D is stable with respect to humidity.

In certain embodiments, Form D of Compound A may be characterized by its stability profile. In certain embodiments, Form D material is stable, e.g., its XRPD pattern remains substantially unchanged, upon compression. For example, in certain embodiments, Form D is stable following compression at about 2000 psi pressure for about one minute. In certain embodiments, Form D is stable following exposure to an environment of about 40° C. and about 75% RH environment for about four weeks, although, in certain embodiments, the resulting peak intensity of the Form D XRPD pattern is reduced. In certain embodiments, this reduction in XRPD peak intensity results from the formation of amorphous material comprising Compound A. In certain embodiments, Form D converts to Form B of Compound A upon exposure to a solvent system comprising, e.g., heptane, ethanol and/or water at about 40° C. for about four weeks. In certain embodiments, Form D converts to Form C of Compound A upon exposure to a solvent system comprising toluene at about 40° C. for about four weeks.

In certain embodiments, Form D of Compound A may be characterized by particle analysis. In certain embodiments, Form D is characterized as a white powder. In certain embodiments, a sample of Form D comprises particles having a flake-like morphology. In certain embodiments, a sample of Form D comprises particles with a D_{90} of less than about 18 μm.

Certain embodiments herein provide Form D of Compound A which is substantially pure. Certain embodiments herein provide Form D of Compound A which is substantially free of other solid forms comprising Compound A including, e.g., Forms A, B, C, E, F, G and/or an amorphous solid form comprising Compound A as provided herein. Certain embodiments herein provide Form D as a mixture of solid forms comprising Compound A, including, e.g., a mixture comprising one or more of the following: Forms A, B, C, E, F, G and an amorphous solid form comprising Compound A as provided herein.

4.1.5. Form E of Compound A

Certain embodiments herein provide the Form E crystal form of Compound A. In certain embodiments, Form E of Compound A can be obtained from various solvents, including, but not limited to, solvent systems comprising acetone, acetonitrile, heptane, methylene chloride, and mixtures comprising two or more thereof. For example, in certain embodiments, Form E can be obtained by crystallization from a solvent system comprising acetonitrile, e.g., by a process comprising the evaporation of acetonitrile, followed by isolation of Form E.

In certain embodiments, Form E of Compound A may be characterized by X-ray powder diffraction analysis. A representative XRPD pattern of Form E of Compound A is provided in FIG. **17**. In certain embodiments, Form E of Compound A is characterized by XRPD peaks located at one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve of the following approximate positions: 7.6, 9.2, 11.4, 15.5, 16.5, 17.9, 19.6, 20.5, 21.6, 22.8, 23.8, 26.6 degrees 2θ. In certain embodiments, Form E of Compound A is characterized by an XRPD pattern which matches the pattern exhibited in FIG. **17**. In certain embodiments, Form E of Compound A is characterized by an XRPD pattern having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 peaks matching peaks in the representative Form E pattern provided herein.

In certain embodiments, Form E of Compound A may be characterized by thermal analysis. A representative DSC plot for Form E of Compound A is shown in FIG. **18**. In certain embodiments, Form E is characterized by a DSC plot comprising an endothermic event with an onset temperature of about 95° C. A representative TGA plot for Form E of Compound A is shown in FIG. **19**. In certain embodiments, Form E is characterized by a TGA plot comprising a mass loss of less than about 8%, e.g., about 4.0%, of the total mass of the sample upon heating from about 25° C. to about 110° C. In certain embodiments, the TGA mass loss event comprises the loss of the solvent acetonitrile, as indicated, e.g., by TG-IR analysis. In certain embodiments, Form E of Compound A is solvated. In certain embodiments, Form E is an acetonitrile solvate. In certain embodiments, the crystal lattice of Form E comprises about 2.5 molar equivalents of acetonitrile per mole of Compound A.

In certain embodiments, Form E of Compound A may be characterized by moisture sorption analysis. A representative moisture sorption isotherm plot is shown in FIG. **20**. In certain embodiments, when the RH is increased from about 0% to about 95% RH, Form E exhibits a mass change of less than about 10%, e.g., about 5.1%, of the starting mass of the sample. In certain embodiments, mass gained upon adsorption is lost when the RH is decreased back to about 0% RH. In certain embodiments, Form E is hygroscopic. In certain embodiments, the XRPD pattern of Form E material is substantially unchanged following the adsorption/desorption analysis. In certain embodiments, Form E is stable with respect to humidity.

In certain embodiments, Form E of Compound A may be characterized by its stability profile. In certain embodiments, Form E material is stable, e.g., its XRPD pattern remains substantially unchanged, upon compression. For example, in

certain embodiments, Form E is stable following compression at about 2000 psi pressure for about one minute.

In certain embodiments, Form E of Compound A may be characterized by particle analysis. In certain embodiments, Form E is characterized as a white powder. In certain embodiments, a sample of Form E comprises particles having a flake-like morphology. In certain embodiments, a sample of Form E comprises particles with a D_{90} of less than about 18 μm.

Certain embodiments herein provide Form E of Compound A which is substantially pure. Certain embodiments herein provide Form E of Compound A which is substantially free of other solid forms comprising Compound A including, e.g., Forms A, B, C, D, F, G and/or an amorphous solid form comprising Compound A as provided herein. Certain embodiments herein provide Form E as a mixture of solid forms comprising Compound A, including, e.g., a mixture comprising one or more of the following: Forms A, B, C, D, F, G and an amorphous solid form comprising Compound A as provided herein.

4.1.6. Form F of Compound A

Certain embodiments herein provide the Form F crystal form of Compound A. In certain embodiments, Form F of Compound A can be obtained from various solvents, including, but not limited to, solvent systems comprising acetone, ethanol, water, and mixtures comprising two or more thereof. For example, in certain embodiments, Form F can be obtained by crystallization from a solvent system comprising ethanol and/or water, e.g., by a process comprising contacting a solid form comprising Compound A with a solvent system comprising ethanol and/or water, followed by isolation of Form F.

In certain embodiments, Form F of Compound A may be characterized by X-ray powder diffraction analysis. A representative XRPD pattern of Form F of Compound A is provided in FIG. **21**. In certain embodiments, Form F of Compound A is characterized by XRPD peaks located at one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve of the following approximate positions: 8.1, 8.6, 15.6, 17.3, 19.3, 21.4, 22.8, 24.6, 25.4, 25.9, 26.6, 27.7 degrees 2θ. In certain embodiments, Form F of Compound A is characterized by an XRPD pattern which matches the pattern exhibited in FIG. **21**. In certain embodiments, Form F of Compound A is characterized by an XRPD pattern having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 peaks matching peaks in the representative Form F pattern provided herein.

In certain embodiments, Form F of Compound A may be characterized by thermal analysis. A representative DSC plot for Form F of Compound A is shown in FIG. **22**. In certain embodiments, Form F is characterized by a DSC plot comprising an endothermic event with an onset temperature of about 145° C. A representative TGA plot for Form F of Compound A is shown in FIG. **23**. In certain embodiments, Form F is characterized by a TGA plot comprising a mass loss of less than about 1%, e.g., about 0.1%, of the total mass of the sample upon heating from about 25° C. to about 180° C. In certain embodiments, Form F of Compound A does not contain substantial amounts of either water or other solvent in the crystal lattice. In certain embodiments, Form F is unsolvated. In certain embodiments, Form F is anhydrous.

In certain embodiments, Form F of Compound A may be characterized by moisture sorption analysis. A representative moisture sorption isotherm plot is shown in FIG. **24**. In certain embodiments, when the RH is increased from about 0% to about 95% RH, Form F exhibits a mass change of less than about 1%, e.g., about 0.2%, of the starting mass of the sample. In certain embodiments, mass gained upon adsorption is lost when the RH is decreased back to about 0% RH. In certain embodiments, Form F is substantially nonhygroscopic. In certain embodiments, the XRPD pattern of Form F material is substantially unchanged following the adsorption/desorption analysis. In certain embodiments, Form F is stable with respect to humidity.

In certain embodiments, Form F of Compound A may be characterized by its stability profile. In certain embodiments, Form F material is stable, e.g., its XRPD pattern remains substantially unchanged, upon compression. For example, in certain embodiments, Form F is stable following compression at about 2000 psi pressure for about one minute. In certain embodiments, Form F is stable following exposure to a solvent system comprising, e.g., ethanol, acetone or mixtures thereof, for about two days at about 25° C.

In certain embodiments, Form F of Compound A may be characterized by particle analysis. In certain embodiments, Form F is characterized as a white powder. In certain embodiments, a sample of Form F comprises particles having a flake-like morphology. In certain embodiments, a sample of Form F comprises particles with a D_{90} of less than about 18 μm.

Certain embodiments herein provide Form F of Compound A which is substantially pure. Certain embodiments herein provide Form F of Compound A which is substantially free of other solid forms comprising Compound A including, e.g., Forms A, B, C, D, E, G and/or an amorphous solid form comprising Compound A as provided herein. Certain embodiments herein provide Form F as a mixture of solid forms comprising Compound A, including, e.g., a mixture comprising one or more of the following: Forms A, B, C, D, E, G and an amorphous solid form comprising Compound A as provided herein.

4.1.7. Form G of Compound A

Certain embodiments herein provide the Form G crystal form of Compound A. In certain embodiments, Form G of Compound A can be obtained from various solvents, including, but not limited to, solvent systems comprising ethyl acetate. For example, in certain embodiments, Form G can be obtained by crystallization from a solvent system comprising ethyl acetate, e.g., by a process comprising contacting a solid form comprising Compound A with a solvent system comprising ethyl acetate, followed by isolation of Form G.

In certain embodiments, Form G of Compound A may be characterized by X-ray powder diffraction analysis. A representative XRPD pattern of Form G of Compound A is provided in FIG. **25**. In certain embodiments, Form G of Compound A is characterized by XRPD peaks located at one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve of the following approximate positions: 7.9, 9.5, 11.7, 15.7, 16.8, 18.1, 19.7, 21.8, 22.8, 25.1, 25.8, 26.7 degrees 2θ. In certain embodiments, Form G of Compound A is characterized by an XRPD pattern which matches the pattern exhibited in FIG. **25**. In certain embodiments, Form G of Compound A is characterized by an XRPD pattern having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 peaks matching peaks in the representative Form G pattern provided herein.

In certain embodiments, Form G of Compound A may be characterized by thermal analysis. A representative DSC plot for Form G of Compound A is shown in FIG. **26**. In certain embodiments, Form G is characterized by a DSC plot comprising an endothermic event with an onset temperature of about 109° C. A representative TGA plot for Form G of Compound A is shown in FIG. **27**. In certain embodiments, Form G is characterized by a TGA plot comprising a mass loss of less than about 8%, e.g., about 3.8%, of the total mass

of the sample upon heating from about 25° C. to about 110° C. In certain embodiments, the TGA mass loss event comprises the loss of the solvent ethyl acetate, as indicated, e.g., by TG-IR analysis. In certain embodiments, Form G of Compound A is solvated. In certain embodiments, Form G is an ethyl acetate solvate. In certain embodiments, the crystal lattice of Form G comprises about three molar equivalents of ethyl acetate per mole of Compound A.

In certain embodiments, Form G of Compound A may be characterized by moisture sorption analysis. A representative moisture sorption isotherm plot is shown in FIG. **28**. In certain embodiments, when the RH is increased from about 0% to about 95% RH, Form G exhibits a mass change of less than about 1%, e.g., about 0.4%, of the starting mass of the sample. In certain embodiments, mass gained upon adsorption is lost when the RH is decreased back to about 0% RH. In certain embodiments, Form G is substantially nonhygroscopic. In certain embodiments, the XRPD pattern of Form G material is substantially unchanged following the adsorption/desorption analysis. In certain embodiments, Form G is stable with respect to humidity.

In certain embodiments, Form G of Compound A may be characterized by its stability profile. In certain embodiments, Form G material is stable, e.g., its XRPD pattern remains substantially unchanged, upon compression. For example, in certain embodiments, Form F is stable following compression at about 2000 psi pressure for about one minute. In certain embodiments, Form G converts to Form B upon exposure to a solvent system comprising, e.g., ethanol, acetone or mixtures thereof, for about two days at about 25° C.

In certain embodiments, Form G of Compound A may be characterized by particle analysis. In certain embodiments, Form G is characterized as a white powder. In certain embodiments, a sample of Form G comprises particles having a flake-like morphology. In certain embodiments, a sample of Form G comprises particles with a D_{90} of less than about 18 μm.

Certain embodiments herein provide Form G of Compound A which is substantially pure. Certain embodiments herein provide Form G of Compound A which is substantially free of other solid forms comprising Compound A including, e.g., Forms A, B, C, D, E, F and/or an amorphous solid form comprising Compound A as provided herein. Certain embodiments herein provide Form G as a mixture of solid forms comprising Compound A, including, e.g., a mixture comprising one or more of the following: Forms A, B, C, D, E, F and an amorphous solid form comprising Compound A as provided herein.

4.2. METHODS OF TREATMENT

The invention encompasses methods of treating, preventing and managing diseases or disorders ameliorated by the reduction of levels of TNF-α in a patient which comprise administering to a patient in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of one or more solid forms comprising Compound A, such as, e.g., Form A of Compound A, Form B of Compound A, Form C of Compound A, Form D of Compound A, Form E of Compound A, Form F of Compound A, Form G of Compound A, or an amorphous form of Compound A, as provided herein.

Disorders ameliorated by the inhibition of TNF-α include, but are not limited to: heart disease, such as congestive heart failure, cardiomyopathy, pulmonary edema, endotoxin-mediated septic shock, acute viral myocarditis, cardiac allograft rejection, and myocardial infarction; solid tumors, including but not limited to, sarcoma, carcinomas, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, Kaposi's sarcoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma; and blood-borne tumors including but not limited to, acute lymphoblastic leukemia "ALL", acute lymphoblastic B-cell leukemia, acute lymphoblastic T-cell leukemia, acute myeloblastic leukemia "AML", acute promyelocytic leukemia "APL", acute monoblastic leukemia, acute erythroleukemic leukemia, acute megakaryoblastic leukemia, acute myelomonocytic leukemia, acute nonlymphocyctic leukemia, acute undifferentiated leukemia, chronic myelocytic leukemia "CML", chronic lymphocytic leukemia "CLL", hairy cell leukemia, multiple myeloma and acute and chronic leukemias, for example, lymphoblastic, myelogenous, lymphocytic, and myelocytic leukemias.

Specific methods of the invention further comprise the administration of an additional therapeutic agent (i.e., a therapeutic agent other than Compound A). Examples of additional therapeutic agents include, but are not limited to, anticancer drugs such as, but are not limited to: alkylating agents, nitrogen mustards, ethylenimines, methylmelamines, alkyl sulfonates, nitrosoureas, triazenes, folic acid analogs, pyrimidine analogs, purine analogs, vinca alkaloids, epipodophyllotoxins, antibiotics, topoisomerase inhibitors and anticancer vaccines.

Specific additional therapeutic agents include, but are not limited to: acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cisplatin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; dactinomycin; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; docetaxel; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; fluorocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; ilmofosine; interleukin II (including recombinant interleukin II, or

rIL2), interferon alfa-2a; interferon alfa-2b; interferon alfa-n1; interferon alfa-n3; interferon beta-I a; interferon gamma-I b; iproplatin; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedepa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; paclitaxel; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; rogletimide; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; triciribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin hydrochloride. Other anti-cancer drugs include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; antidorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-aminotriazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorlns; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl spiromustine; docetaxel; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence

derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

Embodiments herein further encompass a method of treating or preventing diseases or disorders ameliorated by the inhibition of PDE4 in a patient which comprise administering to a patient in need of such treatment or prevention one or more solid forms comprising Compound A. Disorders ameliorated by the inhibition of PDE4 include, but are not limited to, asthma, inflammation, chronic or acute obstructive pulmonary disease, chronic or acute pulmonary inflammatory disease, inflammatory bowel disease, Crohn's Disease, Behcet's Disease, colitis, ulcerative colitis and arthritis or inflammation due to reperfusion. In a preferred embodiment, the disease or disorder to be treated or prevented is chronic obstructive pulmonary disease.

Specific methods of the invention can comprise the administration of an additional therapeutic agent such as, but not limited to, anti-inflammatory drugs, antihistamines and decongestants. Examples of such additional therapeutic agents include, but are not limited to: antihistamines including, but not limited to, ethanolamines, ethylenediamines, piperazines, and phenothiazines; antiinflammatory drugs; NSAIDS, including, but not limited to, aspirin, salicylates, acetominophen, indomethacin, sulindac, etodolac, fenamates, tolmetin, ketorolac, diclofenac, ibuprofen, naproxen, fenoprofen, ketoprofen, flurbiprofen, oxaprozin, piroxicam, meloxicam, pyrazolon derivatives; and steroids including, but not limited to, cortical steroids and adrenocortical steroids.

Specific methods of the invention avoid or reduce drug-drug interactions and other adverse effects associated with agents used in the treatment of such disorders, including racemic substituted phenylethylsulfones. Without being limited by any theory, certain solid forms comprising Compound A may further provide an overall improved therapeutic effectiveness, or therapeutic index, over racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, including solid forms thereof.

As stated above, certain solid forms comprising Compound A may be used in the treatment or prevention of a wide range of diseases and conditions. The magnitude of a prophylactic or therapeutic dose of a particular active ingredient of the invention in the acute or chronic management of a disease or condition may vary with the nature and severity of the disease or condition and the route by which the active ingredient is administered. The dose, and perhaps the dose frequency, will also vary according to the age, body weight, and response of the individual patient. Suitable dosing regimens can be readily selected by those skilled in the art with due consideration of such factors. In general, the recommended daily dose range for the conditions described herein lie within the range of from about 1 mg to about 1,000 mg per day, given as a single once-a-day dose preferably as divided doses throughout a day. More specifically, the daily dose is administered twice daily in equally divided doses. Specifically, a daily dose range may be from about 5 mg to about 500 mg per day, more specifically, between about 10 mg and about 200 mg per day. Specifically, the daily dose may be administered in 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 50 mg, or 100 mg dosage forms. In managing the patient, the therapy should be initiated at a lower dose, perhaps about 1 mg to about 25 mg, and increased if necessary up to about 200 mg to about 1,000 mg per day as either a single dose or divided doses, depending on the patient's global response. Alternatively, the daily dose is from 0.01 mg/kg to 100 mg/kg.

It may be necessary to use dosages of the active ingredient outside the ranges disclosed herein in some cases, as will be apparent to those of ordinary skill in the art. Furthermore, it is noted that the clinician or treating physician will know how and when to interrupt, adjust, or terminate therapy in conjunction with individual patient response.

The phrases "therapeutically effective amount", "prophylactically effective amount" and "therapeutically or prophylactically effective amount," as used herein encompass the above described dosage amounts and dose frequency schedules. Different therapeutically effective amounts may be applicable for different diseases and conditions, as will be readily known by those of ordinary skill in the art. Similarly, amounts sufficient to treat or prevent such disorders, but insufficient to cause, or sufficient to reduce, adverse effects associated with racemic 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione are also encompassed by the above described dosage amounts and dose frequency schedules.

4.3. PHARMACEUTICAL COMPOSITIONS

Pharmaceutical compositions and single unit dosage forms comprising one or more solid forms comprising Compound A are provided herein. Also provided herein are methods for preparing pharmaceutical compositions and single unit dosage forms comprising one or more solid forms comprising Compound A. For example, in certain embodiments, individual dosage forms comprising a solid form provided herein or prepared using solid form provided herein may be suitable for oral, mucosal (including rectal, nasal, or vaginal), parenteral (including subcutaneous, intramuscular, bolus injection, intraarterial, or intravenous), sublingual, transdermal, buccal, or topical administration.

In certain embodiments, pharmaceutical compositions and dosage forms provided herein comprise one or more solid forms comprising Compound A. Certain embodiments herein provide pharmaceutical compositions and dosage forms comprising a solid form comprising Compound A, such as, e.g., Forms A, B, C, D, E, F, G or an amorphous solid form comprising Compound A as provided herein, wherein the solid form comprising Compound A substantially pure. Certain embodiments herein provide pharmaceutical compositions and dosage forms comprising a solid form comprising Compound A, such as, e.g., Forms A, B, C, D, E, F, G or an amorphous solid form comprising Compound A as provided

herein, which is substantially free of other solid forms comprising Compound A including, e.g., Forms A, B, C, D, E, F, G and/or an amorphous solid form comprising Compound A as provided herein. Certain embodiments herein provide pharmaceutical compositions and dosage forms comprising a mixture of solid forms comprising Compound A, including, e.g., a mixture comprising one or more of the following: Forms A, B, C, D, E, F and an amorphous solid form comprising Compound A as provided herein. Pharmaceutical compositions and dosage forms provided herein typically also comprise one or more pharmaceutically acceptable excipient, diluent or carrier.

A particular pharmaceutical composition encompassed by this embodiment comprises one or more solid forms comprising Compound A and at least one additional therapeutic agent. Examples of additional therapeutic agents include, but are not limited to: anti-cancer drugs and anti-inflammation therapies including, but not limited to, those provided herein.

Single unit dosage forms of the invention are suitable for oral, mucosal (e.g., nasal, sublingual, vaginal, buccal, or rectal), parenteral (e.g., subcutaneous, intravenous, bolus injection, intramuscular, or intraarterial), or transdermal administration to a patient. Examples of dosage forms include, but are not limited to: tablets; caplets; capsules, such as soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suppositories; ointments; cataplasms (poultices); pastes; powders; dressings; creams; plasters; solutions; patches; aerosols (e.g., nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a patient, including suspensions (e.g., aqueous or non-aqueous liquid suspensions, oil-in-water emulsions, or a water-in-oil liquid emulsions), solutions, and elixirs; liquid dosage forms suitable for parenteral administration to a patient; and sterile solids (e.g., crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms suitable for parenteral administration to a patient.

The composition, shape, and type of dosage forms of the invention will typically vary depending on their use. For example, a dosage form used in the acute treatment of inflammation or a related disorder may contain larger amounts of one or more of the active ingredients it comprises than a dosage form used in the chronic treatment of the same disease. Similarly, a parenteral dosage form may contain smaller amounts of one or more of the active ingredients it comprises than an oral dosage form used to treat the same disease or disorder. These and other ways in which specific dosage forms encompassed by this invention will vary from one another will be readily apparent to those skilled in the art. See, e.g., *Remington's Pharmaceutical Sciences,* 18th ed., Mack Publishing, Easton Pa. (1990).

Typical pharmaceutical compositions and dosage forms comprise one or more excipients. Suitable excipients are well known to those skilled in the art of pharmacy, and non-limiting examples of suitable excipients are provided herein. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a patient. For example, oral dosage forms such as tablets may contain excipients not suited for use in parenteral dosage forms. The suitability of a particular excipient may also depend on the specific active ingredients in the dosage form.

Lactose-free compositions of the invention can comprise excipients that are well known in the art and are listed, for example, in the U.S. Pharmocopia (USP) SP (XXI)/NF (XVI). In general, lactose-free compositions comprise an active ingredient, a binder/filler, and a lubricant in pharmaceutically compatible and pharmaceutically acceptable amounts. Preferred lactose-free dosage forms comprise an active ingredient, microcrystalline cellulose, pre-gelatinized starch, and magnesium stearate.

This invention further encompasses anhydrous pharmaceutical compositions and dosage forms comprising active ingredients, since water can facilitate the degradation of some compounds. For example, the addition of water (e.g., 5%) is widely accepted in the pharmaceutical arts as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. See, e.g., Jens T. Carstensen, *Drug Stability: Principles & Practice,* 2d. Ed., Marcel Dekker, NY, N.Y., 1995, pp. 379-80. In effect, water and heat accelerate the decomposition of some compounds. Thus, the effect of water on a formulation can be of great significance since moisture and/or humidity are commonly encountered during manufacture, handling, packaging, storage, shipment, and use of formulations.

Anhydrous pharmaceutical compositions and dosage forms of the invention can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms that comprise lactose and at least one active ingredient that comprises a primary or secondary amine are preferably anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected.

An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions are preferably packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples of suitable packaging include, but are not limited to, hermetically sealed foils, plastics, unit dose containers (e.g., vials), blister packs, and strip packs.

The invention further encompasses pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will decompose. Such compounds, which are referred to herein as "stabilizers," include, but are not limited to, antioxidants such as ascorbic acid, pH buffers, or salt buffers.

Like the amounts and types of excipients, the amounts and specific types of active ingredients in a dosage form may differ depending on factors such as, but not limited to, the route by which it is to be administered to patients. However, typical dosage forms provided herein lie within the range of from about 1 mg to about 1,000 mg per day, given as a single once-a-day dose in the morning but preferably as divided doses throughout the day. More specifically, the daily dose is administered twice daily in equally divided doses. Specifically, a daily dose range may be from about 5 mg to about 500 mg per day, more specifically, between about 10 mg and about 200 mg per day. In managing the patient, the therapy may be initiated at a lower dose, perhaps about 1 mg to about 25 mg, and increased if necessary up to about 200 mg to about 1,000 mg per day as either a single dose or divided doses, depending on the patient's global response.

4.3.1. Oral Dosage Forms

Pharmaceutical compositions of the invention that are suitable for oral administration can be presented as discrete dosage forms, such as, but are not limited to, tablets (e.g., chewable tablets), caplets, capsules, and liquids (e.g., flavored syrups). Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of

pharmacy well known to those skilled in the art. See generally *Remington's Pharmaceutical Sciences,* 18th ed., Mack Publishing, Easton Pa. (1990).

Typical oral dosage forms of the invention are prepared by combining the active ingredient(s) in an intimate admixture with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of preparation desired for administration. For example, excipients suitable for use in oral liquid or aerosol dosage forms include, but are not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents. Examples of excipients suitable for use in solid oral dosage forms (e.g., powders, tablets, capsules, and caplets) include, but are not limited to, starches, sugars, micro-crystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid excipients are employed. If desired, tablets can be coated by standard aqueous or nonaqueous techniques. Such dosage forms can be prepared by any of the methods of pharmacy. In general, pharmaceutical compositions and dosage forms are prepared by uniformly and intimately admixing the active ingredients with liquid carriers, finely divided solid carriers, or both, and then shaping the product into the desired presentation if necessary.

For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as powder or granules, optionally mixed with an excipient. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

Examples of excipients that can be used in oral dosage forms of the invention include, but are not limited to, binders, fillers, disintegrants, and lubricants. Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gelatin, natural and synthetic gums such as acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre-gelatinized starch, hydroxypropyl methyl cellulose, (e.g., Nos. 2208, 2906, 2910), microcrystalline cellulose, and mixtures thereof.

Examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pregelatinized starch, and mixtures thereof. The binder or filler in pharmaceutical compositions of the invention is typically present in from about 50 to about 99 weight percent of the pharmaceutical composition or dosage form.

Suitable forms of microcrystalline cellulose include, but are not limited to, the materials sold as AVICEL-PH-101™, AVICEL-PH-103™, AVICEL RC-581™, AVICEL-PH-105™ (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, Pa.), and mixtures thereof. A specific binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC-581 ™. Suitable anhydrous or low moisture excipients or additives include AVICEL-PH-103™ and Starch 1500 LM™.

Disintegrants are used in the compositions of the invention to provide tablets that disintegrate when exposed to an aqueous environment. Tablets that contain too much disintegrant may disintegrate in storage, while those that contain too little may not disintegrate at a desired rate or under the desired conditions. Thus, a sufficient amount of disintegrant that is neither too much nor too little to detrimentally alter the release of the active ingredients should be used to form solid oral dosage forms of the invention. The amount of disintegrant used varies based upon the type of formulation, and is readily discernible to those of ordinary skill in the art. Typical pharmaceutical compositions comprise from about 0.5 to about 15 weight percent of disintegrant, specifically from about 1 to about 5 weight percent of disintegrant.

Disintegrants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, agar-agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrilin potassium, sodium starch glycolate, potato or tapioca starch, pre-gelatinized starch, other starches, clays, other algins, other celluloses, gums, and mixtures thereof.

Lubricants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, and mixtures thereof. Additional lubricants include, for example, a syloid silica gel (AEROSIL 200™, manufactured by W.R. Grace Co. of Baltimore, Md.), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Plano, Tex.), CAB-O-SIL™ (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, Mass.), and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about one weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated.

4.3.2. Delayed Release Dosage Forms

Solid forms comprising Compound A as provided herein can be administered by controlled release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Pat. Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; and 4,008,719, 5,674,533, 5,059,595, 5,591,767, 5,120,548, 5,073,543, 5,639,476, 5,354,556, and 5,733,566, each of which is incorporated herein by reference. Such dosage forms can be used to provide slow or controlled-release of one or more active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the active ingredients of the invention. The invention thus encompasses single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled-release.

All controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition,

controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the drug, and can thus affect the occurrence of side (e.g., adverse) effects.

Most controlled-release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled-release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, temperature, enzymes, water, or other physiological conditions or compounds.

4.3.3. Parenteral Dosage Forms

Parenteral dosage forms can be administered to patients by various routes including, but not limited to, subcutaneous, intravenous (including bolus injection), intramuscular, and intraarterial. Because their administration typically bypasses patients' natural defenses against contaminants, parenteral dosage forms are preferably sterile or capable of being sterilized prior to administration to a patient. Examples of parenteral dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions.

Suitable vehicles that can be used to provide parenteral dosage forms of the invention are well known to those skilled in the art. Examples include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

Compounds that increase the solubility of one or more of the active ingredients disclosed herein can also be incorporated into the parenteral dosage forms of the invention.

4.3.4. Transdermal, Topical, and Mucosal Dosage Forms

Transdermal, topical, and mucosal dosage forms of the invention include, but are not limited to, ophthalmic solutions, sprays, aerosols, creams, lotions, ointments, gels, solutions, emulsions, suspensions, or other forms known to one of skill in the art. See, e.g., *Remington's Pharmaceutical Sciences,* 16th and 18th eds., Mack Publishing, Easton Pa. (1980 & 1990); and *Introduction to Pharmaceutical Dosage Forms,* 4th ed., Lea & Febiger, Philadelphia (1985). Dosage forms suitable for treating mucosal tissues within the oral cavity can be formulated as mouthwashes or as oral gels. Further, transdermal dosage forms include "reservoir type" or "matrix type" patches, which can be applied to the skin and worn for a specific period of time to permit the penetration of a desired amount of active ingredients.

Suitable excipients (e.g., carriers and diluents) and other materials that can be used to provide transdermal, topical, and mucosal dosage forms encompassed by this invention are well known to those skilled in the pharmaceutical arts, and depend on the particular tissue to which a given pharmaceutical composition or dosage form will be applied. With that fact in mind, typical excipients include, but are not limited to, water, acetone, ethanol, ethylene glycol, propylene glycol, butane-1,3-diol, isopropyl myristate, isopropyl palmitate, mineral oil, and mixtures thereof to form lotions, tinctures, creams, emulsions, gels or ointments, which are non-toxic and pharmaceutically acceptable. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well known in the art. See, e.g., *Remington's Pharmaceutical Sciences,* 16th and 18th eds., Mack Publishing, Easton Pa. (1980 & 1990).

Depending on the specific tissue to be treated, additional components may be used prior to, in conjunction with, or subsequent to treatment with active ingredients of the invention. For example, penetration enhancers can be used to assist in delivering the active ingredients to the tissue. Suitable penetration enhancers include, but are not limited to: acetone; various alcohols such as ethanol, oleyl, and tetrahydrofuryl; alkyl sulfoxides such as dimethyl sulfoxide; dimethyl acetamide; dimethyl formamide; polyethylene glycol; pyrrolidones such as polyvinylpyrrolidone; Kollidon grades (Povidone, Polyvidone); urea; and various water-soluble or insoluble sugar esters such as Tween 80™ (polysorbate 80) and Span 60™ (sorbitan monostearate).

The pH of a pharmaceutical composition or dosage form, or of the tissue to which the pharmaceutical composition or dosage form is applied, may also be adjusted to improve delivery of one or more active ingredients. Similarly, the polarity of a solvent carrier, its ionic strength, or tonicity can be adjusted to improve delivery. Compounds such as stearates can also be added to pharmaceutical compositions or dosage forms to advantageously alter the hydrophilicity or lipophilicity of one or more active ingredients so as to improve delivery. In this regard, stearates can serve as a lipid vehicle for the formulation, as an emulsifying agent or surfactant, and as a delivery-enhancing or penetration-enhancing agent. Different solid forms comprising the active ingredients can be used to further adjust the properties of the resulting composition.

4.3.5. Kits

This invention encompasses kits which, when used by the medical practitioner, can simplify the administration of appropriate amounts of active ingredients to a patient.

A typical kit of the invention comprises a unit dosage form of compound A, or a pharmaceutically acceptable solid form or prodrug thereof, and a unit dosage form of a second active ingredient. Examples of second active ingredients include, but are not limited to, those listed herein.

Kits of the invention can further comprise devices that are used to administer the active ingredient(s). Examples of such devices include, but are not limited to, syringes, drip bags, patches, and inhalers.

Kits of the invention can further comprise pharmaceutically acceptable vehicles that can be used to administer one or more active ingredients. For example, if an active ingredient is provided in a solid form that must be reconstituted for parenteral administration, the kit can comprise a sealed container of a suitable vehicle in which the active ingredient can be dissolved to form a particulate-free sterile solution that is suitable for parenteral administration. Examples of pharmaceutically acceptable vehicles include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

5. EXAMPLES

The present application incorporates by reference the entirety of U.S. Pat. No. 6,962,940 (issued Nov. 8, 2005), including the Examples provided therein.

5.1. Example 1

Synthesis of 2-[1-(3-Ethoxy-4-Methoxyphenyl)-2-Methylsulfonylethyl]-4-Acetylaminoisoindoline-1,3-Dione

A stirred solution of 1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethylamine (1.0 g, 3.7 mmol) and 3-acetamidophthalic anhydride (751 mg, 3.66 mmol) in acetic acid (20 mL) was heated at reflux for 15 h. The solvent was removed in vacuo to yield an oil. Chromatography of the resulting oil yielded the product as a yellow solid (1.0 g, 59% yield): mp, 144° C.; ^{1}H NMR ($CDCl_3$) δ: 1.47 (t, J=7.0 Hz, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.88 (s, 3H, CH_3), 3.75 (dd, J=4.4, 14.3 Hz, 1H, CH), 3.85 (s, 3H, CH3), 4.11 (q, J=7 Hz, 2H, CH_2), 5.87 (dd, J=4.3, 10.5 Hz, 1H, NCH), 6.82-6.86 (m, 1H, Ar), 7.09-7.11 (m, 2H, Ar), 7.47 (d, J=7 Hz, 1H, Ar), 7.64 (t, J=8 Hz, 1H, Ar), 8.74 (d, J=8 Hz, 1H, Ar), 9.49 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ: 14.61, 24.85, 41.54, 48.44, 54.34, 55.85, 64.43, 111.37, 112.34, 115.04, 118.11, 120.21, 124.85, 129.17, 130.96, 136.01, 137.52, 148.54, 149.65, 167.38, 169.09, 169.40; Anal Calc'd. for $C_{22}H_{24}NO_7S$: C, 57.38; H, 5.25; N, 6.08. Found: C, 57.31; H, 5.34; N, 5.83.

5.2. Example 2

Synthesis of $(+)_2$-[1-(3-Ethoxy-4-Methoxyphenyl)-2-Methylsulfonylethyl]-4-Acetylaminoisoindoline-1,3-Dione

Preparation of 3-aminopthalic acid

10% Pd/C (2.5 g), 3-nitrophthalic acid (75.0 g, 355 mmol) and ethanol (1.5 L) were charged to a 2.5 L Parr hydrogenator under a nitrogen atmosphere. Hydrogen was charged to the reaction vessel for up to 55 psi. The mixture was shaken for 13 hours, maintaining hydrogen pressure between 50 and 55 psi. Hydrogen was released and the mixture was purged with nitrogen 3 times. The suspension was filtered through a celite bed and rinsed with methanol. The filtrate was concentrated in vacuo. The resulting solid was reslurried in ether and isolated by vacuum filtration. The solid was dried in vacuo to a constant weight, affording 54 g (84% yield) of 3-aminopthalic acid as a yellow product. ^{1}H-NMR (DMSO-d6) δ: 3.17 (s, 2H), 6.67 (d, 1H), 6.82 (d, 1H), 7.17 (t, 1H), 8-10 (br, s, 2H); ^{13}C-NMR (DMSO-d6) δ: 112.00, 115.32, 118.20, 131.28, 135.86, 148.82, 169.15, 170.09.

Preparation of 3-acetamidophthalic anhydride

A 1 L 3-necked round bottom flask was equipped with a mechanical stirrer, thermometer, and condenser and charged with 3-aminophthalic acid (108 g, 596 mmol) and acetic anhydride (550 mL). The reaction mixture was heated to reflux for 3 hours and cooled to about 25° C. and further to 0-5° C. for another 1 hour. The crystalline solid was collected by vacuum filtration and washed with ether. The solid product was dried in vacuo at ambient temperature to a constant weight, giving 75 g (61% yield) of 3-acetamidopthalic anhydride as a white product. ^{1}H-NMR ($CDCl_3$) δ: 2.21 (s, 3H), 7.76 (d, 1H), 7.94 (t, 1H), 8.42 (d, 1H), 9.84 (s, 1H).

Resolution of 2-(3-ethoxy-4-methoxyphenyl-1-(methylsulphonyl)-eth-2-ylamine

A 3 L 3-necked round bottom flask was equipped with a mechanical stirrer, thermometer, and condenser and charged with 2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine (137.0 g, 500 mmol), N-acetyl-L-leucine (52 g, 300 mmol), and methanol (1.0 L). The stirred slurry was heated to reflux for 1 hour. The stirred mixture was allowed to cool to ambient temperature and stirring was continued for another 3 hours at ambient temperature. The slurry was filtered and washed with methanol (250 L). The solid was air-dried and then dried in vacuo at ambient temperature to a constant weight, giving 109.5 g (98% yield) of the crude product (85.8% ee). The crude solid (55.0 g) and methanol (440 mL) were brought to reflux for 1 hour, cooled to room temperature and stirred for an additional 3 hours at ambient temperature. The slurry was filtered and the filter cake was washed with methanol (200 mL). The solid was air-dried and then dried in vacuo at 30° C. to a constant weight, yielding 49.6 g (90% recovery) of (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine-N-acetyl-L-leucine salt (98.4% ee). Chiral HPLC (1/99 EtOH/20 mM KH_2PO_4 @ pH 7.0, Ultron Chiral ES-OVS from Agilent Technologies, 150 mm×4.6 mm, 0.5 mL/min., @ 240 nm): 18.4 min (S-isomer, 99.2%), 25.5 min (R-isomer, 0.8%).

Preparation of Compound A

A 500 mL 3-necked round bottom flask was equipped with a mechanical stirrer, thermometer, and condenser. The reaction vessel was charged with (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-yl amine N-acetyl-L-leucine salt (25 g, 56 mmol, 98% ee), 3-acetamidophthalic anhydride (12.1 g, 58.8 mmol), and glacial acetic acid (250 mL). The mixture was refluxed over night and then cooled to <50° C. The solvent was removed in vacuo, and the residue was dissolved in ethyl acetate. The resulting solution was washed with water (250 mL×2), saturated aqueous $NaHCO_3$ (250 mL×2), brine (250 mL×2), and dried over sodium sulphate. The solvent was evaporated in vacuo, and the residue recrystallized from a binary solvent containing ethanol (150 mL) and acetone (75 mL). The solid was isolated by vacuum filtration and washed with ethanol (100 mL×2). The product was dried in vacuo at 60° C. to a constant weight, affording 19.4 g (75% yield) of S-{2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetamidoisoindoline-1,3-dione} with 98% ee. Chiral HPLC (15/85 EtOH/20 mM KH_2PO_4 @ pH 5, Ultron Chiral ES-OVS from Agilent Technology, 150 mm×4.6 mm, 0.4 mL/min, @ 240 nm): 25.4 min (S-isomer, 98.7%), 29.5 min (R-isomer, 1.2%). ^{1}H-NMR ($CDCl_3$) δ: 1.47 (t, 3H), 2.26 (s, 3H), 2.87 (s, 3H), 3.68-3.75 (dd, 1H), 3.85 (s, 3H), 4.07-4.15 (q, 2H), 4.51-4.61 (dd, 1H), 5.84-5.90 (dd, 1H), 6.82-8.77 (m, 6H), 9.46 (s, 1H); ^{13}C-NMR (DMSO-d6) δ: 14.66, 24.92, 41.61, 48.53, 54.46, 55.91, 64.51, 111.44, 112.40, 115.10, 118.20, 120.28, 124.94, 129.22, 131.02, 136.09, 137.60, 148.62, 149.74, 167.46, 169.14, 169.48.

A reaction scheme illustrating a preparation of the (+) enantiomer of Compound A is provided as FIG. **29**.

5.3. Example 3

TNF-α Inhibition

Human Whole Blood LPS-induced TNF-α Assay

The ability of compounds to inhibit LPS-induced TNF-α production by human whole blood was measured essentially as described below for the LPS-induced TNF-α assay in human PBMC, except that freshly drawn whole blood was

used instead of PBMC. (Muller et al., 1999, *Bioorg. & Med. Chem. Lett.*, 9:1625-1630.) Human whole blood LPS-induced TNF-α IC_{50}=294 nM for Compound A.

Mouse LPS-induced Serum TNF-α Inhibition

Compounds were tested in this animal model according to previously described methods (Corral et al., 1996, *Mol. Med.*, 2:506-515). Mouse LPS-induced serum TNF-α inhibition (ED_{50}, mg/kg, p.o.)=0.05 for Compound A.

LPS-induced TNF-α Production

Lipopolysaccharide (LPS) is an endotoxin produced by gram-negative bacteria such as *E. coli* which induces production of many pro-inflammatory cytokines, including TNF-α. In peripheral blood mononuclear cells (PBMC), the TNF-α produced in response to LPS is derived from monocytes, which comprise approximately 5-20% of the total PBMC. Compounds were tested for the ability to inhibit LPS-induced TNF-α production from human PBMC as previously described (Muller et al., 1996, *J. Med. Chem.*, 39:3238). PBMC from normal donors were obtained by Ficoll Hypaque (Pharmacia, Piscataway, N.J., USA) density centrifugation. Cells were cultured in RPMI (Life Technologies, Grand Island, N.Y., USA) supplemented with 10% AB±human serum (Gemini Bio-products, Woodland, Calif., USA), 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Life Technologies).

PBMC (2×10^5 cells) were plated in 96-well flat-bottom Costar tissue culture plates (Corning, N.Y., USA) in triplicate. Cells were stimulated with LPS (Sigma, St. Louis, Mo., USA) at 100 ng/ml in the absence or presence of compounds. Compounds (Celgene Corp., Warren, N.J., USA) were dissolved in DMSO (Sigma) and further dilutions were done in culture medium immediately before use. The final DMSO concentration in all samples was 0.25%. Compounds were added to cells one hour before LPS stimulation. Cells were incubated for 18-20 hours at 37° C. in 5% CO_2 and supernatants were then collected, diluted with culture medium and assayed for TNF-α levels by ELISA (Endogen, Boston, Mass., USA). LPS-induced TNF-α IC_{50}=77 nM for Compound A.

IL-1β-induced TNF-α Production

During the course of inflammatory diseases, TNF-α production is often stimulated by the cytokine IL-1β, rather than by bacterially derived LPS. Compounds were tested for the ability to inhibit IL-1β-induced TNF-α production from human PBMC as described above for LPS-induced TNF-α production, except that the PBMC were isolated from source leukocyte units (Sera-Tec Biologicals, North Brunswick, N.J., USA) by centrifugation on Ficoll-Paque Plus (Amersham Pharmacia, Piscataway, N.J., USA), plated in 96-well tissue culture plates at 3×10^5 cells/well in RPMI-1640 medium (BioWhittaker, Walkersville, Md., USA) containing 10% heat-inactivated fetal bovine serum (Hyclone), 2 mM L-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin (complete medium), pretreated with compounds at 10, 2, 0.4, 0.08, 0.016, 0.0032, 0.00064, and 0 μM in duplicate at a final DMSO concentration of 0.1% at 37° C. in a humidified incubator at 5% CO_2 for one hour, then stimulated with 50 ng/ml recombinant human IL-1β (Endogen) for 18 hours. IL-β-induced TNF-α IC_{50}=83 nM for Compound A.

5.4. Example 4

PDE Selectivity

PDE1, 2, 3, 5, and 6 Enzyme Assays

The specificity of compounds for PDE4 was assessed by testing at a single concentration (10 μM) against bovine PDE1, human PDE2, PDE3, and PDE5 from human platelets (Hidaka and Asano, 1976, *Biochem. Biophys. Acta*, 429:485, and Nicholsen et al., 1991, *Trends Pharmaco. Sci.*, 12:19), and PDE6 from bovine retinal rod outer segments (Baehr et al., 1979, *J. Biol. Chem.*, 254:11669, and Gillespie et al. 1989, *Mol. Pharm.*, 36:773). Results are listed in Table 1.

PDE7 Enzyme Assay

PDE7 is a cAMP-selective PDE expressed mainly in T cells and in skeletal muscle. T cell-derived cytokines such as IL-2 and IFN-γ are potentially regulatable via PDE7 inhibition. PDE7 was purified from Hut78 human T cells by anion exchange chromatography as previously described (Bloom and Beavo, 1996, *Proc. Natl. Acad. Sci. USA*, 93:14188-14192). Compounds were tested against the PDE7 preparation in the presence of 10 nM cAMP as described for PDE4 in Table 1.

5.5. Example 5

PDE4 Inhibition

PDE4 (U937 Cell-derived) Enzyme Assay

PDE4 enzyme was purified from U937 human monocytic cells by gel filtration chromatography as previously described (Muller et al., 1998, *Bioorg. & Med. Chem. Lett.* 8:2669-2674). Phosphodiesterase reactions were carried out in 50 mM Tris HCl pH 7.5, 5 mM $MgCl_2$, 1 μM cAMP, 10 nM [^{3}H]-cAMP for 30 min at 30° C., terminated by boiling, treated with 1 mg/ml snake venom, and separated using AG-1XS ion exchange resin (BioRad) as described (Muller et al., 1998, *Bioorg. & Med. Chem. Lett.* 8:2669-2674). Reactions consumed less than 15% of available substrate. Results are listed in Table 1.

TABLE 1

PDE Specificity			
	Racemic Compound	Compound A	Compound B*
PDE Inhibition			
PDE4 IC_{50} (from U937 cells) (nM)	81.8	73.5	611
PDE1 (% inhib at 10 μM)	9%	23%	27%
PDE2 (% inhib at 10 μM)	19%	6%	10%
PDE3 (% inhib at 10 μM)	21%	20%	31%
PDE5 (% inhib at μM)	3%	3%	−9%
PDE6 (% inhib at 10 μM)	ND	−6%	10%
PDE7 IC_{50} (nM)	22110	20500	ND
PDE Specificity Ratios from above data (*fold)			
PDE4/PDE1	>2700	>500	>50
PDE4/PDE2	>800	>10000	>260
PDE4/PDE3	>670	>1200	>45
PDE4/PDE5	>12000	>30000	>39000
PDE4/PDE6	ND	>40000	>250
PDE7 IC_{50}/PDE4 IC_{50}	270	279	ND

*Compound B is the (−) enantiomer of Compound A.

5.6. Example 6

Human T Cell Assays

SEB-induced IL-2 and IFN-γ Production

Staphylococcal Enterotoxin B (SEB) is a superantigen derived from gram-positive bacteria *Staphylococcus aureus*. SEB provides a convenient physiological stimulus specific for T cells expressing particular T cell receptor Vβ chains. Human PBMC (consisting of approximately 50% T cells) were isolated from source leukocyte units as described above and plated in 96-well tissue culture plates at 3×10^5 cells/well in complete medium, pretreated with compounds at 10, 2, 0.4, 0.08, 0.016, 0.0032, 0.00064, and 0 μM in duplicate at a final DMSO concentration of 0.1% at 37° C. in a humidified incubator at 5% CO_2 for 1 hour, then stimulated with 100 ng/ml SEB (Sigma Chemical Co., St. Louis, Mo., USA) for 18 hours. IL-2 and IFN-γ levels were measured by ELISA (R&D Systems, Minneapolis, Minn., USA). IL-2 IC_{50}=291 nM for Compound A. IFN-γ IC_{50}=46 nM for Compound A.

5.7. Example 7

cAMP Elevation Assays

PGE_2-induced cAMP Elevation

Prostaglandin E_2 (PGE_2) binds to prostanoid receptors on monocytes, T cells and other leukocytes and consequently elevates intracellular cAMP levels, resulting in inhibition of cellular responses. The combination of PGE_2 and a PDE4 inhibitor synergistically elevates cAMP levels in these cell types, and the elevation of cAMP in PBMC caused by PDE4 inhibitors in the presence of PGE_2 is proportional to the inhibitory activity of that PDE4 inhibitor. Intracellular cAMP was measured in human PBMC as follows. PBMC were isolated as described above and plated in 96-well plates at 1×10^6 cells per well in RPMI-1640. The cells were pretreated with compounds at 100, 10, 1, 0.1, 0.01, and 0 μM in a final concentration of 2% DMSO in duplicate at 37° C. in a humidified incubator at 5% CO_2 for one hour. The cells were then stimulated with PGE_2 (10 μM) (Sigma) for 1 h. The cells were lysed with HCl, 0.1 N final concentration to inhibit phosphodiesterase activity and the plates were frozen at −20° C. The cAMP produced was measured using cAMP (low pH) Immunoassay kit (R&D Systems). PBMC cAMP EC_{50} for racemate is 3.09 μM. PBMC cAMP EC_{50} for Compound A is 1.58 μM.

Elevation of cAMP in human neutrophils was measured as follows. PBMC were removed from source leukocytes (SeraTec Biologicals) by centrifugation on Ficoll-Paque Plus (Amersham Pharmacia). The resulting erythrocyte/polymorphonuclear cell (PMN) pellet was resuspended in Hank's Balanced Salt Solution (BioWhittaker) and mixed with an equal volume of 3% Dextran T-500 (Amersham Pharmacia) in 0.9% saline. Erythrocytes were allowed to sediment for 20 minutes, and the PMN were removed and centrifuged at 120 rpm for 8 minutes at 4° C. The remaining erythrocytes were lysed in cold 0.2% saline for 30 seconds, and the cells restored to isotonicity by the addition of an equal volume of 1.6% saline. The PMN were centrifuged at 1200 rpm for 8 minutes at 4° C., then resuspended in RPMI-1640 and assayed for cAMP elevation as described for PBMC above. PMN were found to be approximately 74% CD 18/CD11b$^+$, 71% CD16$^+$ CD9$^+$ neutrophils by flow cytometry on a FACSCalibur (Becton Dickinson, San Jose, Calif., USA). Results are shown in Table 2.

fMLF-induced LTB4 Production

N-formyl-methionine-leucine-phenylalanine (fMLF) is a bacterially derived peptide that activates neutrophils to rapidly degranulate, migrate, adhere to endothelial cells, and release leukotriene LTB4, a product of arachidonic acid metabolism and itself a neutrophil chemoattractant. Compounds were tested for the ability to block fMLF-induced neutrophil LTB4 production as previously described (Hatzelmann and Schudt, 2001, *J. Pharm. Exp. Ther.,* 297:267-279), with the following modifications. Neutrophils were isolated as described above and resuspended in phosphate-buffered saline without calcium or magnesium (BioWhittaker) containing 10 mM HEPES pH 7.2 and plated in 96-well tissue culture plates at a concentration of 1.7×10^6 cells/well. Cells were treated with 50 μM thimerosal (Sigma)/1 mM $CaCl_2$/1 mM $MgCl_2$ for 15 minutes at 37° C. 5% CO_2, then treated with compounds at 1000, 200, 40, 8, 1.6, 0.32, 0.064, and 0 nM in a final DMSO concentration of 0.01% in duplicate for 10 minutes. Neutrophils were stimulated with 1 μM fMLF for 30 minutes, then lysed by the addition of methanol (20% final concentration) and frozen in a dry ice/isopropanol bath for 10 minutes. Lysates were stored at −70° C. until the LTB4 content was measured by competitive LTB4 ELISA (R&D Systems). Results are shown in Table 2.

Zymosan-induced IL-8 Production

Zymosan A, or the heat-killed yeast *Saccharomyces cerevisiae*, binds to the adhesion molecule Mac-1 on the neutrophil surface and triggers phagocytosis, cell activation and IL-8 production. Zymosan-induced IL-8 production was measured as previously described (Au et al., 1998, *Brit. J. Pharm.,* 123:1260-1266) with the following modifications. Human neutrophils were purified as described above, plated in 96-well tissue culture plates at 3×10^5 cells/well in complete medium, treated with compounds at 10, 2, 0.4, 0.08, 0.016, 0.0032, 0.00064, and 0 μM in duplicate in a final DMSO concentration of 0.1% for 1 hour at 37° C. 5% CO_2. Neutrophils were then stimulated with unopsonized, boiled Zymosan A (Sigma) at 2.5×10^5 particles/well for 18 hours. Supernatants were harvested and tested for IL-8 by ELISA (R&D Systems). Results are shown in Table 2.

fMLF-induced CD18/CD11b Expression

CD18/CD11b (Mac-1) expression on neutrophils was measured as previously described (Derian et al., 1995, *J. Immunol.,* 154:308-3 17) with the following modifications. Neutrophils were isolated as described above, then resuspended in complete medium at 1×10^6 cells/ml, pretreated with compounds at 10, 1, 0.1, 0.01, and 0 μM in duplicate at a final DMSO concentration of 0.1% for 10 minutes at 37° C. 5% CO_2. Cells were then stimulated with 30 nM fMLF for 30 minutes and then chilled to 4° C. Cells were treated with rabbit IgG (Jackson ImmunoResearch Labs, West Grove, Pa., USA) (10 μg/1×10^6 cells) to block Fc receptors, stained with CD18-FITC and CD11b-PE (Becton Dickinson), and analyzed by flow cytometry on a FACSCalibur. CD18/CD11b expression (mean fluorescence) in the absence of stimulation was subtracted from all samples to obtain inhibition curves and calculate IC_{50} values. Results are shown in Table 2.

fMLF-induced Adhesion to HUVEC

Human umbilical vein endothelial cells (HUVEC) were used as a substrate for neutrophil adhesion as previously described (Derian et al., 1995, *J. Immunol.,* 154:308-317) with the following modifications. HUVEC cells were obtained from Anthrogenesis (Cedar Knolls, N.J., USA), and neutrophils were not treated with cytochalasin B. Cells were treated with compounds at 10, 1, 0.1, 0.01, 0.001, and 0 μM in a final DMSO concentration of 0.1% in duplicate for 10 minutes, stimulated with 500 nM fMLF for 30 minutes, and

washed twice with PBS before measuring fluorescence on an FLX800 plate reader (Bio-Tek Instruments, Winooski, Vt., USA). Results are shown in Table 2.

TABLE 2

Assay results

Human Neutrophil Assays (all values in nM)	Racemic Compound	Compound A
PGE_2-induced cAMP EC_{50}	12589	4570
fMLF-induced LTB4 IC_{50}	20.1	2.48
Zymosan-induced IL-8 IC_{50}	ND	94
fMLF-induced CD18 expression IC_{50}	ND	390
fMLF-induced CD11b expression IC_{50}	ND	74
fMLF-induced adhesion to HUVEC IC_{50}	ND	150

5.8. Example 8

Aqueous Solubility

Equilibrium solubilities were measured in pH 7.4 aqueous buffer. The pH 7.4 buffer was prepared by adjusting the pH of a 0.07 M NaH_2PO_4 solution to 7.4 with 10 N NaOH. The ionic strength of the solution was 0.15. At least 1 mg of powder was combined with 1 ml of buffer to make >1 mg/ml mixture. These samples were shaken for >2 hours and left to stand overnight at room temperature. The samples were then filtered through a 0.45-μm Nylon syringe filter that was first saturated with the sample. The filtrate was sampled twice, consecutively. The filtrate was assayed by HPLC against standards prepared in 50% methanol. Compound A has 3.5-fold greater aqueous solubility than the racemic mixture. Measured solubility Compound A=0.012 mg/mL; racemic mixture=0.0034 mg/mL.

5.9. Example 9

LPS-Induced Lung Neutrophilia Ferret Model

The conscious ferret model has been used to investigate anti-inflammatory, emetic and behavioral effects of PDE4 inhibitors when administered by the oral (p.o.) route. From these experiments, a therapeutic index (TI) for each PDE4 inhibitor may be determined. The TI has been calculated by dividing the threshold dose for causing emetic episodes and behavioral changes by the anti-inflammatory dose (dose that causes 50% inhibition of the LPS-induced neutrophilia).

Animal Husbandry

Male ferrets (*Mustela Putorius* Euro, weighing 1-2 kg). Ferrets were supplied either by Bury Green Farm or Misay Consultancy. Following transport, the animals were allowed to acclimatize in the holding rooms for a period of not less than seven days. The diet comprised SDS diet C pelleted food given ad lib with Whiskers™ cat food given three times per week. Water was pasteurized animal grade drinking water and was changed daily.

Dosing with PDE4 Inhibitor

PDE4 inhibitors were administered orally (p.o.), at doses initially of 1-10 g/kg, but subsequently up to 30 mg/kg in order to establish whether the TI was 10 or higher, and/or at lower doses to establish the minimum dose to cause 50% inhibition of neutrophilia. Ferrets were fasted overnight but allowed free access to water. The animals were orally dosed with vehicle or PDE4 inhibitor using a 15 cm dosing needle that was passed down the back of the throat into the oesophagus. After dosing, the animals were returned to holding cages fitted with Perspex doors to allow observation, and given free access to water. After dosing, the animals were constantly observed and any emesis or behavioral changes were recorded. The animals were allowed access to food 60 to 90 minutes after p.o. dosing.

Exposure to LPS

Thirty minutes after p.o. dosing with compound or vehicle control, the ferrets were placed into sealed Perspex containers and exposed to an aerosol of LPS (100 μg/ml) for 10 minutes. Aerosols of LPS were generated by a nebulizer (DeVilbiss, USA) and this was directed into the Perspex exposure chamber. Following a 10 minute exposure period, the animals were returned to the holding cages and allowed free access to water, and at a later stage, food. Observation continued for a period of at least 2.5 hours post p.o. dosing and emetic episodes and behavioral changes were recorded.

Bronchoalveolar Lavage

Six hours after LPS exposure the animals were killed by overdose of sodium pentobarbitone administered intraperitoneally. The trachea was then cannulated with polypropylene tubing and the lungs lavaged twice with 20 ml heparinized (10 units/ml) phosphate buffered saline (PBS).

Blood Sampling/Tissue Removal

A terminal blood sample (10 ml) was removed by transthoracic cardiac puncture. The blood was spun at 2,500 rpm for 15 minutes and the plasma was removed and stored at −20° C. The brain also removed and frozen at −20° C. for analysis of compound content.

Cell Counts

The bronchoalveolar lavage (BAL) samples were centrifuged at 1,500 rpm for 5 minutes. The supernatant was removed and the resulting cell pellet re-suspended in 1 ml PBS. A cell smear of the re-suspended fluid was prepared and stained with Leishmans stain to allow differential cell counting. A total cell count was made using the remaining re-suspended sample. From this, the total number of neutrophils in the BAL was determined.

Parameters Measured

1. % Inhibition of LPS-induced pulmonary neutrophilia.

2. Emetic episodes—the number of vomits and retches were counted.

3. Behavioral changes—the following behavioral effects were noted: salivation, panting, mouth clawing, flattened posture, ataxia, arched back and backward walking. Any behavioral changes were semi-quantified by applying a severity rating (mild, moderate or severe).

4. The TI was calculated as the highest dose found to not cause emetic episodes divided by the lowest dose found to inhibit pulmonary neutrophilia by 50% or more.

The effect of Compound A on LPS-induced neutrophilia in the lungs of conscious ferrets is demonstrated in FIG. **30**.

Emesis and Behavioral Changes

Following p.o. dosing of the PDE4, the ferrets were observed for at least two hours and emetic episodes (vomits and retches) and behavioral changes were recorded.

No emetic episodes (retching or vomiting) were observed in the ferrets pre-treated p.o. with the relevant vehicle (acetone/cremophor/distilled water). In a small proportion of the control-treated animals (7/22), mild behavioral changes (lip licking and backward walking) were seen.

Compound A (0.1-3 mg/kg, p.o.), caused no emetic episodes (retching and vomiting). Some behavioral changes (flattened posture, lip licking and backward walking) were observed and classified as mild. At 10 mg/kg in 2/6 ferrets, some retching but no frank emesis was observed along with salivation and behavioral changes (scored as mild or moderate). At the highest dose tested (30 mg/kg) moderate to

marked emesis was observed in 3/4 animals along with pronounced behavioral changes. These data are summarized in Table 3.

TABLE 3

Conscious ferret: Emetic episodes and behavioral changes following oral administration of Compound A

Treatment/dose (mg/kg)	Vomits	Retches	Salivation	Panting	Mouth clawing	Flattened posture	Ataxia	Lip licking	Backward walking
Vehicle (acetone/ cremophor/ dist. H_2O)	None	None	None	None	None	None	None	Mild (6/22)	Mild (7/22)
Compound A (0.1 mg/kg)	None	None	None	None	None	Mild (2/5)	None	Mild (4/5)	Mild (3/5)
Compound A (0.3 mg/kg)	None	None	None	None	None	Mild (2/6)	None	Mild (3/6)	Mild (4/6)
Compound A (1.0 mg/kg)	None	None	None	None	None	Mild (2/6)	None	Mild (6/6)	Mild (4/6)
Compound A (3.0 mg/kg)	None	None	None	None	Mild (1/8)	Marked (7/8)	None	Mild (2/8)	Moderate (5/8)
Compound A (10 mg/kg)	None	Mild (2/6)	Mild (1/6)	None	Mild (1/6)	Marked (6/6)	None	Moderate (5/6)	Marked (6/6)
Compound A (30 mg/kg)	Moderate (3/4)	Marked (3/4)	Moderate (3/4)	Mild (1/4)	Marked (4/4)	Marked (4/4)	Mild (3/4)	Moderate (4/4)	Mild (2/4)

Animals were observed for up to three hours following dosing. Numbers in parentheses refer to the number of animals that responded. The numbers of animals in each group range from 4 to 22.

Therapeutic Index Calculation

From these experiments, a therapeutic index (TI) was determined for each compound by dividing the threshold dose for inducing emetic episodes by the ED_{50} value for inhibiting the pulmonary neutrophilia. The TI calculation is summarized in Table 4. Compound A had a TI of 12, causing no emetic episodes at an anti-inflammatory dose of 1 mg/kg.

TABLE 4

Summary of the effective doses (ED_{50}) for inhibition of LPS-induced pulmonary neutrophilia and induction of emesis and the therapeutic index derived from these values

Compound	Inhibition of LPS-induced neutrophilia (ED_{50} mg/kg)	Threshold emetic dose (mg/kg)	Therapeutic index
Compound A	0.8	10	12

5.10. Example 10

Biological Activity of Compound A in Patients with Severe Plaque-Type Psoriasis

Compound A is a novel oral agent that downregulates pro-inflammatory cytokine production in human cellular models. Compound A has been shown to decrease TNF-α, IL-12 and IFN-γ production as well as elevate production of IL-10. Psoriasis is strongly associated with dysregulation of cytokines and chemokines allowing for potential therapies with immunomodulatory compounds. This Phase 2, open-label, single arm, pilot study was designed to assess the biological activity of Compound A in patients with severe plaque-type psoriasis. Additional assessments for clinical outcomes were performed to evaluate the potential efficacy of Compound A in treating severe plaque-type psoriasis.

Compound A was administered 20 mg orally daily for 29 days with an additional 28-day observational follow-up period for patient safety. Skin punch biopsy specimens (6 mm) from target plaques were obtained at baseline, Day 15 and Day 29. A nonlesional skin biopsy was also taken at baseline. The primary pharmacodynamic endpoint was the percent change from baseline in epidermal thickness at Day 29. Epidermal skin thickness measurements and immunohistochemical analysis were carried out by a blinded reviewer to evaluate CD11c, CD83, K16, ICAM-1, HLA-DR, and fillagrin. Biopsy specimens were analyzed by RT-PCR for: TNF-α, p40-IL12/IL23, IL-10, IFN-γ, IP10, IL-2, IL-8, iNOS, p19-IL23, K16, CD 83, and hARP. PASI, PGA, and BSA measurements were performed to explore clinical efficacy during the 29-day treatment phase of the study. Adverse event reporting, clinical laboratory evaluations, physical examinations, ECG and vital sign measurements assessed safety. A total of 19 patients were enrolled: 15 patients had complete sets of evaluable biopsies and 17 patients had complete efficacy assessments.

Assessment of the change in epidermal thickness was the primary endpoint in this study. Nineteen patients were enrolled in the study, of which 15 had complete sets of evaluable biopsies at baseline and Day 29. Seventeen of the 19 subjects had clinical efficacy parameters measured at Baseline and Day 29. Eight (53.3%) of the patients with evaluable biopsies at baseline and Day 29 demonstrated a 20% reduction in epidermal skin thickness. The mean reduction of epidermal thickness among all 15 subjects with evaluable biopsies at baseline and Day 29 was 20.5% (p=0.015). FIG. **31** displays the change in epidermal thickness from baseline to Day 29 among subjects with evaluable biopsies.

Key inflammatory markers including epidermal and dermal T-cells, CD83+ and CD11c cells were evaluated in biopsy specimens. Results for 8 patients who responded showed a decrease of epidermal and dermal T-cells by 42.56% and 28.79% respectively in responders (≧20% epidermal thickness reduction). Mean reductions from baseline in epidermal and dermal CD83+ cells were 32.50% and 25.86% respectively in responders. CD11c cells were reduced by 40.16% in the epidermis and 18.50% in the dermis in responders. Table 5 lists reductions in key skin biopsy inflammatory markers in responders and nonresponders. In addition, one patient with

abnormal K16 at baseline had normal K16 at Day 29. Three patients with abnormal ICAM-1 at baseline had normal ICAM-1 at Day 29. Two patients with abnormal HLA-DR had normal HLA-DR at Day 29 and three patients with abnormal fillagrin at baseline had normal fillagrin at Day 29.

TABLE 5

Percentage Reduction of Key Inflammatory Markers at Day 29

Cell		Epidermis	Dermis
T-cells	Responder	−42.56%	−28.79%
	Nonresponder	+8.74%	−17.34%
CD83+	Responder	−32.50%	−25.86%
	Nonresponder	−16.31%	+0.46%
CD11c	Responder	−40.16%	−18.50%
	Nonresponder	−2.54%	−21.19%

Biopsy specimens were evaluated for mRNA gene expression of key inflammatory markers by RT-PCR including: TNFα, p40-IL12/IL23, IL-10, IFNγ, IP10, IL-2, IL-8, iNOS, p19-IL23, K16 and CD83. The mRNA expression of iNOS was reduced 66.5% (p=0.025) in lesional skin after 29 days of treatment with Compound A. Reductions and increases in mRNA expression of other inflammatory markers showed overall trends of improvement. FIG. **32** graphically displays the change in iNOS expression during the study.

A total of 17 of the 19 subjects enrolled completed the 29-day treatment phase and had complete clinical efficacy assessments. Fourteen (73.7%) of the 19 subjects enrolled demonstrated improvement in their PASI with 3 (15.8%) of these patients showing a >50% reduction from baseline in their total Psoriasis Area and Severity Index (PASI) score at Day 29. FIG. **33** displays the percentage change in PASI scores among evaluable patients from baseline at Day 29. Additionally, 9 (52.9%) of the 17 evaluable patients demonstrated improvement in the static Physician's Global Assessment (sPGA) and 10 (58.8%) of the 17 evaluable patients showed a reduction from baseline in their psoriasis body surface area (BSA) after 29 days of treatment with Compound A. Safety was evaluated during treatment and follow-up phases through monitoring of adverse events, ECGs, laboratory tests, physical exams and vital signs. No deaths were reported nor did any patient prematurely discontinue due to an adverse event. Most common treatment-related adverse events included headache (26.3%), and nausea (15.8%).

In this clinical study, Compound A 20 mg p.o. QD for 29 days was safe in subjects with severe plaque type psoriasis. The primary endpoint was reached with 8 (53.3%) of 15 subjects achieving a 20% reduction in epidermal thickness at Day 29. Reductions of key inflammatory markers in skin biopsies were noted including dermal and epidermal T-cells, CD83+ and CD11c cells. RT-PCR analysis revealed a statistically significant reduction of 66.5% in iNOS mRNA in skin biopsies at Day 29. A positive clinical efficacy signal was noted after 29 days of treatment with Compound A. 73.7% of enrolled patients demonstrated improvement in their psoriasis symptoms with 15.8% of these patients showing >50% reduction from baseline in their PASI score at Day 29. 47.4% of enrolled patients showed an improvement in their sPGA and 52.6% of enrolled patients showed a reduction from baseline in their psoriasis body surface area (BSA) at Day 29.

5.11. Example 11

A Phase 2 Study Demonstrating the Efficacy and Safety of Compound A in Subjects with Moderate-to-Severe Psoriasis

This phase 2, multicenter, randomized, double-blind, placebo-controlled, parallel-group, dose-comparison study evaluated the efficacy and safety of Compound A in subjects with moderate to severe plaque-type psoriasis who were candidates for systemic therapy.

This study included a 12-week treatment phase followed by a 4-week observational follow-up phase. A total of 260 subjects were randomized to receive Compound A 20 mg BID, Compound A 20 mg QD, or placebo for 12 weeks. The primary endpoint for this study was the proportion of subjects treated with Compound A who achieved a 75% reduction in Psoriasis Area and Severity Index score ("PASI-75") at week 12/last treatment in reference to the baseline visit. Last treatment is defined as the last PASI assessment completed during the 12-week treatment phase.

At week 12/last treatment, a significantly higher proportion of subjects treated with 20 mg BID (24%) achieved a PASI-75 compared with the placebo group (10%; P=0.023). Of the subjects receiving 20 mg BID or placebo, 57% versus 23% achieved PASI-50 at week 12/last treatment, respectively; whereas 14% versus 6% achieved PASI-90, respectively. At week 12/last treatment, subjects achieved a mean decrease of 52% versus 17% in PASI from baseline in the 20 mg BID versus placebo groups, respectively. Subjects receiving Compound A continued to improve over time, showing the greatest mean percent reduction in PASI score at week 12. Overall, the adverse event profiles were similar across all three treatment groups. The majority of adverse events reported were mild. No study drug-related serious adverse events were reported in this study. No subjects in the 20 mg BID group experienced psoriasis flare during the observational follow-up period.

In this clinical study, Compound A was shown to be well tolerated and safe in subjects with moderate to severe plaque-type psoriasis. The proportions of subjects that achieved 50%, 75%, and 90% improvement in PASI demonstrate the clinical activity of Compound A after 12 weeks of treatment.

5.12. Example 12

Solid Form Screening Studies

5.12.1. Experimental Methodology

Solubility Studies. A weighed sample of Compound A (about 100 mg) was treated with about 2 mL of the test solvent. The solvents used were either reagent or HPLC grade. The resulting mixture was agitated for at least 24 hours at about 25° C. When all of the solids were dissolved by visual inspection, the estimated solubilities were calculated. The solubilities were estimated from these experiments based on the total volume of solvent used to give a solution. The actual solubilities may be greater than those calculated due to the use of large amount of solvent or to a slow rate of dissolution. If dissolution did not occur during the experiment, the solubility was measured gravimetrically. A known volume of filtrate was evaporated to dryness and the weight of the residue was measured.

Solution Evaporation Studies. Solution evaporation was performed for solvents in which the solubility of Compound A was more than about 50 mg/mL, such as acetone, acetonitrile, methylene chloride and tetrahydrofuran. Solid samples were obtained by slowly evaporating the solvents at about 25° C. or about 50° C. in an open vial under nitrogen.

Equilibration Studies. Equilibration experiments were carried out by adding an excess of Compound A to about 2 mL of a test solvent. The resulting mixture was agitated for at least 24 hours at about 25° C. or about 50° C. Upon reaching equilibrium, the saturated solution was removed and allowed to evaporate slowly in an open vial under nitrogen at about 25° C. or about 50° C., respectively. The slurry resulting from the equilibration was filtered and dried in the air.

Cooling Crystallization Studies. Cooling crystallization studies were performed. The solid was dissolved in a solvent at an elevated temperature, about 65° C., and allowed to cool to about 25° C. Samples that did not crystallize at about 25° C. were placed in a refrigerator (about 0-5° C.). Solids were isolated by decantation and allowed to dry in the air.

Solvent/Anti-Solvent Precipitation Studies. Precipitations were carried out by solvent/anti-solvent combinations. The solid was dissolved in a solvent in which Compound A had a relatively high solubility, and then a selected solvent in which Compound A had a relatively low solubility (i.e., an anti-solvent) was added to the solution. A precipitate formed immediately in some solvent/anti-solvent systems. If the precipitation did not occur immediately, the resulting mixture was allowed to cool in a refrigerator (about 0-5° C.) until a precipitate formed. The precipitate was then isolated by decantation and allowed to dry in the air.

Interconversion Studies. Interconversion experiments were performed by making slurries of a solid form in a saturated solvent. The slurries were agitated for at least 2 days at about 25° C. The saturated solution was removed by filtration and the solid was dried in the air.

Compression Studies. Compression tests were performed by pressing the sample under 2000 psi force for at least one minute with Carver Mini C presser. The sample was then analyzed by XRPD.

Hygroscopicity studies. The hygroscopicity of various solid forms was studied using a Surface Measurement Systems DVS instrument. Typically a sample size of between about 10-50 mg was loaded into the DVS instrument sample pan and the sample was analyzed on a DVS automated sorption analyzer at about 25° C. The relative humidity was increased in increments of about 10% from about 0% to about 95% RH. The relative humidity was then decreased in a similar manner to accomplish a full adsorption/desorption cycle. The mass was recorded at periodic intervals throughout the experiment.

5.12.2. Characterization Methodology

Samples generated as described in the solid form screen were typically analyzed by X-Ray Powder Diffraction (XRPD). XRPD was conducted on a Thermo ARL X'TRA™ X-ray powder diffractometer using Cu Kα radiation at 1.54 Å. The instrument was equipped with a fine focus X-ray tube. The voltage and amperage of X-ray generator were set at 45 kV and 40 mA, respectively. The divergence slices were set at 4 mm and 2 mm and the measuring slices were set at 0.5 mm and 0.2 mm. The diffracted radiation was detected by a peltier-cooled Si(Li) solid-state detector. Typically, a theta-two theta continuous scan at 2.40°/min (0.5 sec/0.02° step) from 1.5 °2θ to 40° 2θ was used. A sintered alumina standard was used to check the peak position. In general, positions of XRPD peaks are expected to individually vary on a measurement-by-measurement basis by about ±0.2°2θ. In general, as understood in the art, two XRPD patterns match one another if the characteristic peaks of the first pattern are located at approximately the same positions as the characteristic peaks of the second pattern. As understood in the art, determining whether two XRPD patterns match or whether individual peaks in two XRPD patterns match may require consideration of individual variables and parameters such as, but not limited to, preferred orientation, phase impurities, degree of crystallinity, particle size, variation in diffractometer instrument setup, variation in XRPD data collection parameters, and/or variation in XRPD data processing, among others. The determination of whether two patterns match may be performed by eye and/or by computer analysis. Examples of XRPD patterns collected and analyzed using these methods and parameters are provided herein, e.g., as FIG. **1**, FIG. **5**, FIG. **9**, FIG. **13**, FIG. **17**, FIG. **21** and FIG. **25**.

Differential Scanning Calorimetry (DSC) analyses were performed on a TA Instruments Q1000™. About 5 mg of sample was placed into a tared DSC pan and the weight of the sample was accurately recorded. Typically, the sample was heated under nitrogen at a rate of about 10° C./min from about 25° C. up to a final temperature of about 200° C. Typically, thermal events were reported as extrapolated onset temperatures. Examples of DSC thermograms collected and analyzed using these methods and parameters are provided herein, e.g., as FIG. **2**, FIG. **6**, FIG. **10**, FIG. **14**, FIG. **18**, FIG. **22** and FIG. **26**.

Thermal Gravimetric Analyses (TGA) were performed on a TA Instruments Q500™. Calcium oxalate was used for calibration. About 10 mg of sample was placed on a pan, accurately weighed and loaded into the TGA furnace. The sample was heated under nitrogen at a rate of about 10° C./min from about 25° C. up to a final temperature of about 200° C. Examples of TGA thermograms collected and analyzed using these methods and parameters are provided herein, e.g., as FIG. **3**, FIG. **7**, FIG. **11**, FIG. **15**, FIG. **19**, FIG. **23** and FIG. **27**.

Solvation solvents were identified and quantified by TG-IR experiments using a TA Instruments Q500™ TGA interfaced with a Thermo Nicolet AEM Fourier transform IR spectrophotometer. Typically a sample size of about 20-50 mg was weighed into an aluminum pan and heated to about 200° C. During the TGA run, the vapor was transferred to the cell through a heated transfer line. The temperature of both transfer line and the cell were set at about 225° C. IR spectra were collected every 10-second repeat time. Volatiles were identified from a search of the Aldrich vapor phase spectral library and the library match results are presented to show the identified vapor.

Morphology and particle size analysis of the samples were carried out using an Olympus microscope. The instrument was calibrated with USP standards. D_{90} values were determined using the software Image Plus—Material Plus. The D_{90} value represents the 90th percentile of the particle size distribution as measured by length; i.e., 90% of the particles have a length of this value or less.

5.12.3. Solid Form Screening Study Results

Solid forms comprising Compound A which were prepared during the solid form screening studies included Forms A, B, C, D, E, F, G and an amorphous form. Representative XRPD patterns, DSC plots, TGA plots and DVS plots for each of Forms A, B, C, D, E, F and G are provided herein as FIG. **1**-FIG. **28**.

Solubility Studies. The approximate solubility of Form B of Compound A in various solvents at about 25° C. was determined. Results are shown in Table 6. Form B was found to be most soluble in acetone, acetonitrile, methylene chlo-

ride, methyl ethyl ketone and tetrahydrofuran (greater than about 50 mg/mL) followed by ethyl acetate (about 30.15 mg/mL). Form B was also found to have low solubility in several solvents including n-butanol, heptane, 2-propanol, toluene and water (less than about 1 mg/mL).

Solution Evaporation Studies. Results from solution evaporation studies performed at about 25° C. and about 50° C. are summarized in Table 7.

Equilibration Studies. Results from equilibration studies performed at about 25° C. and about 50° C. are summarized in Table 8.

Cooling Crystallization Studies. Results from cooling crystallization studies are summarized in Table 9. Cooling crystallization studies yielded crystalline material from numerous solvents, including acetone, acetonitrile, n-butyl acetate, ethyl acetate, methanol, methylene chloride, methyl ethyl ketone (MEK) and tetrahydrofuran (THF). The crystalline materials obtained were typically characterized by XRPD, DSC and TGA.

Solvent/Anti-Solvent Precipitation Studies. Results from solvent/anti-solvent precipitation studies are summarized in Table 10. When heptane, water and toluene were added to Form B in THF solution at about 40° C., precipitates formed immediately. When heptane, methyl t-butyl ether (MTBE), toluene and water were added to Form B in acetonitrile solution separately at about 25° C., either a clear solution or a mixture formed. Crystalline material from MTBE/acetonitrile, water/acetonitrile and toluene/acetonitrile was obtained after stirring overnight. However, no crystallization occurred for heptane/acetonitrile mixture. When water was added to Form B in methanol solution at about 50° C., precipitates formed immediately and when heptane and toluene were added to Form B in methanol solution separately at about 50° C., either a clear solution or a mixture formed. Crystalline material from toluene/methanol and heptane/methanol was obtained after stirring overnight. When toluene was added to Form B in methylene chloride solution at about 25° C., precipitates formed immediately and when MTBE was added to Form B in methylene chloride solution at about 25° C., a clear solution was obtained. Crystalline material from MTBE/methylene chloride was obtained after stirred overnight. However, no crystallization occurred when heptane was added to Form B in methylene chloride solution. When heptane was added to Form B in MEK solution at about 50° C., precipitates formed immediately and when MTBE and toluene were added to Form B in MEK solution separately at about 50° C., clear solutions were obtained. Crystalline material from MTBE/MEK and toluene/MEK was obtained after stirring overnight. When heptane was added to Form B in n-butyl acetate solution at about 50° C., precipitates formed immediately and when MTBE and toluene were added to Form B in MEK solution separately at about 50° C., clear solutions were obtained. Crystalline material from MTBE/n-butyl acetate and toluene/n-butyl acetate was obtained after stirring overnight. When water and toluene were added to Form B in acetone solution separately at about 40° C., precipitates formed immediately and when ethanol and 2-propanol were added to Form B in acetone solution separately at about 40° C., clear solutions were obtained. Crystalline material from ethanol/acetone and 2-propanol/acetone were obtained after stirring overnight. Crystalline materials obtained were identified by XRPD, DSC, TGA.

Stability Studies. Stability study results are summarized in Table 11. The stabilities of Forms A, B, C and D were studied by exposing the solid samples to the stress condition of 40° C./75% RH for four weeks. Moreover, the stabilities of Forms A, B, C and D in different solvents were studied by equilibration in different solvents at 40° C. for four weeks. The slurries then were filtered and dried in the air. Solid samples obtained from the stability experiments were analyzed by XRPD and DSC.

Interconversion Studies. Results from interconversion studies are summarized in Table 12.

Compression Studies. Compression tests were performed on Forms A, B, C, D, E, F and G of Compound A. Each form studied was found to be substantially physically stable as observed by XRPD analysis.

Hygroscopicity Studies. Hygroscopicity (moisture sorption/desorption) studies were performed on Forms A, B, C, D, E, F and G. Each of the solid samples were analyzed by XRPD after undergoing a full adsorption/desorption cycle in the DVS system. XRPD results indicated that none of the forms analyzed underwent substantial solid-state transformation as a result of DVS analysis.

TABLE 6

Solubility Study on Form B

Solvent System	Approximate Solubility (mg/ml)
Acetone	>50
Acetonitrile	>50
n-Butanol	>0.72
n-Butyl acetate	9.75
Absolute ethanol	1.38
Ethyl acetate	30.15
Heptane	0.41
Methylene chloride	>50
Methyl ethyl ketone	>50
Methanol	4.05
Methyl t-butyl ether	1.17
2-Propanol	0.81
Tetrahydrofuran	>50
Toluene	0.90
Water	0.69
Ethanol:Water (1:1)	2.86

TABLE 7

Solution Evaporation Studies

Starting Form	Solvent System	Evaporation Temp. (° C.)	XRPD Analysis	DSC thermal events
B	Acetone	25	Form B	
B	Acetonitrile	25	Form B + Form E	77.28° C.; 151.84° C.
B	n-Butyl acetate	25	Form B	
B	Ethyl acetate	25	Form B	
B	Methylene chloride	25	Form D	93.11° C.
B	Methyl ethyl ketone	25	Form B	
B	Tetrahydrofuran	25	Form B	
B	Ethanol:Water (1:1)	25	Form B	
A	Acetonitrile	25	Form E	95.42° C. (TGA wt. loss = 3.56%)
A	Methylene chloride	25	Form D	97.23° C.
A	Acetone	50	Form B	
A	Acetonitrile	50	Form B	
A	n-Butyl acetate	50	Form B	
A	Ethyl acetate	50	Form B	
A	Methyl ethyl ketone	50	Form B	
A	Tetrahydrofuran	50	Form B	
A	Ethanol:Water (1:1)	50	Form B	

TABLE 8

Equilibration Studies

Starting Form	Solvent System	Equilib. Temp. ° C.	XRPD Analysis	DSC Thermal Events
B	n-Butanol	25	Form B	
B	n-Butyl acetate	25	Form B	
B	Ethanol	25	Form B	
B	Ethyl acetate	25	Form B	
B	Heptane	25	Form B	
B	Methanol	25	Form B	
B	Methyl t-butyl ether	25	Form B	
B	2-Propanol	25	Form B	
B	Toluene	25	Form C	159.31° C.
B	Toluene (evap. at 60° C.)	25	Form C	Broad multiplet
B	Toluene:Acetone (9:1) (evap. at 100° C.)	25	Form C	Broad multiplet (TGA wt. loss = 5.90%)
B	Water	25	Form B	
B	Water (50 days)	25	Form B	
A	Ethanol	25	Form F	145.06° C. (multiplet)
A	Heptane	25	Form A	
A	Ethyl acetate	25	Form G	108.96° C.
A	Water	25	Form A	
A	Toluene	25	Form C	170.18° C. (TGA wt. loss = 5.86%)
A	Toluene (evap. at 60° C.)	25	Form C	167.84° C.
A	Toluene:Acetone (9:1) (evap. at 100° C.)	25	Form C	Broad multiplet
A	Acetone:Ethanol (1:1)	25	Form B	154.00° C. (main)

TABLE 8-continued

Equilibration Studies

Starting Form	Solvent System	Equilib. Temp. ° C.	XRPD Analysis	DSC Thermal Events
A	Ethanol:Water (1:1)	25	Form F	145.22° C.
A	n-Butanol	50	Form B	
A	n-Butyl acetate	50	Form B	
A	Ethanol	50	Form B	
A	Heptane	50	Form B	
A	Methanol	50	Form B	
A	Methyl t-butyl ether	50	Form B	
A	2-Propanol	50	Form B	
A	Toluene	50	Form C	165.30° C. (multiplet)
A	Water	50	Form B	
A	Ethanol:Water (1:1)	50	Form B	

TABLE 9

Cooling Crystallization Studies

Starting Form	Solvent System	Analysis by XRPD	DSC Thermal Events
B	Acetone	Form E	
B	Acetonitrile	Form E	95.42° C.
B	n-Butyl acetate	Form B	
B	Ethyl acetate	Form B	
B	Methylene Chloride	Form D	100.90° C.
B	Methanol	Form B	
B	Methyl ethyl ketone	Form B	
B	THF	Form H	

TABLE 10

Solvent/Anti-Solvent Precipitation Studies

Starting Form	Solvent*	Anti-Solvent*	Ratio (Solvent:Antisolvent) & Temp.	Analysis by XRPD	DSC Thermal Events
B	Acetone	Ethanol	1:8 at 40° C.	Form B	
B	Acetone	2-Propanol	1:10 at 40° C.	Form B	
B	Acetone	Water	1:4 at 40° C.	Form B	
B	Acetone	Toluene	1:10 at 40° C.	Form C	167.57° C. (broad)
B	Acetonitrile	Heptane	1:8 at 25° C.	Form B	
B	Acetonitrile	MtBE	1:8 at 25° C.	Form B	
B	Acetonitrile	Water	1:6 at 25° C.	Form B	
B	Acetonitrile	Toluene	1:8 at 50° C.	Form C	167.97° C.
B	Methyl ethyl ketone	Heptane	1:3 at 50° C.	Form B	
B	MEK	MtBE	1:4 at 50° C.	Form B	
B	MEK	Toluene	1:3 at 50° C.	Form C	168.22° C.
B	n-Butyl acetate	Heptane	1:4 at 50° C.	Form B	
B	n-Butyl acetate	MtBE	1:4 at 50° C.	Form B	
B	n-Butyl acetate	Toluene	1:4 at 50° C.	Form B	
B	DCM	Heptane	1:8 at 25° C.	Form E + B	89.65° C.; 149.81° C.
B	DCM	MtBE	1:15 at 25° C.	Form B	
B	DCM	Toluene	1:15 at 25° C.	Form B	167.99° C. (multiplet)
B	Methanol	Heptane	1:3 at 50° C.	Form B	
B	Methanol	Water	1:3 at 50° C.	Form B	
B	Methanol	Toluene	1:3 at 50° C.	Form C	168.37° C. (multiplet)
B	Tetrahydrofuran	Heptane	1:6 at 40° C.	Form B	
B	Tetrahydrofuran	Water	1:6 at 40° C.	Form B	
B	Tetrahydrofuran	Toluene	1:6 at 40° C.	Form C	168.64° C. (multiplet)

*Abbreviations:

MEK = methyl ethyl ketone;

DCM = dichloromethane (i.e., methylene chloride);

MtBE = methyl t-butyl ether

TABLE 11

Stability Studies

Starting Form	Test Conditions ("EQ" = equilibrate; "RH" = relative humidity)	Appearance	Analysis by XRPD
Form A	40° C./75% RH; 4 weeks	White solid	Form A
Form B	40° C./75% RH; 4 weeks	White solid	Form B
Form C	40° C./75% RH; 4 weeks	Yellow solid	Form C
Form D	40° C./75% RH; 4 weeks	White solid	Form D
Form A	EQ in ethanol at 40° C. for 4 weeks		Form F
Form A	EQ in heptane at 40° C. for 4 weeks		Form A
Form A	EQ in water at 40° C. for 4 weeks		Form A
Form A	EQ in toluene at 40° C. for 4 weeks		Form C
Form B	EQ in ethanol at 40° C. for 4 weeks		Form B
Form B	EQ in heptane at 40° C. for 4 weeks		Form B
Form B	EQ in water at 40° C. for 4 weeks		Form B
Form B	EQ in toluene at 40° C. for 4 weeks		Form B
Form C	EQ in ethanol at 40° C. for 4 weeks		Form C
Form C	EQ in heptane at 40° C. for 4 weeks		Form C
Form C	EQ in water at 40° C. for 4 weeks		Form C
Form C	EQ in toluene at 40° C. for 4 weeks		Form C
Form D	EQ in ethanol at 40° C. for 4 weeks		Form B
Form D	EQ in heptane at 40° C. for 4 weeks		Form B
Form D	EQ in water at 40° C. for 4 weeks		Form B
Form D	EQ in toluene at 40° C. for 4 weeks		Form C

TABLE 12

Interconversion Studies

Starting Form	Test Conditions ("EQ" = equilibrate)	Analysis by XRPD
Mixture of Forms A, B, C, D, E, F and G	EQ in acetone:ethanol (1:1) at 25° C.	Form B + C + F
Form A	EQ in acetone:ethanol (1:1) at 25° C.	Form B
Form C	EQ in acetone:ethanol (1:1) at 25° C.	Form C
Form D	EQ in acetone:ethanol (1:1) at 25° C.	Form B
Form E	EQ in acetone:ethanol (1:1) at 25° C.	Form B
Form F	EQ in acetone:ethanol (1:1) at 25° C.	Form F
Form G	EQ in acetone:ethanol (1:1) at 25° C.	Form B

5.13. Example 13

200 Mg Dosage Capsule

Table 13 illustrates a batch formulation and single dosage formulation for a single dose unit containing 200 mg of a solid form comprising Compound A, i.e., about 40 percent by weight, in a size #0 capsule.

TABLE 13

Formulation for 200 mg capsule

Material	Percent By Weight	Quantity (mg/tablet)	Quantity (kg/batch)
Compound A	40.0%	200 mg	16.80 kg
Pregelatinized Corn Starch, NF5	9.5%	297.5 mg	24.99 kg
Magnesium Stearate	0.5%	2.5 mg	0.21 kg
Total	100.0%	500 mg	42.00 kg

The pregelatinized corn starch (SPRESS™ B-820) and Compound A components are passed through a 710 μm screen and then are loaded into a Diffusion Mixer with a baffle insert and blended for 15 minutes. The magnesium stearate is passed through a 210 μm screen and is added to the Diffusion Mixer. The blend is then encapsulated in a size #0 capsule, 500 mg per capsule (8400 capsule batch size) using a Dosator type capsule filling machine.

5.14. Example 14

100 Mg Oral Dosage Form

Table 14 illustrates a batch formulation and a single dose unit formulation containing 100 mg of a solid form comprising Compound A.

TABLE 14

Formulation for 100 mg tablet

Material	Percent by Weight	Quantity (mg/tablet)	Quantity (kg/batch)
Compound A	40%	100.00	20.00
Microcrystalline Cellulose, NF	53.5%	133.75	26.75
Pluronic F-68 Surfactant	4.0%	10.00	2.00
Croscarmellose Sodium Type A, NF	2.0%	5.00	1.00
Magnesium Stearate, NF	0.5%	1.25	0.25
Total	100.0%	250.00 mg	50.00 kg

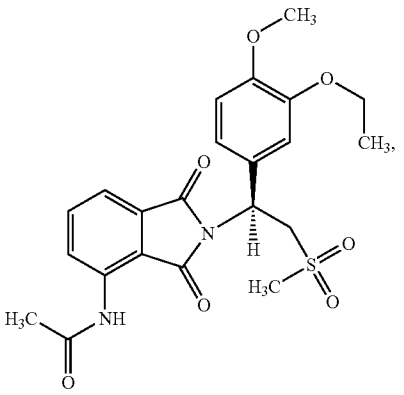
The microcrystalline cellulose, croscarmellose sodium, and Compound A components are passed through a #30 mesh screen (about 430μ to about 655μ). The Pluronic F-68® (manufactured by JRH Biosciences, Inc. of Lenexa, Kans.) surfactant is passed through a #20 mesh screen (about 457% to about 1041μ). The Pluronic F-68® surfactant and 0.5 kgs of croscarmellose sodium are loaded into a 16 qt. twin shell tumble blender and are mixed for about 5 minutes. The mix is then transferred to a 3 cubic foot twin shell tumble blender where the microcrystalline cellulose is added and blended for about 5 minutes. The solid form comprising Compound A is added and blended for an additional 25 minutes. This pre-blend is passed through a roller compactor with a hammer mill attached at the discharge of the roller compactor and moved back to the tumble blender. The remaining croscarmellose sodium and magnesium stearate is added to the tumble blender and blended for about 3 minutes. The final mixture is compressed on a rotary tablet press with 250 mg per tablet (200,000 tablet batch size).

While the invention has been described with respect to the particular embodiments, it will be apparent to those skilled in the art that various changes and modifications may be made without departing from the spirit and scope of the invention as defined in the claims. Such modifications are also intended to fall within the scope of the appended claims.

What is claimed is:

1. A Form B crystal form of the compound of Formula (I):

(I)



which is enantiomerically pure, and which has an X-ray powder diffraction pattern comprising peaks at about 10.1, 13.5, 20.7, and 26.9 degrees 2θ.

2. The crystal form of claim **1**, which has an X-ray powder diffraction pattern further comprising peaks at about 12.4, 15.7, 18.1, and 24.7 degrees 2θ.

3. The crystal form of claim **2**, which has an X-ray powder diffraction pattern further comprising peaks at about 16.3, 22.5, 26.2, and 29.1 degrees 2θ.

4. The crystal form of claim **1**, which has an X-ray powder diffraction pattern matching the pattern depicted in FIG. **5**.

5. The crystal form of claim **1**, which has a differential scanning calorimetry plot comprising an endothermic event with an onset temperature of about 154° C.

6. The crystal form of claim **1**, which has a differential scanning calorimetry plot matching the plot depicted in FIG. **6**.

7. The crystal form of claim **1**, which has a thermal gravimetric analysis plot comprising a mass loss of less than about 1% when heated from about 25° C. to about 140° C.

8. The crystal form of claim **7**, wherein the mass loss is about 0.25%.

9. The crystal form of claim **1**, which has a thermal gravimetric analysis plot matching the plot depicted in FIG. **7**.

10. The crystal form of claim **1**, which exhibits a mass increase of less than about 1% when subjected to an increase in relative humidity from about 0% to about 95% relative humidity.

11. The crystal form of claim **10**, wherein the mass increase is about 0.6%.

12. The crystal form of claim **1**, which has a moisture sorption isotherm plot matching the plot depicted in FIG. **8**.

13. The crystal form of claim **1**, which is stable upon exposure to about 40° C. and about 75% relative humidity for about 4 weeks.

14. The crystal form of any one of claims **1** and **2** to **13**, which is substantially pure.

15. A solid pharmaceutical composition comprising the crystal form of any one of claims **1** and **2** to **13**.

* * * * *

EXHIBIT F

US008455536B2

(12) **United States Patent**
Muller et al.

(10) **Patent No.: US 8,455,536 B2**
(45) **Date of Patent: *Jun. 4, 2013**

(54) **METHODS OF USING (+)-2-[1-(3-ETHOXY-4-METHOXYPHENYL)-2-METHYLSULFONYLETHYL]-4-ACETYLAMINOISOINDOLINE 1,3-DIONE**

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(73) Assignee: **Celgene Corporation**, Summit, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 607 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **12/630,788**

(22) Filed: **Dec. 3, 2009**

(65) **Prior Publication Data**

US 2010/0160405 A1 Jun. 24, 2010

Related U.S. Application Data

(60) Continuation of application No. 12/098,379, filed on Apr. 4, 2008, now Pat. No. 7,659,303, which is a division of application No. 11/170,308, filed on Jun. 28, 2005, now Pat. No. 7,358,272, which is a division of application No. 10/392,195, filed on Mar. 19, 2003, now Pat. No. 6,962,940.

(60) Provisional application No. 60/438,450, filed on Jan. 7, 2003, provisional application No. 60/366,515, filed on Mar. 20, 2002.

(51) **Int. Cl.**
A61K 31/4035 (2006.01)
C07D 209/44 (2006.01)

(52) **U.S. Cl.**
USPC **514/417**; 548/469; 548/478

(58) **Field of Classification Search**
USPC 548/469, 478; 514/417
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,031,450	A	4/1962	Fischer et al.
3,322,755	A	5/1967	Roch et al.
3,920,636	A	11/1975	Takahashi et al.
4,001,237	A	1/1977	Partyka et al.
4,001,238	A	1/1977	Partyka et al.
4,047,404	A	9/1977	Hayashi
4,060,615	A	11/1977	Matier et al.
4,101,548	A	7/1978	Crenshaw et al.
4,162,316	A	7/1979	Nishimura et al.
4,209,623	A	6/1980	Juby
4,880,810	A	11/1989	Lowe, III
4,885,301	A	12/1989	Coates
5,147,875	A	9/1992	Coates et al.
5,354,571	A	10/1994	Morikawa et al.
5,401,774	A	3/1995	Pamukcu et al.
5,439,895	A	8/1995	Lee et al.
5,488,055	A	1/1996	Kumar et al.
5,608,914	A	3/1997	Keesler
5,614,530	A	3/1997	Kumar et al.
5,614,627	A	3/1997	Takase et al.
5,658,940	A	8/1997	Muller et al.
5,698,579	A	12/1997	Muller
5,703,098	A	12/1997	Muller et al.
5,710,170	A	1/1998	Guay et al.
5,728,844	A	3/1998	Muller et al.
5,728,845	A	3/1998	Muller et al.
5,736,570	A	4/1998	Muller et al.
5,798,373	A	8/1998	Warrellow
5,801,195	A	9/1998	Muller et al.
5,849,770	A	12/1998	Iiead et al.
5,877,200	A	3/1999	Muller
5,891,896	A	4/1999	Warrellow et al.
6,011,050	A	1/2000	Muller et al.
6,020,339	A	2/2000	Perrier et al.
6,020,358	A	2/2000	Muller et al.
6,034,089	A	3/2000	Han et al.
6,046,221	A	4/2000	Muller et al.
6,069,156	A	5/2000	Oku et al.
6,162,830	A	12/2000	Connor et al.
6,166,041	A	12/2000	Cavalla et al.
6,177,471	B1	1/2001	Menander et al.
6,204,275	B1	3/2001	Friesen et al.
6,218,400	B1	4/2001	Daugan et al.
6,300,335	B1	10/2001	Campbell et al.

(Continued)

FOREIGN PATENT DOCUMENTS

EP	0 347 146	6/1989
EP	0 349 239 A2	6/1989

(Continued)

OTHER PUBLICATIONS

U.S. Appl. No. 60/454,155, filed Mar. 12, 2003, Muller, G. et al.
U.S. Appl. No. 60/454,149, filed Mar. 12, 2003, Muller, G. et al.
U.S. Appl. No. 60/438,448, filed Jan. 7, 2003, Muller, G. et al.
U.S. Appl. No. 60/436,975, filed Dec. 30, 2002, Muller, G. et al.
U.S. Appl. No. 60/366,515, filed Mar. 20, 2002, Muller, G. et al.
Akazome, M. et al., 1997, "Asymmetric recognition of 1-arylethylamines by (R)-phenylglycyl-(R)-phenylglycine and its mechanism," Tetrahedron: Asymmetry, Elsevier Scince Publishers, Amsterdam, NL, 8(14):2331-2336.
Au et al., 1998, *Brit. J. Pharm.* 123:1260-1266.
Baehr et al., 1979, *J. Biol. Chem.* 254:11669.
Baughman et al., 1990, *J. Lab. Clin. Med.* 115:36-42.
Beavo and Reifsnyder, *Trends in Pharm.*, 11, 150-55, 1990.

(Continued)

Primary Examiner — Golam M M Shameem
(74) *Attorney, Agent, or Firm* — Jones Day

(57) **ABSTRACT**

Stereomerically pure (+)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, substantially free of its (−) isomer, and prodrugs, metabolites, polymorphs, salts, solvates, hydrates, and clathrates thereof are discussed. Also discussed are methods of using and pharmaceutical compositions comprising the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione are disclosed. The methods include methods of treating and/or preventing disorders ameliorated by the reduction of levels of TNF-α or the inhibition of PDE4.

56 Claims, 2 Drawing Sheets

U.S. PATENT DOCUMENTS

6,316,472	B1	11/2001	Frenette et al.	
6,333,354	B1	12/2001	Schudt	
6,962,940	B2	11/2005	Muller et al.	
7,208,516	B2	4/2007	Muller et al.	
7,276,529	B2	10/2007	Muller et al.	
7,358,272	B2	4/2008	Muller et al.	
7,427,638	B2	9/2008	Muller et al.	
7,507,759	B2	3/2009	Muller et al.	
7,659,302	B2 *	2/2010	Muller et al.	514/417
7,659,303	B2 *	2/2010	Muller et al.	514/417

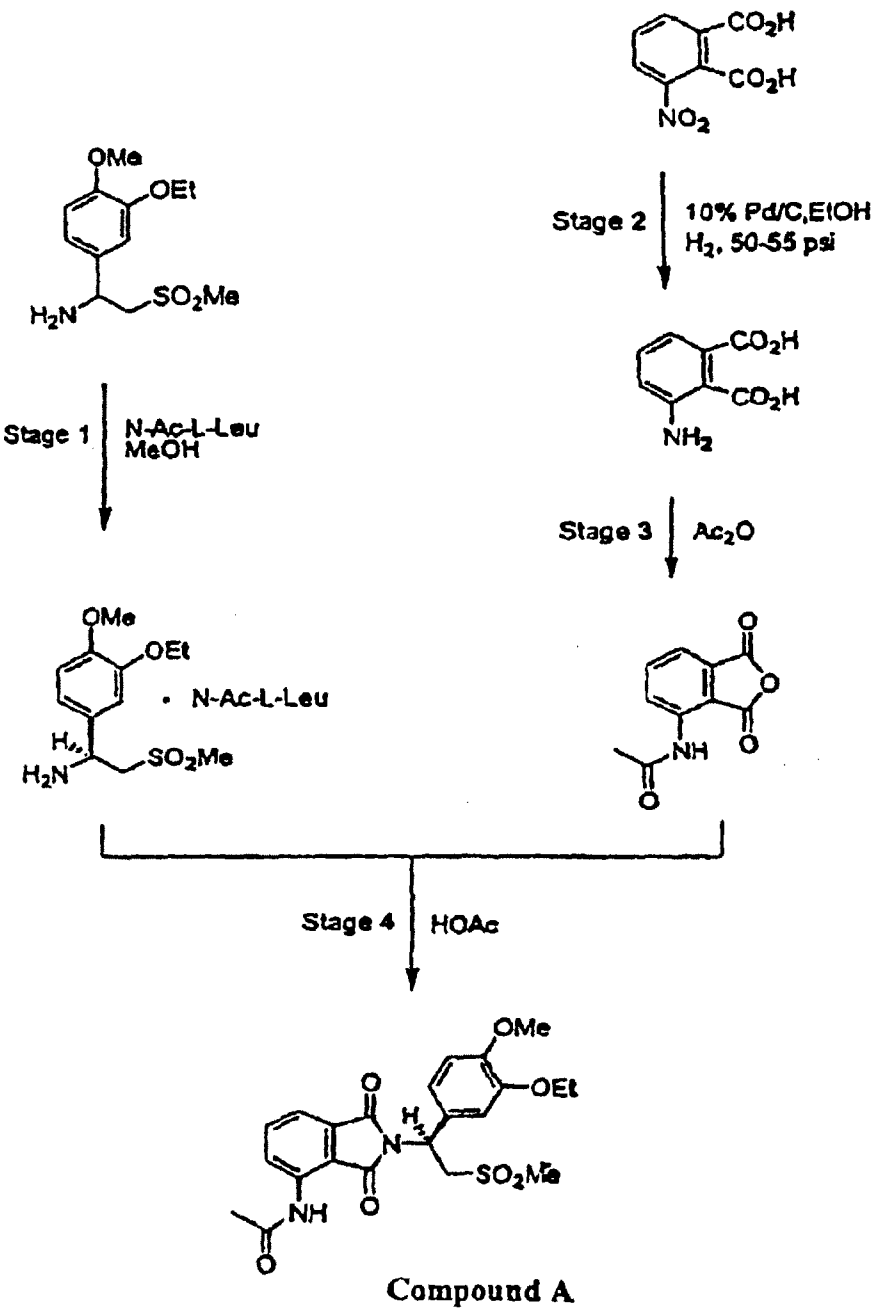
FOREIGN PATENT DOCUMENTS

EP	0 351 058		6/1989
EP	0 352 960	A2	7/1989
EP	0 395 328		4/1990
EP	0 428 268	A2	10/1990
EP	0 463 756	A1	6/1991
EP	0 526 004	A1	7/1992
EP	0 607 439	A1	9/1992
EP	0 722 937	A1	1/1996
EP	0 722 943	A1	1/1996
EP	0 722 944	A1	1/1996
GB	2 063 249	A	9/1980
WO	WO 93/07149		4/1993
WO	WO 93/12095		6/1993
WO	WO 94/01728		1/1994
WO	WO 94/05661		3/1994
WO	WO 94/29277		12/1994
WO	WO 95/19978		7/1995
WO	WO 96/32379		10/1996
WO	WO 97/03070		1/1997
WO	WO 97/03675		2/1997
WO	WO 97/03985		2/1997
WO	WO 97/24334		7/1997
WO	WO 98/06722		2/1998
WO	WO 98/08848		3/1998
WO	WO 98/14448		4/1998
WO	WO 98/16521		4/1998
WO	WO 98/17668		4/1998
WO	WO 98/23597		6/1998
WO	WO 98/38168		9/1998
WO	WO 99/06041		2/1999

OTHER PUBLICATIONS

Bissonnette et al., 1989, *Inflammation* 13:329-339.
Bloom and Beavo 1996, *Proc. Natl. Acad. Sci. USA* 93:14188-14192.
Brackeen, M.F. et al., 1995, "Design and synthesis of conformationally constrained analogues of 4-(3-butoxy-4-methoxybenzyl) imidazolidin-2-one (Ro 20-1724) as potent inhibitors of cAMP-specific phosphodiesterase", *J. Med. Chem.* 38:4848-54.
Carstensen, Jens T., 1995, *Drug Stability: Principles& Practice*, 2nd ed., Marcel Dekker, New York, NY pp. 379-380.
Casini et al., 1964, Farmaco Ed. Sci. 19:563.
Clouse et al., 1989, *J. Immunol.* 142:431-438.
Derian et al., 1995, J. Immunol. 154:308-317.
Duh et al., 1989, *Proc. Nat. Acad. Sci.* 86:5974-5978.
Featherstone, R.L., et al., 2000, "Comparison of phosphodiesterase inhibitors of differing isoenzyme selectivity added to St. Thomas' hospital cardioplegic solution used for hypothermic preservation of rat lungs", Am. J. Respir. Crit. Care Med. 162:850-6.
Gillespie et al., 1989, *Mol. Pharm.* 36:773.
Hidaka and Asano 1976, *Biochem. Biophys. Acta* 429:485.
Hinshaw et al. 1990, *Circ. Shock* 30:2797-292.
Holler et al., 1990, *Blood* 75:1011-1016.
Johnson et al., 1989,*Endocrinology* 124:1424-1427.
List et al., 1990, *J. Clin. Oncol.* 8:1424.MDS.
Luke, G.P. et al., 1999, "Synthesis of (S)-5-(1-aminoethyl)-2-(cyclohexylmethoxy) benzamide," Tetrahedron: Asymmetry, Elsevier Science Publishers, Amsterdam, NL, 10(22):4393-4403.
Merck Manual (1999) 17th ed., 953.
Monté et al., 1990, *Blood* 79:2670.
Muller, George, et al., 1999, *Bioorganic & Medicinal Chemistry Letters* 9; pp. 1625-1630.
Muller et al., 1998, *Bioorg. & Med Chem Lett.* 8:2669-2674.
Muller, et al., 1996, *J. Med. Chem.* 39:3238.
Nicholson et al., 1991, *Trends Pharmaco. Sci.* 12:19.
Shealy et al., 1965, "D- and L-thalidomide." *Chem. Indus.* 12;24:1030-1.
Tierney, et al., ed., 1998, Current Medical Diagnosis & Treatment, 37th ed., Appleton & Lange, pp. 499.
Verghese, et al., *Journal of Pharmacology and Experimental Therapeutics*, 272(3), 1313-1320, 1995.
Van Dullemen et al., 1995, *Gastroenterology*, 109:129-135.
Wilen, S.H., et al., 1977, *Tetrahedron* 33:2725.
Wilen, S.H., 1972, *Tables of Resolving Agents and Optical Resoutions* (E.L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, IN) p. 268.
Wolff, Manfred E., *ed., Burger's Medicinal Chemistry and Drug Discovery*, 5th ed. 1995 172-178, 949-982.
Dredge et al., 2003, "Angiogenesis inhibitors in cancer therapy," *Curr. Opin. Investig. Drugs* 4(6):667-674.
Dredge et al., 2002, "Immunological effects of thalidomide and its chemical and functional analogs," *Crit. Rev. Immunol.* 22(5-6):425-437.
Dredge et al., 2002, "Recent developments in antiangiogenic therapy," *Expert Opin. Biol. Ther.* 2(8):953-966.
Gee et al., 2003, "Selective cytokine inhibitory drugs with enhanced antiangiogenic activity control tumor growth through vascular inhibition," *Cancer Res.* 63(23):8073-8078.
Marriott et al., 2001, "Immunotherapeutic and antitumour potential of thalidomide analogues," *Expert Opin. Biol. Ther.* 1(4):1-8.
Marriott et al., 2002, "Thalidomide and its analogues have distinct and opposing effects on TNF-alpha and TNFR2 during co-stimulation of both CD4(+) and CD8(+) T cells," *Clin. Exp. Immunol.* 130(1):75-84.
Molostvov et al., 2004, "The effects of selective cytokine inhibitory drugs (CC-10004 and CC-1088) on VEGF and IL-6 expression and apoptosis in myeloma and endothelial cell co-cultures," *Br. J. Haematol.* 124(3):366-375.

* cited by examiner



Compound A

FIG. 1

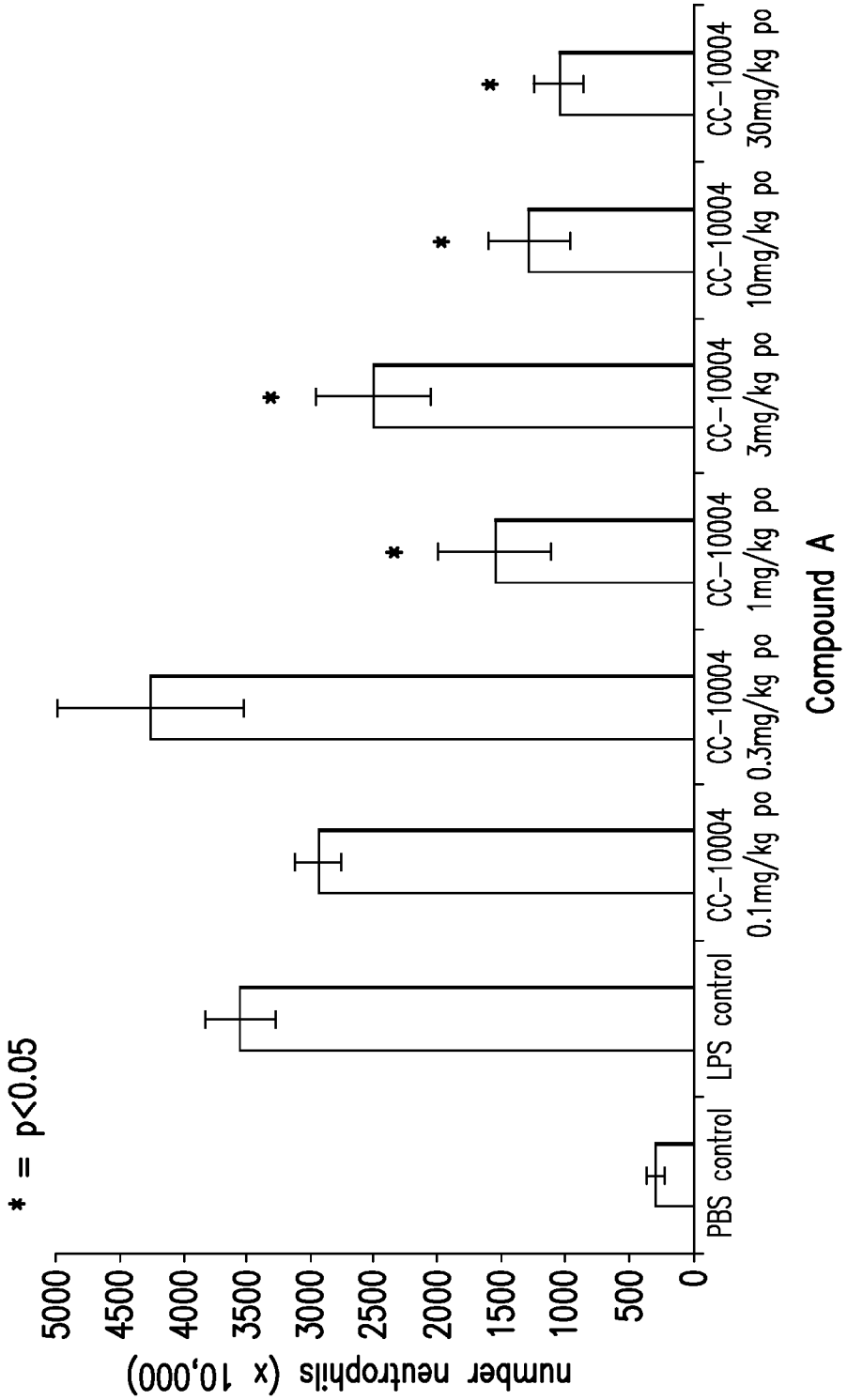

number neutrophils (x 10,000)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
* = p<0.05
PBS control
LPS control
CC-10004 0.1mg/kg po
CC-10004 0.3mg/kg po
CC-10004 1mg/kg po
CC-10004 3mg/kg po
CC-10004 10mg/kg po
CC-10004 30mg/kg po
Compound A

FIG.2

METHODS OF USING (+)-2-[1-(3-ETHOXY-4-METHOXYPHENYL)-2-METHYLSULFONYLETHYL]-4-ACETYLAMINOISOINDOLINE 1,3-DIONE

This application is a CON of 12/098,379 filed Apr. 4, 2008, now U.S. Pat. No. 7,659,303 which claims the benefit of U.S. Provisional Application No. 60/366,515 filed Mar. 20, 2002 and U.S. Provisional Application No. 60/438,450 filed Jan. 7, 2003.

1. FIELD OF INVENTION

The invention relates to methods of using and compositions comprising the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

2. BACKGROUND OF THE INVENTION

Tumor necrosis factor alpha, (TNF-α) is a cytokine that is released primarily by mononuclear phagocytes in response to immunostimulators. TNF-α is capable of enhancing most cellular processes, such as differentiation, recruitment, proliferation, and proteolytic degradation. At low levels, TNF-α confers protection against infective agents, tumors, and tissue damage. But TNF-α also has a role in many diseases. When administered to mammals or humans, TNF-α causes or aggravates inflammation, fever, cardiovascular effects, hemorrhage, coagulation, and acute phase responses similar to those seen during acute infections and shock states. Enhanced or unregulated TNF-α production has been implicated in a number of diseases and medical conditions, for example, cancers, such as solid tumors and blood-born tumors; heart disease, such as congestive heart failure; and viral, genetic, inflammatory, allergic, and autoimmune diseases.

Adenosine 3',5'-cyclic monophosphate (cAMP) also plays a role in many diseases and conditions, such as but not limited to asthma and inflammation, and other conditions (Lowe and Cheng, *Drugs of the Future,* 17(9), 799-807, 1992). It has been shown that the elevation of cAMP in inflammatory leukocytes inhibits their activation and the subsequent release of inflammatory mediators, including TNF-α and NF-κB. Increased levels of cAMP also leads to the relaxation of airway smooth muscle.

It is believed that the primary cellular mechanism for the inactivation of cAMP is the breakdown of cAMP by a family of isoenzymes referred to as cyclic nucleotide phosphodiesterases (PDE) (Beavo and Reitsnyder, *Trends in Pharm.,* 11, 150-155, 1990). There are eleven known PDE families. It is recognized, for example, that the inhibition of PDE type IV is particularly effective in both the inhibition of inflammatory mediator release and the relaxation of airway smooth muscle (Verghese, at, *Journal of Pharmacology and Experimental Therapeutics,* 272(3), 1313-1320, 1995). Thus, compounds that inhibit PDE4 (PDE IV) specifically, may inhibit inflammation and aid the relaxation of airway smooth muscle with a minimum of unwanted side effects, such as cardiovascular or anti-platelet effects. Currently used PDE4 inhibitors lack the selective action at acceptable therapeutic doses.

Cancer is a particularly devastating disease, and increases in blood TNF-α levels are implicated in the risk of and the spreading of cancer. Normally, in healthy subjects, cancer cells fail to survive in the circulatory system, one of the reasons being that the lining of blood vessels acts as a barrier to tumor-cell extravasation. But increased levels of cytokines have been shown to substantially increase the adhesion of cancer cells to endothelium in vitro. One explanation is that cytokines, such as TNF-α, stimulate the biosynthesis and expression of a cell surface receptors called ELAM-1 (endothelial leukocyte adhesion molecule). ELAM-1 is a member of a family of calcium-dependent cell adhesion receptors, known as LEC-CAMs, which includes LECAM-1 and GMP-140. During an inflammatory response, ELAM-1 on endothelial cells functions as a "homing receptor" for leukocytes. Recently, ELAM-1 on endothelial cells was shown to mediate the increased adhesion of colon cancer cells to endothelium treated with cytokines (Rice at, 1989, *Science* 246:1303-1306).

Inflammatory diseases such as arthritis, related arthritic conditions (e.g., osteoarthritis and rheumatoid arthritis), inflammatory bowel disease (e.g., Crohn's disease and ulcerative colitis), sepsis, psoriasis, atopic dermatitis, contact dermatitis, and chronic obstructive pulmonary disease, chronic inflammatory pulmonary diseases are also prevalent and problematic ailments. TNF-α plays a central role in the inflammatory response and the administration of their antagonists block chronic and acute responses in animal models of inflammatory disease.

Enhanced or unregulated TNF-α production has been implicated in viral, genetic, inflammatory, allergic, and autoimmune diseases. Examples of such diseases include but are not limited to: HIV; hepatitis; adult respiratory distress syndrome; bone-resorption diseases; chronic obstructive pulmonary diseases; chronic pulmonary inflammatory diseases; asthma, dermatitis; cystic fibrosis; septic shock; sepsis; endotoxic shock; hemodynamic shock; sepsis syndrome; post ischemic reperfusion injury, meningitis; psoriasis; fibrotic disease; cachexia; graft rejection; auto-immune disease; rheumatoid spondylitis; arthritic conditions, such as rheumatoid arthritis and osteoarthritis; osteoporosis; Crohn's disease; ulcerative colitis; inflammatory-bowel disease; multiple sclerosis; systemic lupus erythrematosus; ENL in leprosy; radiation damage; asthma; and hyperoxic alveolar injury. Tracey et al., 1987, *Nature* 330:662-664 and Hinshaw et al., 1990, *Circ. Shock* 30:279-292 (endotoxic shock); Dezube et al., 1990, *Lancet,* 335:662 (cachexia); Millar et al., 1989, *Lancet* 2:712-714 and Ferrai-Baliviera at, 1989, *Arch. Surg.* 124:1400-1405 (adult respiratory distress syndrome); Bertolini et al., 1986, *Nature* 319:516-518, Johnson et al., 1989, *Endocrinology* 124:1424-1427, Holler at al., 1990, *Blood* 75:1011-1016, and Grau et al., 1989, *N. Engl. J. Med.* 320:1586-1591 (bone resorption diseases); Pignet et al., 1990, *Nature,* 344:245-247, Bissonnette at al., 1989, *Inflammation* 13:329-339 and Baughman et al., 1990, *J. Lab. Clin. Med.* 115:36-42 (chronic pulmonary inflammatory diseases); Elliot et al., 1995, *Int. J. Pharmac.* 17:141-145 (rheumatoid arthritis); von Dullemen et al., 1995, *Gastroenterology,* 109: 129-135 (Crohn's disease); Duh et al., 1989, *Proc. Nat. Acad. Sci.* 86:5974-5978, Poll at al., 1990, *Proc. Nat. Acad. Sci.* 87:782-785, Monto at al., 1990, *Blood* 79:2670, Clouse et al., 1989, *J. Immunol.* 142, 431-438, Poll et al., 1992, *AIDS Res. Hum. Retrovirus,* 191-197, Poli et al. 1990, *Proc. Natl. Acad. Sci.* 87:782-784, Folks at, 1989, PNAS 86:2365-2368 (HIV and opportunistic infections resulting from HIV).

Pharmaceutical compounds that can block the activity or inhibit the production of certain cytokines, including TNF-α, may be beneficial therapeutics. Many small-molecule inhibitors have demonstrated an ability to treat or prevent inflammatory diseases implicated by TNF-α (for a review, see Lowe, 1998 *Exp. Opin. Ther. Patents* 8:1309-1332). One such class of molecules are the substituted phenethylsulfones described in U.S. Pat. No. 6,020,358.

3. SUMMARY OF THE INVENTION

This invention relates to methods of treating diseases and disorders utilizing an enantiomer of a substituted phenethylsulfone compound and pharmaceutically acceptable salts, hydrates, solvates, clathrates, prodrugs and polymorphs

thereof and methods for reducing the level of cytokines and their precursors in mammals. The invention also relates to pharmaceutical compositions comprising an enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione and a pharmaceutically acceptable carrier. The invention further relates to an enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione substantially free of its other enantiomer.

This invention particularly relates to the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione. This compound is believed to have increased potency and other benefits as compared to its racemate 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

The invention encompasses the use of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione for treating or preventing diseases or disorders ameliorated by the inhibition of TNF-α production in mammals. In certain embodiments, this treatment includes the reduction or avoidance of adverse effects. Such disorders include, but are not limited to, cancers, including, but not limited to cancer of the head, thyroid, neck, eye, skin, mouth, throat, esophagus, chest, bone, blood, bone marrow, lung, colon, sigmoid, rectum, stomach, prostate, breast, ovaries, kidney, liver, pancreas, brain, intestine, heart, adrenal, subcutaneous tissue, lymph nodes, heart, and combinations thereof. Specific cancers that can be treated by this method are multiple myeloma, malignant melanoma, malignant glioma, leukemia and solid tumors.

The invention also encompasses the use of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione in the treatment or prevention of heart disease, including, but not limited to congestive heart failure, cardiomyopathy, pulmonary edema, endotoxin-mediated septic shock, acute viral myocarditis, cardiac allograft rejection, and myocardial infarction.

The invention also encompasses the use of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione to treat diseases or disorders ameliorated by the inhibition of PDE4. For example, the compounds and compositions of the invention may be useful to treat or prevent viral, genetic, inflammatory, allergic, and autoimmune diseases. Examples of such diseases include, but are not limited to: HIV; hepatitis; adult respiratory distress syndrome; bone-resorption diseases; chronic obstructive pulmonary diseases; chronic pulmonary inflammatory diseases; dermatitis; inflammatory skin disease, atopic dermatitis, cystic fibrosis; septic shock; sepsis; endotoxic shock; hemodynamic shock; sepsis syndrome; post ischemic reperfusion injury; meningitis; psoriasis; fibrotic disease; cachexia; graft rejection including graft versus host disease; auto-immune disease; rheumatoid spondylitis; arthritic conditions, such as rheumatoid arthritis and osteoarthritis; osteoporosis; Crohn's disease; ulcerative colitis; inflammatory-bowel disease; multiple sclerosis; systemic lupus erythrematosus; erythema nodosum leprosum (ENL) in leprosy, radiation damage; asthma; and hyperoxic alveolar injury.

In yet another embodiment, the stereomerically pure (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is also useful in the treatment or prevention of microbial infections or the symptoms of microbial infections including, but not limited to, bacterial infections, fungal infections, malaria, mycobacterial infection, and opportunistic infections resulting from HIV.

The invention further encompasses pharmaceutical compositions and single unit dosage forms comprising an enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione and pharmaceutically acceptable polymorphs, prodrugs, salts, hydrates, clathrates, and solvates thereof.

In a separate embodiment, the invention encompasses the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

In a further embodiment, the invention encompasses a method of producing a stereomerically pure enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione which comprises contacting 1-(3-Ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethylamine with a chiral amino acid and contacting the product of the first step with N-(1,3-Dioxo-1,3-dihydro-isobenzofuran-4-yl)-acetamide. In a related embodiment the invention encompasses a chiral salt of 1-(3-Ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethylamine.

3.1. BRIEF DESCRIPTION OF THE FIGURES

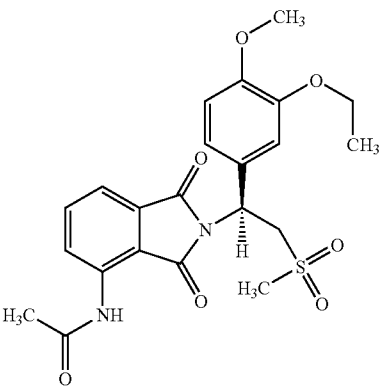
FIG. **1**. illustrates the preparation of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

FIG. **2**. illustrates the effect of the enantiomer of the invention on LPS-induced neutrophilia in the lungs of conscious ferrets.

3.2. DEFINITIONS

As used herein, term "Compound A" refers to an enantiomerically pure form of 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione which comes off of an HPLC column at about 25.4 minutes when that column is a 150 mm×4.6 mm Ultron Chiral ES-OVS chiral HPLC column (Agilent Technology), the eluent is 15:85 ethanol: 20 mM KH_2PO_4 at pH 3.5, and the observation wavelength is 240 nm. The ^1H NMR spectrum of compound A is substantially as follows: δ($CDCl_3$): 1.47 (t, 3H), 2.26 (s, 3H), 2.87 (s, 3H), 3.68-3.75 (dd, 1H), 3.85 (s, 3H), 4.07-4.15 (q, 2H), 4.51-4.61 (dd, 1H), 5.84-5.90 (dd, 1H), 6.82-8.77 (m, 6H), 9.46 (s, 1H). The ^{13}C NMR spectrum of Compound A is substantially as follows δ(DMSO-d_6): 14.66, 24.92, 41.61, 48.53, 54.46, 55.91, 64.51, 111.44, 112.40, 115.10, 118.20, 120.28, 124.94, 129.22, 131.02, 136.09, 137.60, 148.62, 149.74, 167.46, 169.14, 169.48. Compound A dissolved in methanol also rotates plane polarized light in the (+) direction.

Without being limited by theory, Compound A is believed to be S-{2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione}, which has the following structure:



As used herein, the term "patient" refers to a mammal, particularly a human.

As used herein, the term "pharmaceutically acceptable salts" refer to salts prepared from pharmaceutically acceptable non-toxic acids or bases including inorganic acids and bases and organic acids and bases. Suitable pharmaceutically acceptable base addition salts for the compound of the present invention include metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from lysine, N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. Suitable non-toxic acids include, but are not limited to, inorganic and organic acids such as acetic, alginic, anthranilic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethenesulfonic, formic, fumaric, furoic, galacturonic, gluconic, glucuronic, glutamic, glycolic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phenylacetic, phosphoric, propionic, salicylic, stearic, succinic, sulfanilic, sulfuric, tartaric acid, and p-toluenesulfonic acid. Specific non-toxic acids include hydrochloric, hydrobromic, phosphoric, sulfuric, and methanesulfonic acids. Examples of specific salts thus include hydrochloride and mesylate salts.

As used herein and unless otherwise indicated, the term "prodrug" means a derivative of a compound that can hydrolyze, oxidize, or otherwise react under biological conditions (in vitro or in vivo) to provide the compound. Examples of prodrugs include, but are not limited to, derivatives and metabolites of Compound A that include biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Prodrugs can typically be prepared using well-known methods, such as those described by 1 *Burger's Medicinal Chemistry and Drug Discovery,* 172-178, 949-982 (Manfred E. Wolff ed., 5th ed. 1995).

As used herein and unless otherwise indicated, the terms "biohydrolyzable amide," "biohydrolyzable ester," "biohydrolyzable carbamate," "biohydrolyzable carbonate," "biohydrolyzable ureide," "biohydrolyzable phosphate" mean an amide, ester, carbamate, carbonate, ureide, or phosphate, respectively, of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties in vivo, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted in vivo to the biologically active compound. Examples of biohydrolyzable esters include, but are not limited to, lower alkyl esters, alkoxyacyloxy esters, alkyl acylamino alkyl esters, and choline esters. Examples of biohydrolyzable amides include, but are not limited to, lower alkyl amides, α-amino acid amides, alkoxyacyl amides, and alkylaminoalkylcarbonyl amides. Examples of biohydrolyzable carbamates include, but are not limited to, lower alkylamines, substituted ethylenediamines, aminoacids, hydroxyalkylamines, heterocyclic and heteroaromatic amines, and polyether amines.

As used herein and unless otherwise indicated, the term "stereomerically pure" means a composition that comprises one stereoisomer of a compound and is substantially free of other stereoisomers of that compound. For example, a stereomerically pure composition of a compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically pure composition of a compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, more preferably greater than about 90% by weight of one stereoisomer of the compound and less than about 10% by weight of the other stereoisomers of the compound, even more preferably greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, and most preferably greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound.

As used herein and unless otherwise indicated, the term "enantiomerically pure" means a stereomerically pure composition of a compound having one chiral center.

As used herein, term "adverse effects" includes, but is not limited to gastrointestinal, renal and hepatic toxicities, leukopenia, increases in bleeding times due to, e.g., thrombocytopenia, and prolongation of gestation, nausea, vomiting, somnolence, asthenia, dizziness, teratogenicity, extra-pyramidal symptoms, akathisia, cardiotoxicity including cardiovascular disturbances, inflammation, male sexual dysfunction, and elevated serum liver enzyme levels. The term "gastrointestinal toxicities" includes but is not limited to gastric and intestinal ulcerations and erosions. The term "renal toxicities" includes but is not limited to such conditions as papillary necrosis and chronic interstitial nephritis.

As used herein and unless otherwise indicated, the phrases "reduce or avoid adverse effects" and "reducing or avoiding adverse effects" mean the reduction of the severity of one or more adverse effects as defined herein.

It should be noted that if there is a discrepancy between a depicted structure and a name given that structure, the depicted structure is to be accorded more weight. In addition, if the stereochemistry of a structure or a portion of a structure is not indicated with, for example, bold or dashed lines, the structure or portion of the structure is to be interpreted as encompassing all stereoisomers of it.

4. DETAILED DESCRIPTION OF THE INVENTION

This invention relates to stereomerically pure Compound A, which is an enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, substantially free of its other enantiomer, as well as novel methods using, and compositions comprising stereomerically pure Compound A. For example, the present invention encompasses the in vitro and in vivo use of Compound A, and the incorporation of Compound A into pharmaceutical compositions and single unit dosage forms useful in the treatment and prevention of a variety of diseases and disorders. Diseases and disorders which are ameliorated by the reduction of levels of TNF-α or inhibition of PDE4 are well known in the art and are described herein. Specific methods of the invention reduce or avoid the adverse effects associated with compounds used as TNF-α inhibitor. Other specific methods of the invention reduce or avoid the adverse effects associated with use of racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

Specific methods of the invention include methods of treating or preventing diseases and disorders including, but not limited to, solid tumor cancers, blood-born cancers and inflammatory diseases.

Pharmaceutical and dosage forms of the invention, which comprise Compound A or a pharmaceutically acceptable polymorph, prodrug, salt, clathrate, solvate or hydrate thereof, can be used in the methods of the invention.

Without being limited by theory, it is believed that Compound A can inhibit TNF-α production. Consequently, a first

embodiment of the invention relates to a method of inhibiting TNF-α production which comprises contacting a cell exhibiting abnormal TNF-α production with an effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. In a particular embodiment, the invention relates to a method of inhibiting TNF-α production which comprises contacting a mammalian cell exhibiting abnormal TNF-α production with an effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof.

The invention also relates to a method of treating or preventing disorders ameliorated by the reduction of levels of TNF-α in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof.

A further embodiment of the invention relates to a method of treating or preventing cancer, including but not limited to, solid tumor, blood-born tumor, leukemias, and in particular, multiple myeloma in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically effective amount of stereomerically pure compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof; in particular wherein the patient is a mammal.

In another embodiment, the invention relates to a method of inhibiting PDE4 which comprises contacting PDE4 with an effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof.

In another embodiment, the invention relates to a method of controlling cAMP levels in a cell which comprises contacting a cell with an effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. As used herein the term "controlling cAMP levels" includes preventing or reducing the rate of the breakdown of Adenosine 3',5'-cyclic monophosphate (cAMP) in a cell or increasing the amount of Adenosine 3',5'-cyclic monophosphate present in a cell, preferably a mammalian cell, more preferably a human cell. In a particular method, the rate of cAMP breakdown is reduced by about 10, 25, 50, 100, 200, or 500 percent as compared to the rate in comparable cells which have not been contacted with a compound of the invention.

A further embodiment of the invention relates to a method of treating or preventing diseases or disorders ameliorated by the inhibition of PDE4 in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. Disorders ameliorated by the inhibition of PDE4 include, but are not limited to, asthma, inflammation (e.g., inflammation due to reperfusion), chronic or acute obstructive pulmonary diseases, chronic or acute pulmonary inflammatory diseases, inflammatory bowel disease, Crohn's Disease, Bechet's Disease, or colitis.

A further embodiment of the invention relates to a method of treating or preventing depression, asthma, inflammation (e.g., contact dermatitis, atopic dermatitis, psoriasis, rheumatoid arthritis, osteoarthritis, inflammatory skin disease, inflammation due to reperfusion), chronic or acute obstructive pulmonary diseases, chronic or pulmonary inflammatory diseases, inflammatory bowel disease, Crohn's Disease, Bechet's Disease or colitis in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof; in particular wherein the patient is a mammal.

A separate embodiment of the invention encompasses methods of treating or preventing Myelodysplastic syndrome (MDS) which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. MDS refers to a diverse group of hematopoietic stem cell disorders. MDS is characterized by a cellular marrow with impaired morphology and maturation (dysmyelopoiesis), peripheral blood cytopenias, and a variable risk of progression to acute leukemia, resulting from ineffective blood cell production. See The Merck Manual 953 (17th ed. 1999) and List et al., 1990, *J. Clin. Oncol.* 8:1424.MDS

A separate embodiment of the invention encompasses methods of treating or preventing Myeloproliferative disease (MPD) which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. Myeloproliferative disease (MPD) refers to a group of disorders characterized by clonal abnormalities of the hematopoietic stem cell. See e.g., Current Medical Diagnosis & Treatment, pp. 499 (37th ed., Tierney et al. ed, Appleton & Lange, 1998).

The invention also encompasses a method of treating, preventing or managing complex regional pain syndrome, which comprises administering to a patient in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of a stereomerically pure Compound A, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. In a specific embodiment, the administration is before, during or after surgery or physical therapy directed at reducing or avoiding a symptom of complex regional pain syndrome in the patient.

In particular methods of the invention, stereomerically pure Compound A, or a pharmaceutically acceptable polymorph, prodrug, salt, solvate, hydrate, or clathrate thereof, is adjunctively administered with at least one additional therapeutic agent. Examples of additional therapeutic agents include, but are not limited to, anti-cancer drugs, anti-inflammatories, antihistamines and decongestants.

4.1. SYNTHESIS AND PREPARATION

Racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is readily prepared using the methods in U.S. Pat. No. 6,020,358, which is incorporated herein by reference.

Compound A can be isolated from the racemic compound by techniques known in the art. Examples include, but are not limited to, the formation of chiral salts and the use of chiral or high performance liquid chromatography "HPLC" and the formation and crystallization of chiral salts. See, e.g., Jacques, J., et al., *Enantiomers, Racemates and Resolutions* (Wiley-Interscience, New York, 1981); Wilen, S. H., et al., *Tetrahedron* 33:2725 (1977); Eliel, E. L., *Stereochemistry of Carbon Compounds* (McGraw-Hill, NY, 1962); and Wilen, S.

H., *Tables of Resolving Agents and Optical Resolutions* p. 268 (E. L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, Ind., 1972).

In a specific method, Compound A is synthesized from 3-acetamidophthalic anhydride and a chiral amino acid salt of (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine. Chiral amino acid salts of (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine include, but not limited to salts formed with the L isomers of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, ornithine, 4-aminobutyric acid, 2 amino isobutyric acid, 3 amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, and N-acetyl-leucine. A specific chiral amino acid salt is (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine N-acetyl-L-leucine salt, which is resolved from 2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine and N-acetyl-L-leucine in methanol.

4.2. METHODS OF TREATMENT

The invention encompasses methods of treating and preventing diseases or disorders ameliorated by the reduction of levels of TNF-α in a patient which comprise administering to a patient in need of such treatment or prevention a therapeutically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof.

Disorders ameliorated by the inhibition of TNF-α include, but are not limited to: heart disease, such as congestive heart failure, cardiomyopathy, pulmonary edema, endotoxin-mediated septic shock, acute viral myocarditis, cardiac allograft rejection, and myocardial infarction; solid tumors, including but not limited to, sarcoma, carcinomas, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, Kaposi's sarcoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma; and blood-born tumors including but not limited to, acute lymphoblastic leukemia "ALL", acute lymphoblastic B-cell leukemia, acute lymphoblastic T-cell leukemia, acute myeloblastic leukemia "AML", acute promyelocytic leukemia "APL", acute monoblastic leukemia, acute erythroleukemic leukemia, acute megakaryoblastic leukemia, acute myelomonocytic leukemia, acute nonlymphocyctic leukemia, acute undifferentiated leukemia, chronic myelocytic leukemia "CML", chronic lymphocytic leukemia "CLL", hairy cell leukemia, multiple myeloma and acute and chronic leukemias, for example, lymphoblastic, myelogenous, lymphocytic, and myelocytic leukemias.

Specific methods of the invention further comprise the administration of an additional therapeutic agent (i.e., a therapeutic agent other than Compound A). Examples of additional therapeutic agents include, but are not limited to, anticancer drugs such as, but are not limited to: alkylating agents, nitrogen mustards, ethylenimines, methylmelamines, alkyl sulfonates, nitrosoureas, triazenes, folic acid analogs, pyrimidine analogs, purine analogs, ulna alkaloids, epipodophyllotoxins, antibiotics, topoisomerase inhibitors and anti-cancer vaccines.

Specific additional therapeutic agents include, but are not limited to: acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer, carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cisplatin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; dactinomycin; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; docetaxel; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; flurocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; ilmofosine; interleukin II (including recombinant interleukin II, or rIL2), interferon alfa-2a; interferon alfa-2b; interferon alfa-n1; interferon alfa-n3; interferon beta-I a; interferon gamma-I b; iproplatin; irinotecan hydrochloride; lanreotide acetate; tetrazole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedepa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper, mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; paclitaxel; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; rogletimide; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; triciribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vine-

pidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin hydrochloride. Other anti-cancer drugs include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; antidorsalizing morphogenetic protein-I; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor, bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-aminotriazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl spiromustine; docetaxel; docosanol; dolasefron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; element; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor, multiple tumor suppressor 1-based therapy, mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor, platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator, protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor, retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor, stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor, urokinase receptor antagonists; vaprcotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

The invention further encompasses a method of treating or preventing diseases or disorders ameliorated by the inhibition

of PDE4 in a patient which comprise administering to a patient in need of such treatment or prevention a therapeutically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. Disorders ameliorated by the inhibition of PDE4 include, but are not limited to, asthma, inflammation, chronic or acute obstructive pulmonary disease, chronic or acute pulmonary inflammatory disease, inflammatory bowel disease, Crohn's Disease, Bechet's Disease, colitis, ulcerative colitis and arthritis or inflammation due to reperfusion. In a preferred embodiment, the disease or disorder to be treated or prevented is chronic obstructive pulmonary disease.

Specific methods of the invention can comprise the administration of an additional therapeutic agent such as, but not limited to, anti-inflammatory drugs, antihistamines and decongestants. Examples of such additional therapeutic agents include, but are not limited to: antihistamines including, but not limited to, ethanolamines, ethylenediamines, piperazines, and phenothiazines; antiinflammatory drugs; NSAIDS, including, but not limited to, aspirin, salicylates, acetominophen, indomethacin, sulindac, etodolac, fenamates, tolmetin, ketorolac, diclofenac, ibuprofen, naproxen, fenoprofen, ketoprofen, flurbiprofen, oxaprozin, piroxicam, meloxicam, pyrazolon derivatives; and steroids including, but not limited to, cortical steroids and adrenocortical steroids.

Specific methods of the invention avoid or reduce drug-drug interactions and other adverse effects associated with agents used in the treatment of such disorders, including racemic substituted phenylethylsulfones. Without being limited by any theory, stereomerically pure Compound A may further provide an overall improved therapeutic effectiveness, or therapeutic index, over racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione. For example, a smaller amount of the drug may in some circumstances be administered to attain the same level of effectiveness.

As stated above, the active compound of the invention (i.e., Compound A) may be used in the treatment or prevention of a wide range of diseases and conditions. The magnitude of a prophylactic or therapeutic dose of a particular active ingredient of the invention in the acute or chronic management of a disease or condition will vary, however, with the nature and severity of the disease or condition, and the route by which the active ingredient is administered. The dose, and perhaps the dose frequency, will also vary according to the age, body weight, and response of the individual patient. Suitable dosing regimens can be readily selected by those skilled in the art with due consideration of such factors. In general, the recommended daily dose range for the conditions described herein lie within the range of from about 1 mg to about 1000 mg per day, given as a single once-a-day dose preferably as divided doses throughout a day. More specifically, the daily dose is administered twice daily in equally divided doses. Specifically, a daily dose range should be from about 5 mg to about 500 mg per day, more specifically, between about 10 mg and about 200 mg per day. Specifically, the daily dose may be administered in 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 50 mg, or 100 mg dosage forms. In managing the patient, the therapy should be initiated at a lower dose, perhaps about 1 mg to about 25 mg, and increased if necessary up to about 200 mg to about 1000 mg per day as either a single dose or divided doses, depending on the patient's global response. Alternatively, the daily dose is from 0.01 mg/kg to 100 mg/kg.

It may be necessary to use dosages of the active ingredient outside the ranges disclosed herein in some cases, as will be apparent to those of ordinary skill in the art. Furthermore, it is noted that the clinician or treating physician will know how and when to interrupt, adjust, or terminate therapy in conjunction with individual patient response.

The phrases "therapeutically effective amount", "prophylactically effective amount" and "therapeutically or prophylactically effective amount," as used herein encompasses the above described dosage amounts and dose frequency schedules. Different therapeutically effective amounts may be applicable for different diseases and conditions, as will be readily known by those of ordinary skill in the art. Similarly, amounts sufficient to treat or prevent such disorders, but insufficient to cause, or sufficient to reduce, adverse effects associated with racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione are also encompassed by the above described dosage amounts and dose frequency schedules.

4.3. PHARMACEUTICAL COMPOSITIONS

Pharmaceutical compositions and single unit dosage forms comprising Compound A, or a pharmaceutically acceptable polymorph, prodrug, salt, solvate, hydrate, or clathrate thereof, are encompassed by the invention. Individual dosage forms of the invention may be suitable for oral, mucosal (including rectal, nasal, or vaginal), parenteral (including subcutaneous, intramuscular, bolus injection, intraarterial, or intravenous), sublingual, transdermal, buccal, or topical administration.

Pharmaceutical compositions and dosage forms of the invention comprise stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. Pharmaceutical compositions and dosage forms of the invention typically also comprise one or more pharmaceutically acceptable excipients.

A particular pharmaceutical composition encompassed by this embodiment comprises stereomerically pure Compound A, or a pharmaceutically acceptable polymorph, prodrug, salt, solvate, hydrate, or clathrate thereof, and at least one additional therapeutic agent. Examples of additional therapeutic agents include, but are not limited to: anti-cancer drugs and anti-inflammation therapies including, but not limited to, those listed above in section 4.2.

Single unit dosage forms of the invention are suitable for oral, mucosal (e.g., nasal, sublingual, vaginal, buccal, or rectal), parenteral (e.g., subcutaneous, intravenous, bolus injection, intramuscular, or intraarterial), or transdermal administration to a patient. Examples of dosage forms include, but are not limited to: tablets; caplets; capsules, such as soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suppositories; ointments; cataplasms (poultices); pastes; powders; dressings; creams; plasters; solutions; patches; aerosols (e.g., nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a patient, including suspensions (e.g., aqueous or non-aqueous liquid suspensions, oil-in-water emulsions, or a water-in-oil liquid emulsions), solutions, and elixirs; liquid dosage forms suitable for parenteral administration to a patient; and sterile solids (e.g., crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms suitable for parenteral administration to a patient.

The composition, shape, and type of dosage forms of the invention will typically vary depending on their use. For example, a dosage form used in the acute treatment of inflammation or a related disorder may contain larger amounts of one or more of the active ingredients it comprises than a dosage form used in the chronic treatment of the same dis-

ease. Similarly, a parenteral dosage form may contain smaller amounts of one or more of the active ingredients it comprises than an oral dosage form used to treat the same disease or disorder. These and other ways in which specific dosage forms encompassed by this invention will vary from one another will be readily apparent to those skilled in the art. See, e.g., *Remington's Pharmaceutical Sciences,* 18th ed., Mack Publishing, Easton Pa. (1990).

Typical pharmaceutical compositions and dosage forms comprise one or more excipients. Suitable excipients are well known to those skilled in the art of pharmacy, and non-limiting examples of suitable excipients are provided herein. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a patient. For example, oral dosage forms such as tablets may contain excipients not suited for use in parenteral dosage forms. The suitability of a particular excipient may also depend on the specific active ingredients in the dosage form.

Lactose-free compositions of the invention can comprise excipients that are well known in the art and are listed, for example, in the U.S. Pharmocopia (USP)SP (XXI)/NF (XVI). In general, lactose-free compositions comprise an active ingredient, a binder/filler, and a lubricant in pharmaceutically compatible and pharmaceutically acceptable amounts. Preferred lactose-free dosage forms comprise an active ingredient, microcrystalline cellulose, pre-gelatinized starch, and magnesium stearate.

This invention further encompasses anhydrous pharmaceutical compositions and dosage forms comprising active ingredients, since water can facilitate the degradation of some compounds. For example, the addition of water (e.g., 5%) is widely accepted in the pharmaceutical arts as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. See, e.g., Jens T. Carstensen, *Drug Stability: Principles & Practice,* 2d. Ed., Marcel Dekker, NY, NY, 1995, pp. 379-80. In effect, water and heat accelerate the decomposition of some compounds. Thus, the effect of water on a formulation can be of great significance since moisture and/or humidity are commonly encountered during manufacture, handling, packaging, storage, shipment, and use of formulations.

Anhydrous pharmaceutical compositions and dosage forms of the invention can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms that comprise lactose and at least one active ingredient that comprises a primary or secondary amine are preferably anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected.

An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions are preferably packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples of suitable packaging include, but are not limited to, hermetically sealed foils, plastics, unit dose containers (e.g., vials), blister packs, and strip packs.

The invention further encompasses pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will decompose. Such compounds, which are referred to herein as "stabilizers," include, but are not limited to, antioxidants such as ascorbic acid, pH buffers, or salt buffers.

Like the amounts and types of excipients, the amounts and specific types of active ingredients in a dosage form may differ depending on factors such as, but not limited to, the route by which it is to be administered to patients. However, typical dosage forms of the invention comprise compound A, or a pharmaceutically acceptable salt, solvate, clathrate, hydrate, polymoprh or prodrug thereof lie within the range of from about 1 mg to about 1000 mg per day, given as a single once-a-day dose in the morning but preferably as divided doses throughout the day taken with food. More specifically, the daily dose is administered twice daily in equally divided doses. Specifically, a daily dose range should be from about 5 mg to about 500 mg per day, more specifically, between about 10 mg and about 200 mg per day. In managing the patient, the therapy should be initiated at a lower dose, perhaps about 1 mg to about 25 mg, and increased if necessary up to about 200 mg to about 1000 mg per day as either a single dose or divided doses, depending on the patient's global response.

4.3.1. ORAL DOSAGE FORMS

Pharmaceutical compositions of the invention that are suitable for oral administration can be presented as discrete dosage forms, such as, but are not limited to, tablets (e.g., chewable tablets), caplets, capsules, and liquids (e.g., flavored syrups). Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of pharmacy well known to those skilled in the art. See generally, *Remington's Pharmaceutical Sciences,* 18th ed., Mack Publishing, Easton Pa. (1990).

Typical oral dosage forms of the invention are prepared by combining the active ingredient(s) in an intimate admixture with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of preparation desired for administration. For example, excipients suitable for use in oral liquid or aerosol dosage forms include, but are not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents. Examples of excipients suitable for use in solid oral dosage forms (e.g., powders, tablets, capsules, and caplets) include, but are not limited to, starches, sugars, micro-crystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid excipients are employed. If desired, tablets can be coated by standard aqueous or nonaqueous techniques. Such dosage forms can be prepared by any of the methods of pharmacy. In general, pharmaceutical compositions and dosage forms are prepared by uniformly and intimately admixing the active ingredients with liquid carriers, finely divided solid carriers, or both, and then shaping the product into the desired presentation if necessary.

For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as powder or granules, optionally mixed with an excipient. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

Examples of excipients that can be used in oral dosage forms of the invention include, but are not limited to, binders, fillers, disintegrants, and lubricants. Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gelatin, natural and synthetic gums such as acacia, sodium alginate, alginic acid, other alginates, powdered

tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre-gelatinized starch, hydroxypropyl methyl cellulose, (e.g., Nos. 2208, 2906, 2910), microcrystalline cellulose, and mixtures thereof.

Examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof. The binder or filler in pharmaceutical compositions of the invention is typically present in from about 50 to about 99 weight percent of the pharmaceutical composition or dosage form.

Suitable forms of microcrystalline cellulose include, but are not limited to, the materials sold as AVICEL-PH-101, AVICEL-PH-103 AVICEL RC-581, AVICEL-PH-105 (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, Pa.), and mixtures thereof. An specific binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC-581. Suitable anhydrous or low moisture excipients or additives include AVICEL-PH-103™ and Starch 1500 LM.

Disintegrants are used in the compositions of the invention to provide tablets that disintegrate when exposed to an aqueous environment. Tablets that contain too much disintegrant may disintegrate in storage, while those that contain too little may not disintegrate at a desired rate or under the desired conditions. Thus, a sufficient amount of disintegrant that is neither too much nor too little to detrimentally alter the release of the active ingredients should be used to form solid oral dosage forms of the invention. The amount of disintegrant used varies based upon the type of formulation, and is readily discernible to those of ordinary skill in the art. Typical pharmaceutical compositions comprise from about 0.5 to about 15 weight percent of disintegrant, specifically from about 1 to about 5 weight percent of disintegrant.

Disintegrants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, agar-agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrilin potassium, sodium starch glycolate, potato or tapioca starch, pre-gelatinized starch, other starches, clays, other algins, other celluloses, gums, and mixtures thereof.

Lubricants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, and mixtures thereof. Additional lubricants include, for example, a syloid silica gel (AEROSIL 200, manufactured by W.R. Grace Co. of Baltimore, Md.), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Plano, Tex.), CAB-O-SIL (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, Mass.), and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about 1 weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated.

4.3.2. DELAYED RELEASE DOSAGE FORMS

Active ingredients of the invention can be administered by controlled release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Pat. Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; and 4,008,719, 5,674,533, 5,059,595, 5,591,767, 5,120,548, 5,073,543, 5,639,476, 5,354,556, and 5,733,566, each of which is incorporated herein by reference. Such dosage forms can be used to provide slow or controlled-release of one or more active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the active ingredients of the invention. The invention thus encompasses single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled-release.

All controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition, controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the drug, and can thus affect the occurrence of side (e.g., adverse) effects.

Most controlled-release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled-release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, temperature, enzymes, water, or other physiological conditions or compounds.

4.3.3. PARENTERAL DOSAGE FORMS

Parenteral dosage forms can be administered to patients by various routes including, but not limited to, subcutaneous, intravenous (including bolus injection), intramuscular, and intraarterial. Because their administration typically bypasses patients' natural defenses against contaminants, parenteral dosage forms are preferably sterile or capable of being sterilized prior to administration to a patient. Examples of parenteral dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions.

Suitable vehicles that can be used to provide parenteral dosage forms of the invention are well known to those skilled in the art. Examples include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and

polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

Compounds that increase the solubility of one or more of the active ingredients disclosed herein can also be incorporated into the parenteral dosage forms of the invention.

4.3.4. TRANSDERMAL, TOPICAL, AND MUCOSAL DOSAGE FORMS

Transdermal, topical, and mucosal dosage forms of the invention include, but are not limited to, ophthalmic solutions, sprays, aerosols, creams, lotions, ointments, gels, solutions, emulsions, suspensions, or other forms known to one of skill in the art. See, e.g., *Remington's Pharmaceutical Sciences,* 16th and 18th eds., Mack Publishing, Easton Pa. (1980 & 1990); and *Introduction to Pharmaceutical Dosage Forms,* 4th ed., Lea & Febiger, Philadelphia (1985). Dosage forms suitable for treating mucosal tissues within the oral cavity can be formulated as mouthwashes or as oral gels. Further, transdermal dosage forms include "reservoir type" or "matrix type" patches, which can be applied to the skin and worn for a specific period of time to permit the penetration of a desired amount of active ingredients.

Suitable excipients (e.g., carriers and diluents) and other materials that can be used to provide transdermal, topical, and mucosal dosage forms encompassed by this invention are well known to those skilled in the pharmaceutical arts, and depend on the particular tissue to which a given pharmaceutical composition or dosage form will be applied. With that fact in mind, typical excipients include, but are not limited to, water, acetone, ethanol, ethylene glycol, propylene glycol, butane-1,3-diol, isopropyl myristate, isopropyl palmitate, mineral oil, and mixtures thereof to form lotions, tinctures, creams, emulsions, gels or ointments, which are non-toxic and pharmaceutically acceptable. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well known in the art. See, e.g., *Remington's Pharmaceutical Sciences,* 16th and 18th eds., Mack Publishing, Easton Pa. (1980 & 1990).

Depending on the specific tissue to be treated, additional components may be used prior to, in conjunction with, or subsequent to treatment with active ingredients of the invention. For example, penetration enhancers can be used to assist in delivering the active ingredients to the tissue. Suitable penetration enhancers include, but are not limited to: acetone; various alcohols such as ethanol, oleyl, and tetrahydrofuryl; alkyl sulfoxides such as dimethyl sulfoxide; dimethyl acetamide; dimethyl formamide; polyethylene glycol; pyrrolidones such as polyvinylpyrrolidone; Kollidon grades (Povidone, Polyvidone); urea; and various water-soluble or insoluble sugar esters such as Tween 80 (polysorbate 80) and Span 60 (sorbitan monostearate).

The pH of a pharmaceutical composition or dosage form, or of the tissue to which the pharmaceutical composition or dosage form is applied, may also be adjusted to improve delivery of one or more active ingredients. Similarly, the polarity of a solvent carrier, its ionic strength, or tonicity can be adjusted to improve delivery. Compounds such as stearates can also be added to pharmaceutical compositions or dosage forms to advantageously alter the hydrophilicity or lipophilicity of one or more active ingredients so as to improve delivery. In this regard, stearates can serve as a lipid vehicle for the formulation, as an emulsifying agent or surfactant, and as a delivery-enhancing or penetration-enhancing agent. Different salts, hydrates or solvates of the active ingredients can be used to further adjust the properties of the resulting composition.

4.3.5. KITS

Typically, active ingredients of the invention are preferably not administered to a patient at the same time or by the same route of administration. This invention therefore encompasses kits which, when used by the medical practitioner, can simplify the administration of appropriate amounts of active ingredients to a patient.

A typical kit of the invention comprises a unit dosage form of compound A, or a pharmaceutically acceptable salt, solvate, hydrate, clathrate, polymorph or prodrug thereof, and a unit dosage form of a second active ingredient. Examples of second active ingredients include, but are not limited to, those listed in section 4.2 above.

Kits of the invention can further comprise devices that are used to administer the active ingredient(s). Examples of such devices include, but are not limited to, syringes, drip bags, patches, and inhalers.

Kits of the invention can further comprise pharmaceutically acceptable vehicles that can be used to administer one or more active ingredients. For example, if an active ingredient is provided in a solid form that must be reconstituted for parenteral administration, the kit can comprise a sealed container of a suitable vehicle in which the active ingredient can be dissolved to form a particulate-free sterile solution that is suitable for parenteral administration. Examples of pharmaceutically acceptable vehicles include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

5. EXAMPLES

5.1. Example 1

Synthesis of 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione

A stirred solution of 1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethylamine (1.0 g, 3.7 mmol) and 3-acetamidophthalic anhydride (751 mg, 3.66 mmol) in acetic acid (20 mL) was heated at reflux for 15 h. The solvent was removed in vacuo to yield an oil. Chromatography of the resulting oil yielded the product as a yellow solid (1.0 g, 59% yield): mp, 144° C.; ^{1}H NMR ($CDCl_3$) δ1.47 (t, J=7.0 Hz, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.88 (s, 3H, CH_3), 3.75 (dd, J=4.4, 14.3 Hz, 1H, CHH), 3.85 (s, 3H, CH3), 4.11 (q, J=7 Hz, 2H, CH2), 5.87 (dd, J=4.3, 10.5 Hz, 1H, NCH), 6.82-6.86 (m, 1H, Ar), 7.09-7.11 (m, 2H, Ar), 7.47 (d, J=7 Hz, 1H, Ar), 7.64 (t, J=8 Hz, 1H, Ar), 8.74 (d, J=8 Hz, 1H, Ar), 9.49 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ14.61, 24.85, 41.54, 48.44, 54.34, 55.85, 64.43, 111.37, 112.34, 115.04, 118.11, 120.21, 124.85, 129.17, 130.96, 136.01, 137.52, 148.54, 149.65, 167.38, 169.09, 169.40; Anal Calc'd. for $C_{22}H_{24}NO_7S$: C, 57.38; H, 5.25; N, 6.08. Found: C, 57.31; H, 5.34; N, 5.83.

5.2. Example 2

Synthesis of (+)2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione

Preparation of 3-Aminopthalic Acid

10% Pd/C (2.5 g), 3-nitrophthalic acid (75.0 g, 355 mmol) and ethanol (1.5 L) were charged to a 2.5 L Parr hydrogenator, under a nitrogen atmosphere. Hydrogen was charged to the reaction vessel for up to 55 psi. The mixture was shaken for 13 hours, maintaining hydrogen pressure between 50 and 55 psi. Hydrogen was released and the mixture was purged with nitrogen 3 times. The suspension was filtered through a celite bed and rinsed with methanol. The filtrate was concentrated in vacuo. The resulting solid was reslurried in ether and isolated by vacuum filtration. The solid was dried in vacuo to a constant weight, affording 54 g (84% yield) of 3-aminopthalic acid as a yellow product. ^{1}H-NMR (DMSO-d_6) δ: 3.17 (s, 2H), 6.67 (d, 1H), 6.82 (d, 1H), 7.17 (t, 1H), 8-10 (brs, 2H). ^{13}C-NMR (DMSO-d6) δ: 112.00, 115.32, 118.20, 131.28, 135.86, 148.82, 169.15, 170.09.

Preparation of 3-Acetamidophthalic Anhydride

A 1 L 3-necked round bottom flask was equipped with a mechanical stirrer, thermometer, and condenser and charged with 3-aminophthalic acid (108 g, 596 mmol) and acetic anhydride (550 mL). The reaction mixture was heated to reflux for 3 hours and cooled to ambient temperature and Rather to 0-5° C. for another 1 hour. The crystalline solid was collected by vacuum filtration and washed with ether. The solid product was dried in vacuo at ambient temperature to a constant weight, giving 75 g (61% yield) of 3-acetamidopthalic anhydride as a white product. ^{1}H-NMR ($CDCl_3$) δ: 2.21 (s, 3H), 7.76 (d, 1H), 7.94 (t, 1H), 8.42 (d, 1H), 9.84 (s, 1H).

Resolution of 2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine

A 3 L 3-necked round bottom flask was equipped with a mechanical stirrer, thermometer, and condenser and charged with 2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine (137.0 g, 500 mmol), N-acetyl-L-leucine (52 g, 300 mmol), and methanol (1.0 L). The stirred slurry was heated to reflux for 1 hour. The stirred mixture was allowed to cool to ambient temperature and stirring was continued for another 3 hours at ambient temperature. The slurry was filtered and washed with methanol (250 mL). The solid was air-dried and then dried in vacuo at ambient temperature to a constant weight, giving 109.5 g (98% yield) of the crude product (85.8% ee). The crude solid (55.0 g) and methanol (440 mL) were brought to reflux for 1 hour, cooled to room temperature and stirred for an additional 3 hours at ambient temperature. The slurry was filtered and the filter cake was washed with methanol (200 mL). The solid was air-dried and then dried in vacuo at 30° C. to a constant weight, yielding 49.6 g (90% recovery) of (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine-N-acetyl-L-leucine salt (98.4% ee). Chiral HPLC (1/99 EtOH/20 mM KH_2PO_4 @pH 7.0, Ultron Chiral ES-OVS from Agilent Technologies, 150 mm×4.6 mm, 0.5 mL/min., @240 nm): 18.4 min (S-isomer, 99.2%), 25.5 min (R-isomer, 0.8%).

Preparation of Compound A

A 500 mL 3-necked round bottom flask was equipped with a mechanical stirrer, thermometer, and condenser. The reaction vessel was charged with (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-yl amine N-acetyl-L-leucine salt (25 g, 56 mmol, 98% ee), 3-acetamidophthalic anhydride (12.1 g 58.8 mmol), and glacial acetic acid (250 mL). The mixture was refluxed over night and then cooled to <50° C. The solvent was removed in vacuo, and the residue was dissolved in ethyl acetate. The resulting solution was washed with water (250 mL×2), saturated aqueous $NaHCO_3$ (250 mL×2), brine (250 mL×2), and dried over sodium sulphate. The solvent was evaporated in vacuo, and the residue recrystallized from a binary solvent containing ethanol (150 mL) and acetone (75 mL). The solid was isolated by vacuum filtration and washed with ethanol (100 mL×2). The product was dried in vacuo at 60° C. to a constant weight, affording 19.4 g (75% yield) of S-{2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-aminoisoindoline-1,3-dione with 98% ee. Chiral HPLC (15/85 EtOH/20 mM KH_2PO_4 @pH 3.5, Ultron Chiral ES-OVS from Agilent Technology, 150 mm×4.6 mm, 0.4 mL/min., @240 nm); 25.4 min (S-isomer, 98.7%), 29.5 min (R-isomer, 1.2%). ^{1}H-NMR ($CDCl_3$) δ: 1.47 (t, 3H), 2.26 (s, 3H), 2.87 (s, 3H), 3.68-3.75 (dd, 1H), 3.85 (s, 3H), 4.07-4.15 (q, 2H), 4.51-4.61 (dd, 1H), 5.84-5.90 (dd, 1H), 6.82-8.77 (m, 6H), 9.46 (s, 1H). ^{13}C-NMR (DMSO-d6) δ: 14.66, 24.92, 41.61, 48.53, 54.46, 55.91, 64.51, 111.44, 112.40, 115.10, 118.20, 120.28, 124.94, 129.22, 131.02, 136.09, 137.60, 148.62, 149.74, 167.46, 169.14, 169.48.

5.3. Example 3

TNF-α Inhibition

Human Whole Blood LPS-Induced TNF-α Assay

The ability of compounds to inhibit LPS-induced TNF-α production by human whole blood was measured essentially as described below for the LPS-induced TNF-α assay in human PBMC, except that freshly drawn whole blood was used instead of PBMC. (George Muller, at al. 1999, *Bioorganic & Medicinal Chemistry Letters* 9; 1625-1630.) Human whole blood LPS-induced TNF-α IC_{50}-294 nM

Mouse LPS-Induced Serum TNF-α Inhibition

Compounds were tested in this animal model according to previously described methods (Corral et al. 1996, *Mol. Med* 2:506-515). Mouse LPS-induced serum TNF-α inhibition (ED_{50}, mg/kg, p.o.)=0.05.

LPS-Induced TNF-α Production

Lipopolysaccharide (LPS) is an endotoxin produced by gram-negative bacteria such as *E. coli* which induces production of many pro-inflammatory cytokines, including TNF-α. In peripheral blood mononuclear cells (PBMC), the TNF-α produced in response to LPS is derived from monocytes, which comprise approximately 5-20% of the total PBMC. Compounds were tested for the ability to inhibit LPS-induced TNF-α production from human PBMC as previously described (Muller et al. 1996, *J. Med Chem.* 39:3238). PBMC from normal donors were obtained by Ficoll Hypaque (Pharmacia, Piscataway, N.J., USA) density centrifugation. Cells were cultured in RPMI (Life Technologies, Grand Island, N.Y., USA) supplemented with 10% AB±human serum (Gemini Bio-products, Woodland, Calif., USA), 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Life Technologies).

PBMC (2×10^5 cells) were plated in 96-well flat-bottom Costar tissue culture plates (Corning, N.Y., USA) in triplicate. Cells were stimulated with LPS (Sigma, St. Louis, Mo., USA) at 100 ng/ml in the absence or presence of compounds. Compounds (Celgene Corp., Warren, N.J., USA) were dissolved in DMSO (Sigma) and further dilutions were done in culture medium immediately before use. The final DMSO concentration in all samples was 0.25%. Compounds were added to cells 1 hour before LPS stimulation. Cells were incubated for 18-20 hours at 37° C. in 5% CO_2 and superna-

tants were then collected, diluted with culture medium and assayed for TNF-α levels by ELISA (Endogen, Boston, Mass., USA). LPS-induced TNF-α IC_{50}=77 nM.

IL-1β-Induced TNF-α Production

During the course of inflammatory diseases, TNF-α production is often stimulated by the cytokine IL-1β, rather than by bacterially derived LPS. Compounds were tested for the ability to inhibit IL-1β-induced TNF-α production from human PBMC as described above for LPS-induced TNF-α production, except that the PBMC were isolated from source leukocyte units (Sera-Tec Biologicals, North Brunswick, N.J., USA) by centrifugation on Ficoll-Paque Plus (Amersham Pharmacia, Piscataway, N.J., USA), plated in 96-well tissue culture plates at 3×10^5 cells/well in RPMI-1640 medium (BioWhittaker, Walkersville, Md., USA) containing 10% heat-inactivated fetal bovine serum (Hyclone), 2 mM L-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin (complete medium), pretreated with compounds at 10, 2, 0.4, 0.08, 0.016, 0.0032, 0.00064, and 0 μM in duplicate at a final DMSO concentration of 0.1% at 37° C. in a humidified incubator at 5% CO_2 for 1 hour, then stimulated with 50 ng/ml recombinant human IL-1β (Endogen) for 18 hours. IL-β-induced TNF-α IC_{50}=83 nM.

5.4. Example 4

PDE Selectivity

PDE1, 2, 3, 5, and 6 Enzyme Assays

The specificity of compounds for PDE4 was assessed by testing at a single concentration (10 μM) against bovine PDE1, human PDE2, PDE3, and PDE5 from human platelets (Hidaka and Asano 1976, *Biochem. Biophys. Acta* 429:485, and Nicholsen et al. 1991, *Trends Pharmaco. Sci.* 12:19), and PDE6 from bovine retinal rod outer segments (Baehr et al. 1979, *J. Biol. Chem.* 254:11669, and Gillespie et al. 1989, *Mol. Pharm.* 36:773). Results are listed in Table 1.

PDE7 Enzyme Assay

PDE7 is a cAMP-selective PDE expressed mainly in T cells and in skeletal muscle. T cell-derived cytokines such as IL-2 and IFN-γ are potentially regulatable via PDE7 inhibition. PDE7 was purified from Hut78 human T cells by anion exchange chromatography as previously described (Bloom and Beavo 1996, *Proc. Natl. Acad Sci. USA* 93:14188-14192). Compounds were tested against the PDE7 preparation in the presence of 10 nM cAMP as described for PDE4 in Table 1 below.

TABLE 1

	Racemic Compound	Compound A	Compound B*
PDE Inhibition			
PDE4 IC_{50} (from U937 cells) (nM)	81.8	73.5	611
PDE1 (% inhib at 10 μM)	9%	23%	27%
PDE2(% inhib at 10 μM)	19%	6%	10%
PDE3 (% inhib at 10 μM)	21%	20%	31%
PDE5 (% inhib at 10 μM)	3%	3%	−9%
PDE6 (% inhib at 10 μM)	ND	−6%	10%
PDE7 IC_{50} (nM)	22110	20500	ND
PDE Specificity Ratios from above data (*fold)			
PDE4/PDE1	>2700	>500	>50
PDE4/PDE2	>800	>10000	>260
PDE4/PDE3	>670	>1200	>45
PDE4/PDE5	>12000	>30000	>39000
PDE4/PDE6	ND	>40000	>250
PDE7 IC_{50} /PDE4 IC_{50}	270	279	ND

*Compound B is the opposite enantiomer of Compound A.

5.5. Example 5

PDE4 Inhibition

PDE4 (U937 Cell-Derived) Enzyme Assay

PDE4 enzyme was purified from U937 human monocytic cells by gel filtration chromatography as previously described (Muller et al. 1998, *Bioorg. & Med Chem Lett* 8:2669-2674). Phosphodiesterase reactions were carried out in 50 mM Tris HCl pH 7.5, 5 mM $MgCl_2$, 1 μM cAMP, 10 nM [^{3}H]-cAMP for 30 min at 30° C., terminated by boiling, treated with 1 mg/ml snake venom, and separated using AG-1XS ion exchange resin (BioRad) as described (Muller et al. 1998, *Bioorg.* & Med Chem Lett 8:2669-2674). Reactions consumed less than 15% of available substrate. Results are listed in Table 1.

5.6. Example 6

Human T Cell Assays

SEB-Induced IL-2 and IFN-γ Production

Staphylococcal Enterotoxin B (SEB) is a superantigen derived from gram-positive bacteria *Staphylococcus aureus*. SEB provides a convenient physiological stimulus specific for T cells expressing particular T cell receptor Vβ chains. Human PBMC (consisting of approximately 50% T cells) were isolated from source leukocyte units as described above and plated in 96-well tissue culture plates at 3×10^5 cells/well in complete medium, pretreated with compounds at 10, 2, 0.4, 0.08, 0.016, 0.0032, 0.00064, and 0 μM in duplicate at a final DMSO concentration of 0.1% at 37° C. in a humidified incubator at 5% CO_2 for 1 hour, then stimulated with 100 ng/ml SEB (Sigma Chemical Co., St. Louis, Mo., USA) for 18 hours. IL-2 and IFN-γ levels were measured by ELISA (R&D Systems, Minneapolis, Minn., USA). IL-2 IC_{50}=291 nM. IFN-γ IC_{50}=46 nM.

5.7. Example 6

cAMP Elevation Assays

PGE_2-Induced cAMP Elevation

Prostaglandin E_2 (PGE_2) binds to prostanoid receptors on monocytes, T cells and other leukocytes and consequently elevates intracellular cAMP levels, resulting in inhibition of cellular responses. The combination of PGE_2 and a PDE4 inhibitor synergistically elevates cAMP levels in these cell types, and the elevation of cAMP in PBMC caused by PDE4 inhibitors in the presence of PGE_2 is proportional to the inhibitory activity of that PDE4 inhibitor. Intracellular cAMP was measured in human PBMC as follows. PBMC were isolated as described above and plated in 96-well plates at 1×10^6 cells per well in RPMI-1640. The cells were pretreated with compounds at 100, 10, 1, 0.1, 0.01, and 0 μM in a final concentration of 2% DMSO in duplicate at 37° C. in a humidified incubator at 5% CO_2 for one hour. The cells were then stimulated with PGE_2 (10 μM) (Sigma) for 1 h. The cells were lysed with HCl, 0.1N final concentration to inhibit phosphodiesterase activity and the plates were frozen at −20° C. The cAMP produced was measured using cAMP (low pH) Immunoassay kit (R&D Systems). PBMC cAMP EC_{50} for racemate is 3.09 μM. PBMC cAMP EC_{50} for Compound A is 1.58 μM.

Elevation of cAMP in human neutrophils was measured as follows. PBMC were removed from source leukocytes (Sera-Tec Biologicals) by centrifugation on Ficoll—Paque Plus (Amersham Pharmacia). The resulting erythrocyte/polymorphonuclear cell (PMN) pellet was resuspended in Hank's Balanced Salt Solution (BioWhittaker) and mixed with an

equal volume of 3% Dextran T-500 (Amersham Pharmacia) in 0.9% saline. Erythrocytes were allowed to sediment for 20 minutes, and the PMN were removed and centrifuged at 120 rpm for 8 minutes at 4° C. The remaining erythrocytes were lysed in cold 0.2% saline for 30 seconds, and the cells restored to isotonicity by the addition of an equal volume of 1.6% saline. The PMN were centrifuged at 1200 rpm for 8 minutes at 4° C., then resuspended in RPMI-1640 and assayed for cAMP elevation as described for PBMC above. PMN were found to be approximately 74% $CD18/CD11b^+$, 71% $CD16^+$ $CD9^+$ neutrophils by flow cytometzy on a FACSCalibur (Becton Dickinson, San Jose, Calif., USA). Results are shown in Table 2.

fMLF-Induced LTB4 Production

N-formyl-methionine-leucine-phenylalanine (fMLF) is a bacterially derived peptide that activates neutrophils to rapidly degranulate, migrate, adhere to endothelial cells, and release leukotriene LTB4, a product of arachidonic acid metabolism and itself a neutrophil chemoattractant. Compounds were tested for the ability to block fMLF-induced neutrophil LTB4 production as previously described (Hatzelmann and Schudt 2001, *J. Pharm. Exp. Ther.* 297:267-279), with the following modifications. Neutrophils were isolated as described above and resuspended in phosphate-buffered saline without calcium or magnesium (BioWhittaker) containing 10 mM HEPES pH7.2 and plated in 96-well tissue culture plates at a concentration of 1.7×10^6 cells/well. Cells were treated with 50 μM thimerosal (Sigma)/1 mM $CaCl_2$/1 mM $MgCl_2$ for 15 minutes at 37° C. 5% CO_2, then treated with compounds at 1000, 200, 40, 8, 1.6, 0.32, 0.064, and 0 nM in a final DMSO concentration of 0.01% in duplicate for 10 minutes. Neutrophils were stimulated with 1 μM fMLF for 30 minutes, then lysed by the addition of methanol (20% final concentration) and frozen in a dry ice/isopropanol bath for 10 minutes. Lysates were stored at −70° C. until the LTB4 content was measured by competitive LTB4 ELISA (R&D Systems). Results are shown in Table 2.

Zymosan-Induced IL-8 Production

Zymosan A, or the heat-killed yeast *Saccharomyces cerevisiae*, binds to the adhesion molecule Mac-1 on the neutrophil surface and triggers phagocytosis, cell activation and IL-8 production. Zymosan-induced IL-8 production was measured as previously described (Au et al. 1998, *Brit. J. Pharm.* 123:1260-1266) with the following modifications. Human neutrophils were purified as described above, plated in 96-well tissue culture plates at 3×10^5 cells/well in complete medium, treated with compounds at 10, 2, 0.4, 0.08, 0.016, 0.0032, 0.00064, and 0 μM in duplicate in a final DMSO concentration of 0.1% for 1 hour at 37° C. 5% CO_2. Neutrophils were then stimulated with unopsonized, boiled Zymosan A (Sigma) at 2.5×10^5 particles/well for 18 hours. Supernatants were harvested and tested for IL-8 by ELISA (R&D Systems). Results are shown in Table 2.

fMLF-Induced CD18/CD11b Expression

CD18/CD11b (Mac-1) expression on neutrophils was measured as previously described (Derian et al. 1995, *J. Immunol.:* 154:308-317) with the following modifications. Neutrophils were isolated as described above, then resuspended in complete medium at 1×10^6 cells/ml, pretreated with compounds at 10, 1, 0.1, 0.01, and 0 μM in duplicate at a final DMSO concentration of 0.1% for 10 minutes at 37° C. 5% CO_2. Cells were then stimulated with 30 nM fMLF for 30 minutes and then chilled to 4° C. Cells were treated with rabbit IgG (Jackson ImmunoResearch Labs, West Grove, Pa., USA) (10 μg/1×10^6 cells) to block Fc receptors, stained with CD18-FITC and CD11b-PE (Becton Dickinson), and analyzed by flow cytometry on a FACSCalibur. CD18/CD11b expression (mean fluorescence) in the absence of stimulation was subtracted from all samples to obtain inhibition curves and calculate IC_{50}s. Results are shown in Table 2.

fMLF-Induced Adhesion to HUVEC

Human umbilical vein endothelial cells (HUVEC) were used as a substrate for neutrophil adhesion as previously described (Derian et al. 1995, *J. Immunol.:* 154:308-317) with the following modifications. HUVEC cells were obtained from Anthrogenesis (Cedar Knolls, N.J., USA), and neutrophils were not treated with cytochalasin B. Cells were treated with compounds at 10, 1, 0.1, 0.01, 0.001, and 0 μM in a final DMSO concentration of 0.1% in duplicate for 10 minutes, stimulated with 500 nM fMLF for 30 minutes, and washed twice with PBS before measuring fluorescence on an FLX800 plate reader (Bio-Tek Instruments, Winooski, Vt., USA). Results are shown in Table 2.

TABLE 2

Human Neutrophil Assays (all values in nM)	Racemic Compound	Compound A
PGE_2-induced cAMP EC_{50}	12589	4570
fMLF-induced LTB4 IC_{50}	20.1	2.48
Zymosan-induced IL-8 IC_{50}	ND	94
fMLF-induced CD18 expression IC_{50}	ND	390
fMLF-induced CD11b expression IC_{50}	ND	74
fMLF-induced adhesion to HUVEC IC_{50}	ND	150

5.8. Example 8

Aqueous Solubility

Equilibrium solubilities were measured in pH 7.4 aqueous buffer. The pH 7.4 buffer was prepared by adjusting the pH of a 0.07 M NaH_2PO_4 solution to 7.4 with 10 N NaOH. The ionic strength of the solution was 0.15. At least 1 mg of powder was combined with 1 ml of buffer to make >1 mg/ml mixture. These samples were shaken for >2 hours and left to stand overnight at room temperature. The samples were then filtered through a 0.45-μm Nylon syringe filter that was first saturated with the sample. The filtrate was sampled twice, consecutively. The filtrate was assayed by HPLC against standards prepared in 50% methanol. Compound A has 3.5-fold greater aqueous solubility than the racemic mixture. Measured solubility Compound A=0.012 mg/mL; racemic mixture=0.0034 mg/mL.

5.9. Example 8

LPS—Induced Lung Neutrophilia Ferret Model

The conscious ferret model has been used to investigate anti-inflammatory, emetic and behavioral effects of PDE4 inhibitors when administered by the oral (p.o.) route. From these experiments, a therapeutic index (TI) for each PDE4 inhibitor may be determined. The TI has been calculated by dividing the threshold dose for causing emetic episodes and behavioral changes by the anti-inflammatory dose (dose that causes 50% inhibition of the LPS-induced neutrophilia).

Animal Husbandry

Male ferrets (Mustela Pulorius Euro, weighing 1-2 kg). Ferrets were supplied either by Bury Green Farm or Misay Consultancy. Following transport, the animals were allowed to acclimatize in the holding rooms for a period of not less than 7 days. The Diet comprised SDS diet C pelleted food

given ad lib with Whiskers cat food given 3 times per week. Water was pasteurized animal grade drinking water and was changed daily.

Dosing with PDE4 Inhibitor

PDE4 inhibitors were administered orally (p.o.), at doses initially of 1-10 mg/kg, but subsequently up to 30 mg/kg in order to establish whether the TI was 10 or higher, and/or at lower doses to establish the minimum dose to cause 50% inhibition of neutrophilia. Ferrets were fasted overnight but allowed free access to water. The animals were orally dosed with vehicle or PDE4 inhibitor using a 15 cm dosing needle that was passed down the back of the throat into the oesophagus. After dosing, the animals were returned to holding cages fitted with Perspex doors to allow observation, and given free access to water. After dosing, the animals were constantly observed and any emesis or behavioural changes were recorded. The animals were allowed access to food 60-90 minutes after p.o. dosing

Exposure to LPS

Thirty minutes after p.o. dosing with compound or vehicle control, the ferrets were placed into sealed Perspex containers and exposed to an aerosol of LPS (100 μg/ml) for 10 minutes. Aerosols of LPS were generated by a nebulizer (DeVilbiss, USA) and this was directed into the Perspex exposure chamber. Following a 10 minute exposure period, the animals were returned to the holding cages and allowed free access to water, and at a later stage, food. Observation continued for a period of at least 2.5 hours post p.o. dosing and emetic episodes and behavioral changes were recorded.

Bronchoalveolar Lavage

Six hours after LPS exposure the animals were killed by overdose of sodium pentobarbitone administered intraperitoneally. The trachea was then cannulated with polypropylene tubing and the lungs lavaged twice with 20 ml heparinized (10 units/ml) phosphate buffered saline (PBS).

Blood Sampling/Tissue Removal

A terminal blood sample (10 ml) was removed by transthoracic cardiac puncture. The blood was spun at 2500 rpm for 15 minutes and the plasma removed and stored at −20° C. The brain also removed and frozen at −20° C. for analysis of compound content.

Cell Counts

The bronchoalveolar lavage (BAL) samples were centrifuged at 1500 rpm for 5 minutes. The supernatant was removed and the resulting cell pellet re-suspended in 1 ml PBS. A cell smear of the re-suspended fluid was prepared and stained with Leishmans stain to allow differential cell counting. A total cell count was made using the remaining re-suspended sample. From this, the total number of neutrophils in the BAL was determined.

Parameters Measured:

1. % Inhibition of LPS-induced pulmonary neutrophilia.

2. Emetic episodes—the number of vomits and retches were counted.

3. Behavioral changes—the following behavioral effects were noted: salivation, panting, mouth clawing, flattened posture, ataxia, arched back and backward walking. Any behavioral changes were semi-quantified by applying a severity rating (mild, moderate or severe).

4. The TI was calculated as the highest dose found to not cause emetic episodes divided by the lowest dose found to inhibit pulmonary neutrophilia by 50% or more.

The effect of Compound A on LPS-induced neutrophilia in the lungs of conscious ferrets is demonstrated in FIG. **1**.

Emesis and Behavioral Changes

Following p.o. dosing of the PDE4, the ferrets were observed for at least 2 hours and emetic episodes (vomits and retches) and behavioral changes were recorded.

No emetic episodes (retching or vomiting) wore observed in the ferrets pre-treated p.o. with the relevant vehicle (acetone/cremophor/distilled water). In a small proportion of the control-treated animals (7/22), mild behavioral changes (lip licking and backward walking) were seen.

Compound A (0.1-3 mg/kg, p.o.), caused no emetic episodes (retching and vomiting). Some behavioral changes (flattened posture, lip licking and backward walking) were observed and classified as mild. At 10 mg/kg in 2/6 ferrets, some retching but no frank emesis was observed along with salivation and behavioral changes (scored as mild or moderate). At the highest dose tested (30 mg/kg) moderate to marked emesis was observed in ¾ animals along with pronounced behavioral changes. These data are summarized in Table III.

TABLE III

Conscious ferret: Emetic episodes and behavioural changes following oral administration of Compound A.

Treatment/dose (mg/kg)	Vomits	Retches	Salivation	Panting	Mouth clawing	Flattened posture	Ataxla	Lip licking	Backward walking
Vehicle (acetone/cremophor/ dist. H2O)	None	None	None	None	None	None	None	Mild (6/22)	Mild (7/22)
Compound A (0.1 mg/kg)	None	None	None	None	None	Mild (2/5)	None	Mild (4/5)	Mild (3/5)
Compound A (0.3 mg/kg)	None	None	None	None	None	Mild (2/6)	None	Mild (3/6)	Mild (4/6)
Compound A (1.0 mg/kg)	None	None	None	None	None	Mild (2/6)	None	Mild (6/6)	Mild (4/6)
Compound A (3.0 mg/kg)	None	None	None	None	Mild (1/8)	Marked (7/8)	None	Mild (2/8)	Moderate (5/8)
Compound A (10 mg/kg)	None	Mild (2/6)	Mild (1/6)	None	Mild (1/6)	Marked (6/6)	None	Moderate (5/6)	Marked (6/6)
Compound A (30 mg/kg)	Moderate (3/4)	Marked (3/4)	Moderate (3/4)	Mild (1/4)	Marked (4/4)	Marked (4/4)	Mild (3/4)	Moderate (4/4)	Mild (2/4)

Animals were observed for up to 3 hours following dosing. Numbers in parentheses refer to the number of animals that responded The numbers of animals in each group range from 4-22.

Therapeutic Index Calculation

From these experiments, a therapeutic index (TI) was determined for each compound by dividing the threshold dose for inducing emetic episodes by the ED_{50} value for inhibiting the pulmonary neutrophilia. The TI calculation is summarized in Table IV. Compound A had a TI of 12, causing no emetic episodes at an anti-inflammatory dose of 1 ms/kg.

TABLE IV

Summary of the effective doses (ED_{50}) for inhibition of LPS-induced pulmonary neutrophilia and induction of emesis and the therapeutic index derived from these values.

Compound	Inhibition of LPS-induced neutrophilia (ED_{50} mg/kg)	Threshold emetic dose (mg/kg)	Therapeutic Index
Compound A	0.8	10	12

5.10. Example 9

200 mg Dosage Capsule

Table V illustrates a batch formulation and single dosage formulation for a 200 mg Compound A single dose unit, i.e., about 40 percent by weight, in a size #0 capsule.

TABLE V

Formulation for 200 mg capsule

Material	Percent By Weight	Quantity (mg/tablet)	Quantity (kg/batch)
Compound A	40.0%	200 mg	16.80 kg
Pregelatinized Corn Starch, NF5	9.5%	297.5 mg	24.99 kg
Magnesium Stearate	0.5%	2.5 mg	0.21 kg
Total	100.0%	500 mg	42.00 kg

The pregelatinized corn starch (SPRESS B-820) and Compound A components are passed through a 710 μm screen and then are loaded into a Diffusion Mixer with a baffle insert and blended for 15 minutes. The magnesium stearate is passed through a 210 μm screen and is added to the Diffusion Mixer. The blend is then encapsulated in a size #0 capsule, 500 mg per capsule (8400 capsule batch size) using a Dosator type capsule filling machine.

5.11. Example 10

100 mg Oral Dosage Form

Table VI illustrates a batch formulation and a single dose unit formulation containing 100 mg of Compound A.

TABLE VI

Formulation for 100 mg tablet

Material	Percent by Weight	Quantity (mg/tablet)	Quantity (kg/batch)
Compound A	40%	100.00	20.00
Microcrystalline Cellulose, NF	53.5%	133.75	26.75
Pluronic F-68 Surfactant	4.0%	10.00	2.00
Croscarmellose Sodium Type A, NF	2.0%	5.00	1.00
Magnesium Stearate, NF	0.5%	1.25	0.25
Total	100.0%	250.00 mg	50.00 kg

The microcrystalline cellulose, croscarmellose sodium, and Compound A components are passed through a #30 mesh screen (about 430μ to about 655μ). The Pluronic F-68® (manufactured by JRH Biosciences, Inc. of Lenexa, Kans.) surfactant is passed through a #20 mesh screen (about 457μ to about 1041μ). The Plutonic F-68® surfactant and 0.5 kgs of croscarmellose sodium are loaded into a 16 qt. twin shell tumble blender and are mixed for about 5 minutes. The mix is then transferred to a 3 cubic foot twin shell tumble blender where the microcrystalline cellulose is added and blended for about 5 minutes. The thalidomide is added and blended for an additional 25 minutes. This pre-blend is passed through a roller compactor with a hammer mill attached at the discharge of the roller compactor and moved back to the tumble blender. The remaining croscarmellose sodium and magnesium stearate is added to the tumble blender and blended for about 3 minutes. The final mixture is compressed on a rotary tablet press with 250 mg per tablet (200,000 tablet batch size).

8.12. Example 11

Aerosol Dosage Form

A concentrate is prepared by combining Compound A, and a 12.6 kg portion of the trichloromonofluoromethane in a sealed stainless steel vessel equipped with a high shear mixer. Mixing is carried out for about 20 minutes. The bulk suspension is then prepared in the sealed vessel by combining the concentrate with the balance of the propellants in a bulk product tank that is temperature controlled to 21° to 27° C. and pressure controlled to 2.8 to 4.0 BAR. 17 ml aerosol containers which have a metered valve which is designed to provide 100 inhalations of the composition of the invention. Each container is provided with the following:

Compound A	0.0120 g
trichloromonofluoromethane	1.6939 g
dichlorodifluoromethane	3.7175 g
dichlorotetrafluoroethane	1.5766 g
total	7.0000 g

While the invention has been described with respect to the particular embodiments, it will be apparent to those skilled in the art that various changes and modifications may be made without departing from the spirit and scope of the invention as defined in the claims. Such modifications are also intended to fall within the scope of the appended claims.

What is claimed is:

1. A method of treating psoriasis, which comprises orally administering to a patient having psoriasis about 10 mg to about 200 mg per day of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acety-

laminoisoindoline-1,3-dione, wherein the compound is administered in the form of a tablet or capsule as either a single dose or a divided dose.

2. The method of claim **1**, wherein about 10 mg to about 100 mg of the compound is administered per day.

3. The method of claim **1**, wherein the compound is administered twice daily in equally divided doses.

4. The method of claim **1**, wherein the stereomerically pure compound comprises greater than about 90% by weight of (+) isomer based on the total weight percent of the compound.

5. The method of claim **1**, wherein the stereomerically pure compound comprises greater than about 95% by weight of (+) isomer based on the total weight percent of the compound.

6. The method of claim **1**, wherein the stereomerically pure compound comprises greater than about 97% by weight of (+) isomer based on the total weight percent of the compound.

7. The method of claim **1**, wherein the compound is administered in capsule form.

8. The method of claim **7**, wherein the capsule contains about 10 mg of the compound.

9. The method of claim **7**, wherein the capsule contains about 20 mg of the compound.

10. The method of claim **7**, wherein the capsule contains about 25 mg of the compound.

11. The method of claim **7**, wherein the capsule contains about 50 mg of the compound.

12. The method of claim **11**, wherein the compound is administered in tablet form.

13. The method of claim **12**, wherein the tablet contains about 10 mg of the compound.

14. The method of claim **12**, wherein the tablet contains about 20 mg of the compound.

15. The method of claim **12**, wherein the tablet contains about 25 mg of the compound.

16. The method of claim **12**, wherein the tablet contains about 50 mg of the compound.

17. A method of treating rheumatoid arthritis, which comprises administering to a patient having rheumatoid arthritis a therapeutically effective amount of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

18. The method of claim **17**, wherein the compound is administered orally.

19. The method of claim **18**, wherein about 5 mg to about 500 mg of the compound is administered per day.

20. The method of claim **18**, wherein about 10 mg to about 200 mg of the compound is administered per day.

21. The method of claim **18**, wherein about 10 mg to about 100 mg of the compound is administered per day.

22. The method of claim **18**, wherein the compound is administered twice daily in equally divided doses.

23. The method of claim **18**, wherein the stereomerically pure compound comprises greater than about 90% by weight of (+) isomer based on the total weight percent of the compound.

24. The method of claim **18**, wherein the stereomerically pure compound comprises greater than about 95% by weight of (+) isomer based on the total weight percent of the compound.

25. The method of claim **18**, wherein the stereomerically pure compound comprises greater than about 97% by weight of (+) isomer based on the total weight percent of the compound.

26. The method of claim **18**, wherein the compound is administered in capsule form.

27. The method of claim **26**, wherein the capsule contains about 10 mg of the compound.

28. The method of claim **26**, wherein the capsule contains about 20 mg of the compound.

29. The method of claim **26**, wherein the capsule contains about 25 mg of the compound.

30. The method of claim **26**, wherein the capsule contains about 50 mg of the compound.

31. The method of claim **18**, wherein the compound is administered in tablet form.

32. The method of claim **31**, wherein the tablet contains about 10 mg of the compound.

33. The method of claim **31**, wherein the tablet contains about 20 mg of the compound.

34. The method of claim **31**, wherein the tablet contains about 25 mg of the compound.

35. The method of claim **31**, wherein the tablet contains about 50 mg of the compound.

36. A method of treating rheumatoid arthritis, which comprises orally administering to a patient having rheumatoid arthritis about 10 mg to about 100 mg of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonyl-ethyl]-4-acetylaminoisoindoline-1,3-dione comprising greater than about 97% by weight of (+) isomer wherein the compound is administered in the form of a tablet or capsule twice daily in equally divided doses.

37. A method of treating Behcet's Disease, which comprises administering to a patient having Behcet's Disease a therapeutically effective amount of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

38. The method of claim **37**, wherein the compound is administered orally.

39. The method of claim **38**, wherein about 5 mg to about 500 mg of the compound is administered per day.

40. The method of claim **38**, wherein about 10 mg to about 200 mg of the compound is administered per day.

41. The method of claim **38**, wherein about 10 mg to about 100 mg of the compound is administered per day.

42. The method of claim **38**, wherein the compound is administered twice daily in equally divided doses.

43. The method of claim **38**, wherein the stereomerically pure compound comprises greater than about 90% by weight of (+) isomer based on the total weight percent of the compound.

44. The method of claim **38**, wherein the stereomerically pure compound comprises greater than about 95% by weight of (+) isomer based on the total weight percent of the compound.

45. The method of claim **38**, wherein the stereomerically pure compound comprises greater than about 97% by weight of (+) isomer based on the total weight percent of the compound.

46. The method of claim **38**, wherein the compound is administered in capsule form.

47. The method of claim **46**, wherein the capsule contains about 10 mg of the compound.

48. The method of claim **46**, wherein the capsule contains about 20 mg of the compound.

49. The method of claim **46**, wherein the capsule contains about 25 mg of the compound.

50. The method of claim **46**, wherein the capsule contains about 50 mg of the compound.

51. The method of claim **38**, wherein the compound is administered in tablet form.

52. The method of claim **51**, wherein the tablet contains about 10 mg of the compound.

53. The method of claim **51**, wherein the tablet contains about 20 mg of the compound.

54. The method of claim **51**, wherein the tablet contains about 25 mg of the compound.

55. The method of claim **51**, wherein the tablet contains about 50 mg of the compound.

56. A method of treating Behcet's Disease, which comprises orally administering to a patient having Behcet's Disease about 10 mg to about 100 mg of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonyl-ethyl]-4-acetylaminoisoindoline-1,3-dione comprising greater than about 97% by weight of (+) isomer wherein the compound is administered in the form of a tablet or capsule twice daily in equally divided doses.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 8,455,536 B2
APPLICATION NO. : 12/630788
DATED : June 4, 2013
INVENTOR(S) : George W. Muller et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Claims

In claim 12, at column 31, line 27, replace "claim 11", with --- claim 1 ---

Signed and Sealed this
Thirteenth Day of January, 2015

Michelle K. Lee

Michelle K. Lee
Deputy Director of the United States Patent and Trademark Office

EXHIBIT G

US009018243B2

(12) **United States Patent**
Muller et al.

(10) **Patent No.: US 9,018,243 B2**
(45) **Date of Patent: *Apr. 28, 2015**

(54) **SOLID FORMS COMPRISING (+)-2-[1-(3-ETHOXY-4-METHOXYPHENYL)-2-METHYLSULFONYLETHYL]-4-ACETYLAMINOISOINDOLINE-1,3-DIONE, COMPOSITIONS THEREOF, AND USES THEREOF**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **14/102,407**

(22) Filed: **Dec. 10, 2013**

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Related U.S. Application Data

(60) Division of application No. 13/300,458, filed on Nov. 18, 2011, now Pat. No. 8,629,173, which is a division of application No. 12/945,800, filed on Nov. 12, 2010, now Pat. No. 8,093,283, which is a continuation of application No. 12/079,615, filed on Mar. 27, 2008, now Pat. No. 7,893,101, which is a continuation-in-part of application No. 11/106,142, filed on Apr. 13, 2005, now Pat. No. 7,427,638, which is a division of application No. 10/392,195, filed on Mar. 19, 2003, now Pat. No. 6,962,940.

(60) Provisional application No. 60/366,515, filed on Mar. 20, 2002, provisional application No. 60/438,450, filed on Jan. 7, 2003.

(51) **Int. Cl.**

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(52) **U.S. Cl.**

CPC ***C07D 209/48*** (2013.01); ***A61K 31/4035*** (2013.01); *C07B 2200/07* (2013.01); ***C07C 317/28*** (2013.01)

(58) **Field of Classification Search**

USPC 514/417; 548/469

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,031,450	A	4/1962	Fischer et al.
3,322,755	A	5/1967	Roch et al.
3,598,123	A	8/1971	Zaffaroni
3,845,770	A	11/1974	Theeuwes et al.
3,916,899	A	11/1975	Theeuwes et al.
3,920,636	A	11/1975	Takahashi et al.
4,001,237	A	1/1977	Partyka et al.
4,001,238	A	1/1977	Partyka et al.
4,008,719	A	2/1977	Theeuwes et al.
4,047,404	A	9/1977	Hayashi
4,060,615	A	11/1977	Matier et al.
4,101,548	A	7/1978	Crenshaw et al.
4,162,316	A	7/1979	Nishimura et al.
4,209,623	A	6/1980	Juby
4,880,810	A	11/1989	Lowe, III
4,885,301	A	12/1989	Coates
5,059,595	A	10/1991	Le Grazie
5,073,543	A	12/1991	Marshall et al.
5,120,548	A	6/1992	McClelland et al.
5,147,875	A	9/1992	Coates et al.
5,354,556	A	10/1994	Sparks et al.
5,354,571	A	10/1994	Morikawa et al.
5,401,774	A	3/1995	Pamukcu et al.
5,439,895	A	8/1995	Lee et al.
5,488,055	A	1/1996	Kumar et al.
5,591,767	A	1/1997	Mohr et al.
5,608,914	A	3/1997	Keesler
5,614,530	A	3/1997	Kumar et al.
5,614,627	A	3/1997	Takase et al.
5,639,476	A	6/1997	Oshlack et al.
5,658,940	A	8/1997	Muller et al.
5,674,533	A	10/1997	Santus et al.
5,698,579	A	12/1997	Muller
5,703,098	A	12/1997	Muller et al.
5,710,170	A	1/1998	Guay et al.
5,728,844	A	3/1998	Muller et al.
5,728,845	A	3/1998	Muller et al.

(Continued)

FOREIGN PATENT DOCUMENTS

DE	2051871 A1	11/1971
EP	0 347 146	6/1989

(Continued)

OTHER PUBLICATIONS

U.S. Appl. No. 60/454,155, filed Mar. 12, 2003, Muller, et al.

(Continued)

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(74) *Attorney, Agent, or Firm* — Jones Day

(57) **ABSTRACT**

Solid forms comprising (+)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, compositions comprising the solid forms, methods of making the solid forms and methods of their use are disclosed. The methods include methods of treating and/or preventing disorders ameliorated by the reduction of levels of TNF-α or the inhibition of PDE4.

21 Claims, 33 Drawing Sheets

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,733,566	A	3/1998	Lewis	
5,736,570	A	4/1998	Muller et al.	
5,798,373	A	8/1998	Warrellow	
5,801,195	A	9/1998	Muller et al.	
5,849,770	A	12/1998	Head et al.	
5,877,200	A	3/1999	Muller	
5,891,896	A	4/1999	Warrellow et al.	
6,011,050	A	1/2000	Muller et al.	
6,020,339	A	2/2000	Perrier et al.	
6,020,358	A	2/2000	Muller et al.	
6,034,089	A	3/2000	Han et al.	
6,046,221	A	4/2000	Muller et al.	
6,069,156	A	5/2000	Oku et al.	
6,162,830	A	12/2000	Connor et al.	
6,166,041	A	12/2000	Cavalla et al.	
6,177,471	B1	1/2001	Menander et al.	
6,204,275	B1	3/2001	Friesen et al.	
6,218,400	B1	4/2001	Daugan et al.	
6,300,335	B1	10/2001	Campbell et al.	
6,316,472	B1	11/2001	Frenette et al.	
6,333,354	B1	12/2001	Schudt	
6,962,940	B2 *	11/2005	Muller et al.	514/417
7,208,516	B2	4/2007	Muller et al.	
7,276,529	B2	10/2007	Muller et al.	
7,358,272	B2	4/2008	Muller et al.	
7,427,638	B2	9/2008	Muller et al.	
7,507,759	B2	3/2009	Muller et al.	
7,659,302	B2	2/2010	Muller et al.	
7,659,303	B2	2/2010	Muller et al.	
7,799,782	B2 *	9/2010	Munson et al.	514/234.5
7,893,101	B2	2/2011	Muller et al.	
8,093,283	B2	1/2012	Muller et al.	
8,455,536	B2 *	6/2013	Muller et al.	514/417
8,629,173	B2	1/2014	Muller et al.	
2010/0324108	A1	12/2010	Liu	

FOREIGN PATENT DOCUMENTS

EP	0 349 239	6/1989
EP	0 351 058	6/1989
EP	0 352 960	7/1989
EP	0 395 328	4/1990
EP	0 428 268	10/1990
EP	0 463 756	6/1991
EP	0 526 004	7/1992
EP	0 607 439	9/1992
EP	0 722 937	1/1996
EP	0 722 943	1/1996
EP	0 722 944	1/1996
GB	2 063 249	9/1980
WO	WO 93/07149	4/1993
WO	WO 93/12095	6/1993
WO	WO 94/01728	1/1994
WO	WO 94/05661	3/1994
WO	WO 94/29277	12/1994
WO	WO 95/19978	7/1995
WO	WO 96/32379	10/1996
WO	WO 97/03070	1/1997
WO	WO 97/03675	2/1997
WO	WO 97/03985	2/1997
WO	WO 97/24334	7/1997
WO	WO 98/06722	2/1998
WO	WO 98/08848	3/1998
WO	WO 98/14448	4/1998
WO	WO 98/16521	4/1998
WO	WO 98/17668	4/1998
WO	WO 98/23597	6/1998
WO	WO 98/38168	9/1998
WO	WO 99/06041	2/1999
WO	WO 00/25777	5/2000
WO	WO 01/34606	5/2001
WO	WO 01/47915	7/2001
WO	WO 03/080049	10/2003
WO	WO 2010/030345	3/2010

OTHER PUBLICATIONS

U.S. Appl. No. 60/454,149, filed Mar. 12, 2003, Muller, et al.
U.S. Appl. No. 60/438,450, filed Jan. 7, 2003, Muller, et al.
U.S. Appl. No. 60/436,975, filed Dec. 30, 2002, Muller, et al.
U.S. Appl. No. 60/366,515, filed Mar. 20, 2002, Muller, et al.
Akazome, M. et al., "Asymmetric recognition of 1-arylethylamines by (R)-phenylglycyl-(R)-phenylglycine and its mechanism," Tetrahedron: Asymmetry, Elsevier Scince Publishers, Amsterdam, NL, 8(14):2331-2336 (1997).
Andrew et al., Controlling Crystal Architecture in Molecular Solids: The Supramolecular Approach, in Supramolecular Organization and Materials Design, 391, 436 (W. Jones & C. N. R. Rao, eds., 2001).
Au et al., "Effect of PDE4 inhibitors on zymosan-induced IL-8 release from human neutrophils: synergism with prostanoids and salbutamol," Brit. J. Pharm. 123:1260-1266 (1998).
Baehr et al., "Isolation and characterization of cGMP phosphodiesterase from bovine rod outer segments," J. Biol. Chem. 254:11669-11677 (1979).
Balasubramanian et al., "Synthesis of β-amino-sulphones and αβ-unsaturated sulphones, Part II," J. Chem. Soc., 3296-3298 (1955).
Baughman et al., "Release of tumor necrosis factor by alveolar macrophages of patients with sarcoidosis," J. Lab. Clin. Med. 115:36-42 (1990).
Beavo and Reifsnyder, "Primary sequence of cyclic nucleotide phosphodiesterase isozymes and the design of selective inhibitors," Trends in Pharm., 11:150-155 (1990).
Berge et al., "Pharmaceutical Salts," Journal of Pharmaceutical Sciences, 66(1):1-19 (1977).
Bertolini et al., "Stimulation of bone resorption and inhibition of bone formation in vitro by human tumour necrosis factors," Nature, 319(6053)516-518 (1986).
Bissonnette et al., "Pulmonary inflammation and fibrosis in a murine model of asbestosis and silicosis. Possible role of tumor necrosis factor," Inflammation 13:329-339 (1989).
Bloom et al., "Identification and tissue-specific expression of PDE7 phosphodiesterase splice variants," Proc. Natl. Acad. Sci. USA 93:14188-14192 (1996).
Brackeen et al., "Design and Synthesis of Conformationally Constrained Analogues of 4-(3-Butoxy-4-Methoxybenzyl) Imidazolidin-2-one (Ro 20-1724) as Potent Inhibitors of cAMP-Specific Phosphodiesterase", J. Med. Chem. 38:4848-54 (1995).
Britain's, "Polymorphism in Pharmaceutical Solids," Drugs and the Pharmaceutical Science, 95: 348-361 (1999).
Bundgaard, Hans, "Design of Prodrugs: Bioreversible Derivatives for Various Functional Groups and Chemical Entities," Design of Prodrugs, Chapter 1: 1-4 (1985).
Carstensen, Drug Stability: Principles & Practice, 2nd ed., Marcel Dekker, New York, NY pp. 379-380 (1995).
Casini et al., "Preparation of One of the Optical Antipodes of 2-Phthalimidoglutarimide," Farmaco Ed. Sci. 19:563-565 (1964).
Chemburkar et al., "Dealing with the impact of ritonavir polymorphs on the late stages of bulk drug process development," Org. Process Res. Dev., 4:413-417 (2000).
Clouse et al., "Monokine regulation of human immunodeficiency virus-1 expression in a chronically infected human T cell clone," J. Immunol. 142:431-438 (1989).
Craven et al., "Crystal Structures of Two Polymorphs of 5-Ethyl-5-Isoamylbarbituric Acid (Amobarbital)," Acta Crystallographica, Section B: Structural Crystal Chemistry, B25 (PL. 10) (1969).
Craven et al., CAS: 71-117431 (1969).
Derian et al., "Inhibition of chemotactic peptide-induced neutrophil adhesion to vascular endothelium by cAMP modulators," J. Immunol. 154:308-317 (1995).
Dezube et al., "Pentoxifylline and wellbeing in patients with cancer," Lancet, 335:662 (1990).
Dredge et al., "Angiogenesis Inhibitors in Cancer Therapy," Curr. Opin. Investig. Drugs 4(6):667-674 (2003).
Dredge et al., "Recent Developments in Antiangiogenic Therapy," Expert Opin. Biol. Ther. 2(8):953-966 (2002).
Dredge et al., "Immunological effects of thalidomide and its chemical and functional analogs," Crit. Rev. Immunol. 22(5-6):425-437 (2002).

(56) **References Cited**

OTHER PUBLICATIONS

Duh et al., "Tumor necrosis factor alpha activates human immunodeficiency virus type 1 through induction of nuclear factor binding to the NF-kappa B sites in the long terminal repeat," Proc. Nat. Acad. Sci. 86:5974-5978 (1989).
Elliott et al., "TNFα Blockade in rheumatoid arthritis: rationale, clinical outcomes and mechanisms of action," Int. J. Immunopharmac., 17(2):141-145 (1995).
FDA's Guidance for Industry, pp. 1-20 (2000).
Featherstone et al., "Comparison of Phosphodiesterase Inhibitors of Differing Isoenzyme Selectivity Added to St. Thomas' Hospital Cardioplegic Solution Used for Hypothermic Preservation of Rat Lungs", Am. J. Respir. Crit. Care Med. 162:850-856 (2000).
Ferrai-Baliviera et al., "Tumor necrosis factor induces adult respiratory distress syndrome in rats," Arch. Surg., 124(12):1400-1405 (1989).
Folks et al., "Tumor necrosis factor alpha induces expression of human immunodeficiency virus in a chronically infected T-cell clone," Proc. Natl. Acad. Sci., USA, 86(7):2365-2368 (1989).
Gee et al., "Selective Cytokine Inhibitory Drugs With Enhanced Antiangiogenic Activity Control Tumor Growth Through Vascular Inhibition," Cancer Res. 63(23):8073-8078 (2003).
Gillespie et al., "Inhibition and stimulation of photoreceptor phosphodiesterases by dipyridamole and M&B 22,948.," Mol. Pharm. 36:773-781 (1989).
Grant, "Theory and Origin of Polymorphism," Polymorphism in Pharmaceutical Solids, pp. 1-10 (Jan. 1999).
Grau et al., "Tumor necrosis factor and disease severity in children with falciparum malaria," N. Engl. J. Med., 320(24):1586-1591 (1989).
Hidaka and Asano, "Human blood platelet 3': 5'-cyclic nucleotide phosphodiesterase. Isolation of low-Km and high-Km phosphodiesterase," Biochem. Biophys. Acta, 429:485-497 (1976).
Hinshaw et al., "Survival of primates in LD100 septic shock following therapy with antibody to tumor necrosis factor (TNF alpha)," Circ. Shock, 30:279-292 (1990).
Holler et al., "Increased serum levels of tumor necrosis factor alpha precede major complications of bone marrow transplantation," Blood 75:1011-1016 (1990).
International Search Report in Corresponding Application No. PCT/US2008/004021 dated Jan. 14, 2009.
Johnson et al., "Tumors producing human tumor necrosis factor induced hypercalcemia and osteoclastic bone resorption in nude mice," Endocrinology 124:1424-1427 (1989).
Jones et al., "Pharmaceutical Cocrystals: An Emerging Approach to Physical Property Enhancement," Mater. Res. Bull., 31:875-79 (2006).
Lai et al., "One-pot approach for the regioselective synthesis of beta-keto sufones based on acid-catalyzed reaction of sulfonyl chlorides with arylacetylenes and water," Tetrahedron Lett., 46(3):513-515 (2005).
List et al., "The myelodysplastic syndromes: biology and implications for management," J. Clin. Oncol. 8:1424-1441 (1990).
Liu et al., "Synthesis of enantiomerically pure N-tert-butanesulfinyl imines (tert-butanesulfinimines) by the direct condensation of tert-butanesulfinamide with aldehydes and ketones," J. Org. Chem. 64:1278-1284 (1999).
Lowe and Cheng, "The PDE IV family of calcium-independent phosphodiesterase enzymes," Drugs of the Future, 17(9):799-807 (1992).
Lowe et al., "Novel dioxolanes as cholesterol lowering agents," Exp. Opin. Ther. Patents *:1309-1332 (1998).
Luke et al., "Synthesis of (S)-5-(1-Aminoethyl)-2-(Cyclohexylmethoxy) Benzamide," Tetrahedron: Asymmetry, Elsevier Science Publishers, Amsterdam, NL, 10(22):4393-4403 (1999).
Marriott et al., "Immunotherapeutic and Antitumour Potential of Thalidomide Analogues," Expert Opin. Biol. Ther. 1(4):1-8 (2001).
Marriott et al., "Thalidomide and Its Analogues Have Distinct and Opposing Effects on TNF-Alpha and TNFR2 During Co-Stimulation of Both CD4(+) and CD8(+) T cells," Clin. Exp. Immunol. 130(1):75-84 (2002).
Merck Manual ,17th ed., 953 (1999).
Millar et al., "Tumour necrosis factor in bronchopulmonary secretions of patients with adult respiratory distress syndrome," Lancet, 2(8665):712-714 (1989).
Molostvov et al., "The Effects of Selective Cytokine Inhibitory Drugs (CC-10004 and CC-1088) on VEGF and IL-6 Expression and Apoptosis in Myeloma and Endothelial Cell Co-Cultures," Br. J. Haematol.124(3):366-375 (2004).
Monté et al., "Productive human immunodeficiency virus-1 infection of megakaryocytic cells is enhanced by tumor necrosis factor-alpha," Blood 79:2670-2679 (1992).
Muller et al., "Amino-substituted thalidomide analogs: potent inhibitors of TNF-alpha production," Bioorg. Med. Chem. Lett., 9:1625-1630 (1999).
Muller et al., "Structural modifications of thalidomide produce analogs with enhanced tumor necrosis factor inhibitory activity," J. Med. Chem. 39:3238-3240 (1996).
Muller et al., "Thalidomide analogs and PDE4 inhibition," Bioorg. & Med Chem Lett., 8:2669-2674 (1998).
Nicholson et al., "Differential modulation of tissue function and therapeutic potential of selective inhibitors of cyclic nucleotide phosphodiesterase isoenzymes," Trends Pharmaco. Sci., 12:19-27 (1991).
Non-Final Office Action dated Jun. 16, 2010 in U.S. Appl. No. 12/079,615.
Non-Final Office Action dated Mar. 18, 2013 in U.S. Appl. No. 13/300,458.
Notice of Allowance dated Oct. 20, 2010 in U.S. Appl. No. 12/079,615.
Notice of Allowance dated Sep. 23, 2013 in U.S. Appl. No. 13/300,458.
Notice of References Cited from Office Action dated Dec. 3, 2007 in U.S. Appl. No. 11/106,142.
Piguet et al., "Requirement of tumour necrosis factor for development of silica-induced pulmonary fibrosis," Nature, 344(6263):245-247 (1990).
Poli et al., Tumor necrosis factor alpha functions in an autocrine manner in the induction of human immunodeficiency virus expression, Proc. Natl. Acad. Sci USA, 87(2):782-785 (1990).
Poli et al., "The Effect of Cytokines and Pharmacologic Agents on Chronic HIV Infection," AIDS Res. Hum. Retrovirus, 8(2)191-197 (1992).
Rice and Bevilacqua, "An inducible endothelial cell surface glycoprotein mediates melanoma adhesion," Science, 246(4935):1303-1306 (1989).
Shealy et al., "D- and L-thalidomide," Chem. Indus. 12;24:1030-1 (1965).
STN Accession No. 1967:75462 (1966).
Tierney et al., Current Medical Diagnosis & Treatment, 37th ed., Appleton & Lange, p. 499 (1998).
Tracey et al., "Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia," Nature, 330:662-664 (1987).
Trask et al., "Selective Polymorph Transformation Via Solvent-Drop Grinding," Chem. Commun., 880-882 (2005).
U.S. Department of Health and Human Services, Food and Drug Administration, "Guidance for Industry—Impurities: Residual Solvents in New Veterinary Medicinal Products, Active Substances and Excipients," pp. 1-20 (2000).
Van Dullemen et al., "Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2)," Gastroenterology, 109:129-135 (1995).
Velasquez et al.,. "Stereoselective synthesis of β-substituted β-amino sulfones and sulfonamides via addition of sulfonyl anions to chiral N-sulfinyl imines," Organic Lett., 8(4):789-792 (2006).
Verghese et al., "Regulation of ion transport by endothelins in rat colonic mucosa: effects of an ETA antagonist (FR139317) and an ETB agonist (IRL1620)," J. Pharmacol. Exp. Ther., 272(3): 1313-1320 (1995).

(56) **References Cited**

OTHER PUBLICATIONS

Vippagunta et al., "Crystalline solids," Adv. Drug Deliv. Rev., 48:3-26 (2001).
Wilen et al., "Strategies in optical resolutions," Tetrahedron 33:2725-2736 (1977).
Wilen, Tables of Resolving Agents and Optical Resolutions (E.L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, IN) p. 268 (1972).
Wolff, Manfred E., ed., "Burger's Medicinal Chemistry and Drug Discovery," 5th ed., 172-178, 949-982 (1995).
Yu, "Amorphous pharmaceutical solids: preparation, characterization and stabilization," Adv. Drug Deliv. Rev., 48:27-42 (2001).
Zhang et al., "Practical and stereoselective synthesis of β-amino sulfones from alkyl phenyl sulfones and N-(tert-butylsulfinyl) aldimines," Org. Biomol. Chem., 9:6502-6505 (2011).
Corral et al., "Selection of novel analogs of thalidomide with enhanced tumor necrosis factor αinhibitory activity," Mol. Med., 2(4):506-515 (1996).
Hatzelmann and Schudt, "Anti-inflammatory and immunomodulatory potential of the novel PDE4 inhbitor roflumilast in vitro," J. Pharm. Exper. Ther., 297(1):267-279 (2001).
Akoğlu et al., "TNF, soluble IL-2R and soluble CD-8 in Behçet's disease," Journal of Rheumataology, 17(8):1107-1108 (1990).
Simonyi et al., "Stereochemical Aspects of Drug Action. II: Optical Isomerism," in the Practice of Medicinal Chemistry, Ed. Wermuth, Academic Press, London, Chapter 21 (1996).

* cited by examiner

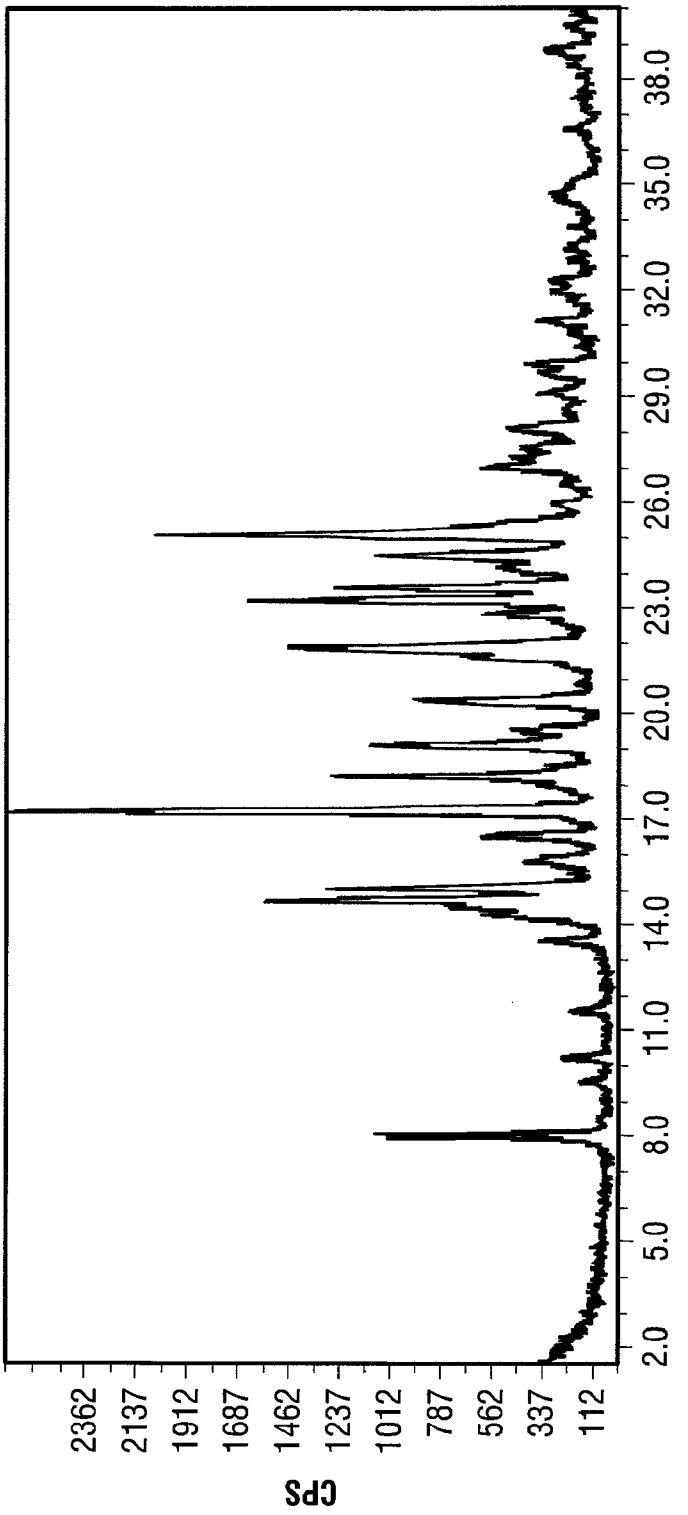

CPS
2362
2137
1912
1687
1462
1237
1012
787
562
337
112
2.0
5.0
8.0
11.0
14.0
17.0
20.0
23.0
26.0
29.0
32.0
35.0
38.0
Deg.

FIG. 1

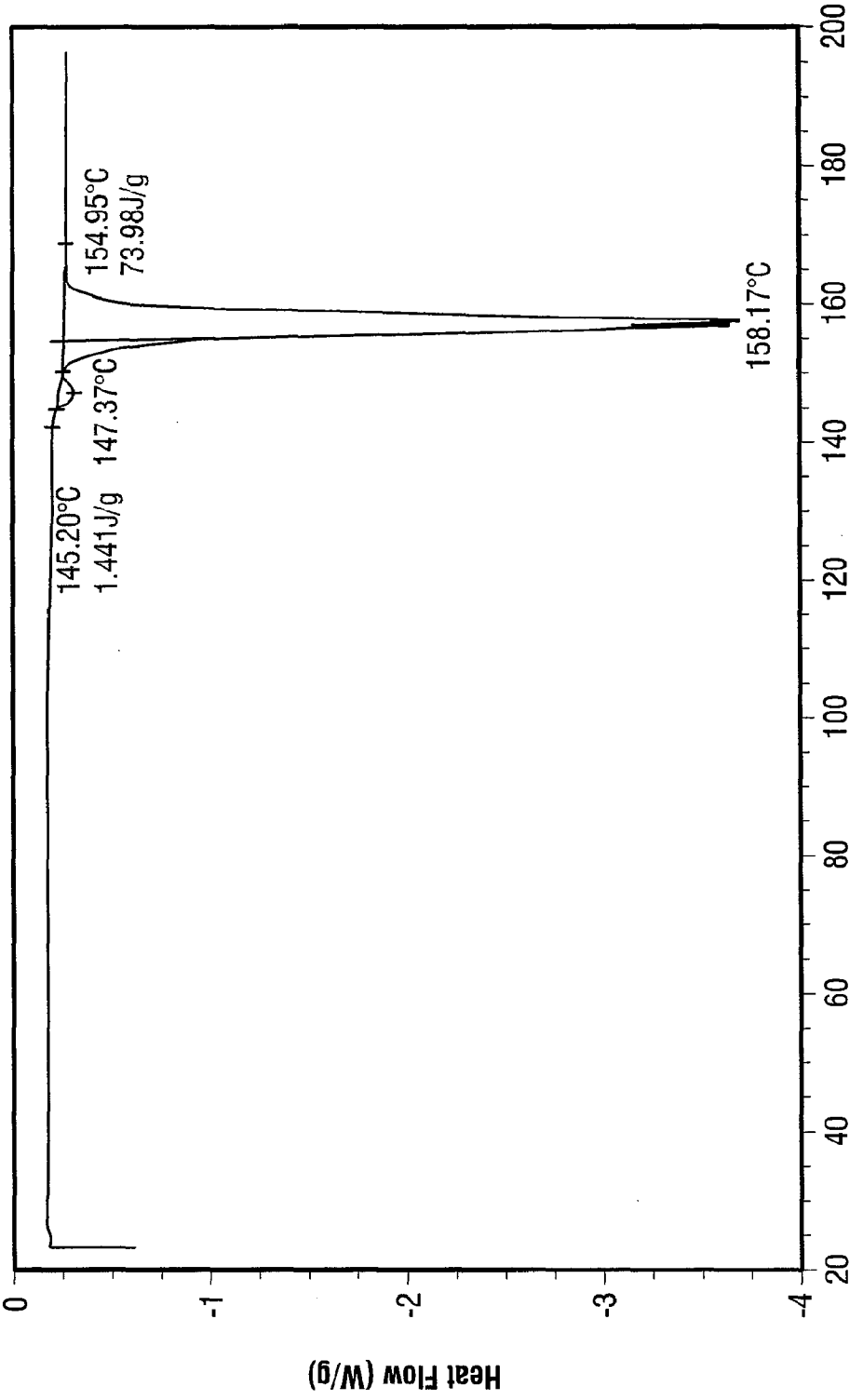

154.95°C
73.98J/g
158.17°C
147.37°C
145.20°C
1.441J/g
Heat Flow (W/g)
0
-1
-2
-3
-4
20
40
60
80
100
120
140
160
180
200
Temperature (°C)

FIG. 2

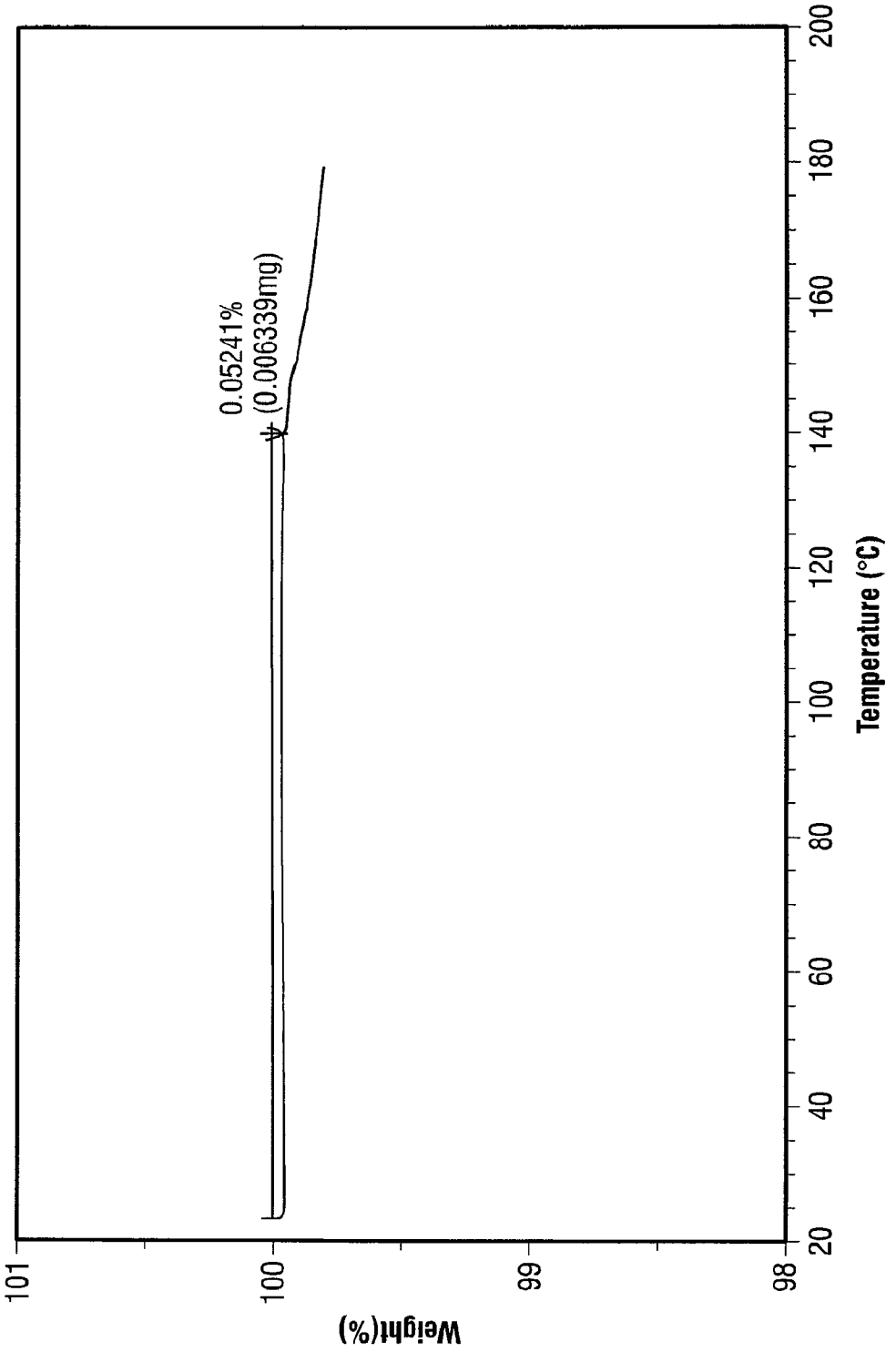

0.05241%
(0.006339mg)
Weight(%)
101
100
99
98
Temperature (°C)
20
40
60
80
100
120
140
160
180
200

FIG. 3

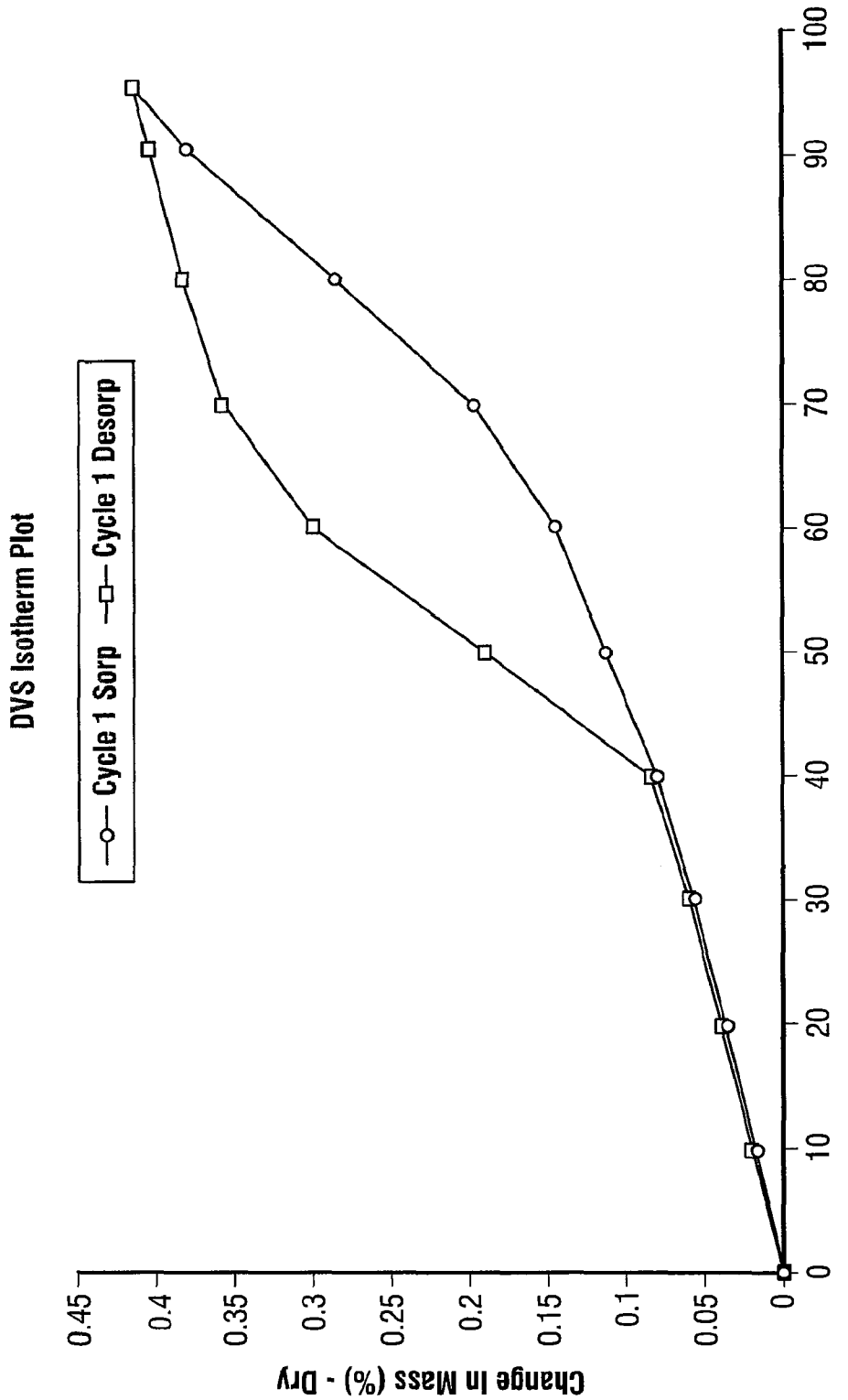

DVS Isotherm Plot
Cycle 1 Sorp
Cycle 1 Desorp
Change In Mass (%) - Dry
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Target RH(%)
0
10
20
30
40
50
60
70
80
90
100

FIG. 4

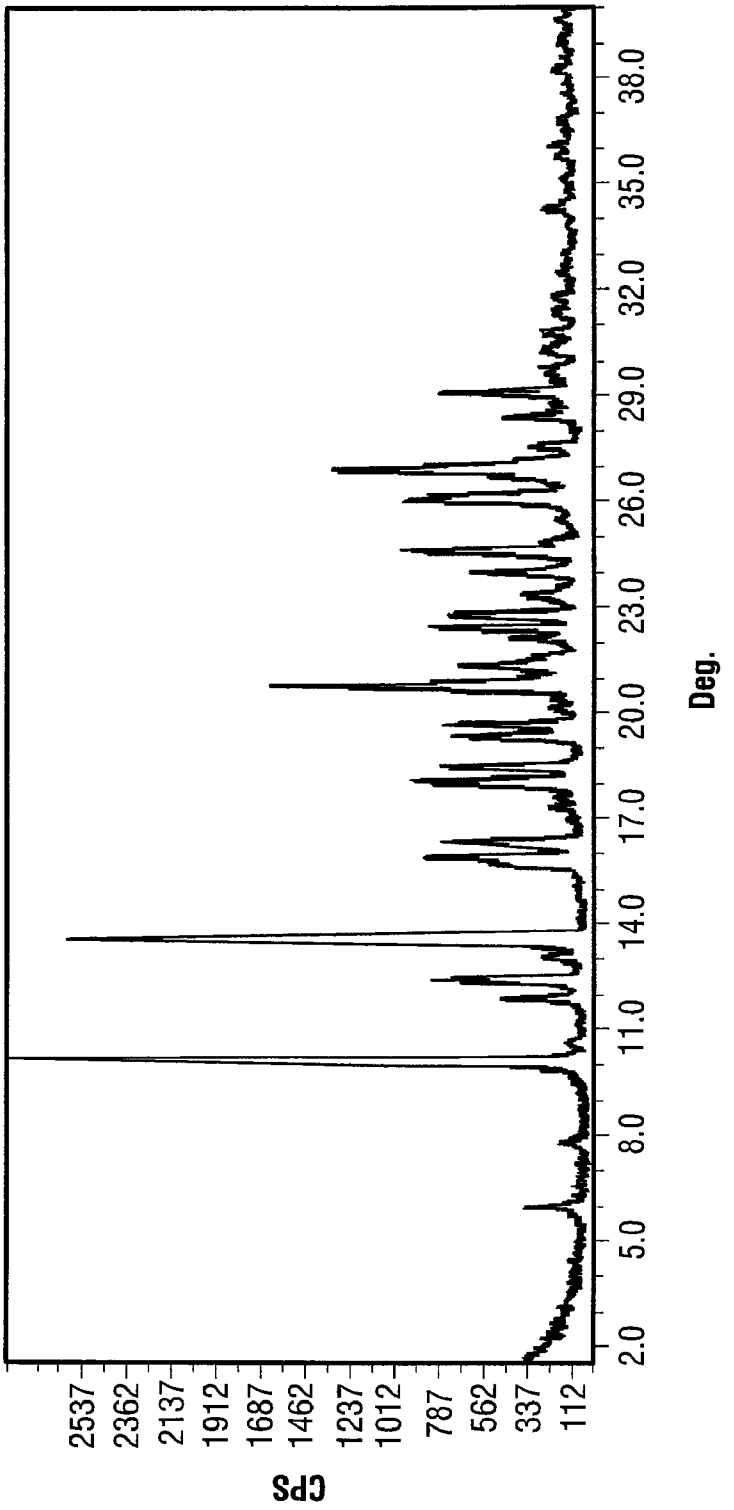

CPS
2537
2362
2137
1912
1687
1462
1237
1012
787
562
337
112
2.0
5.0
8.0
11.0
14.0
17.0
20.0
23.0
26.0
29.0
32.0
35.0
38.0
Deg.

FIG. 5

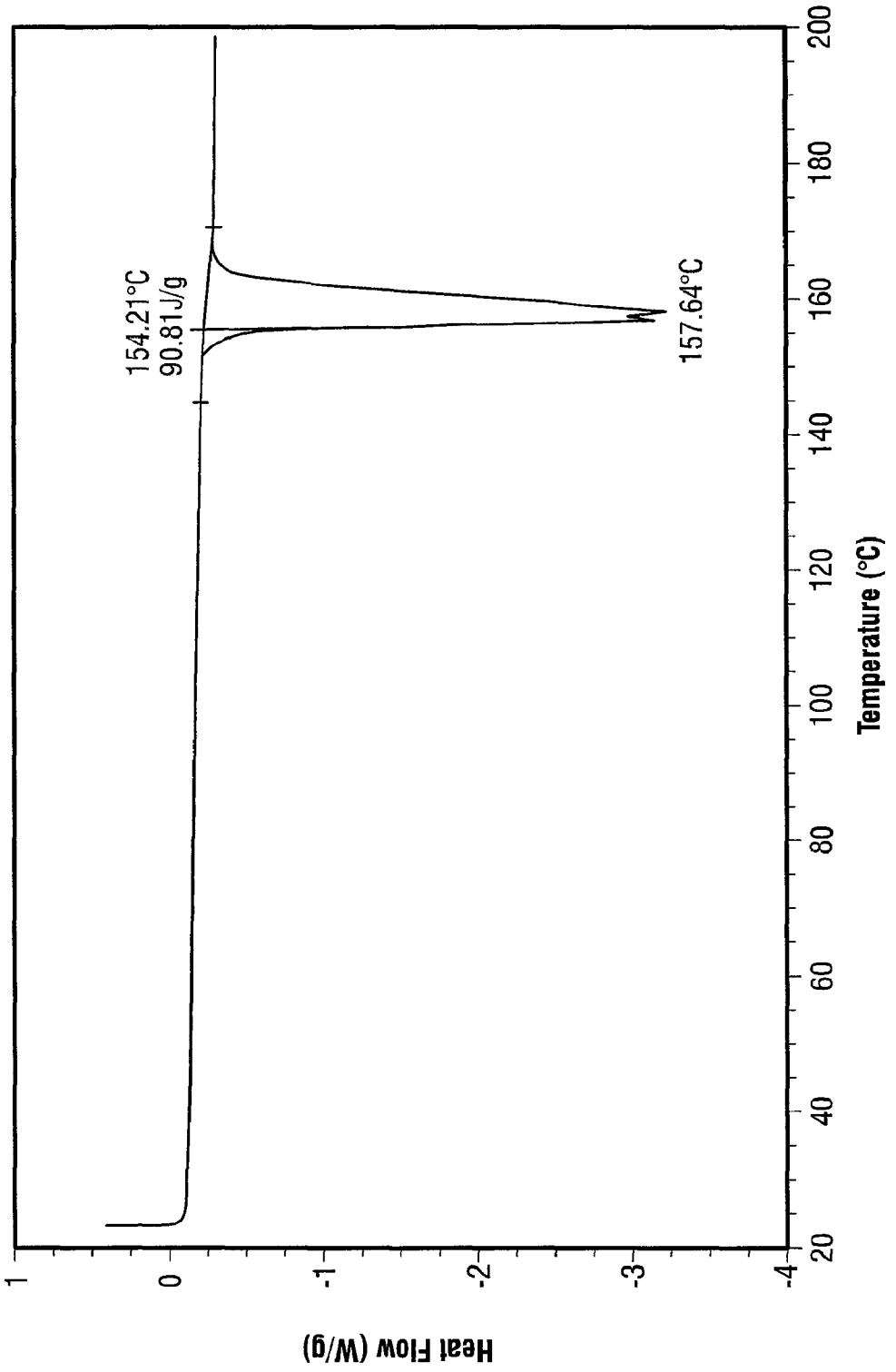

154.21°C
90.81J/g
157.64°C
Heat Flow (W/g)
1
0
-1
-2
-3
-4
20
40
60
80
100
120
140
160
180
200
Temperature (°C)

FIG. 6

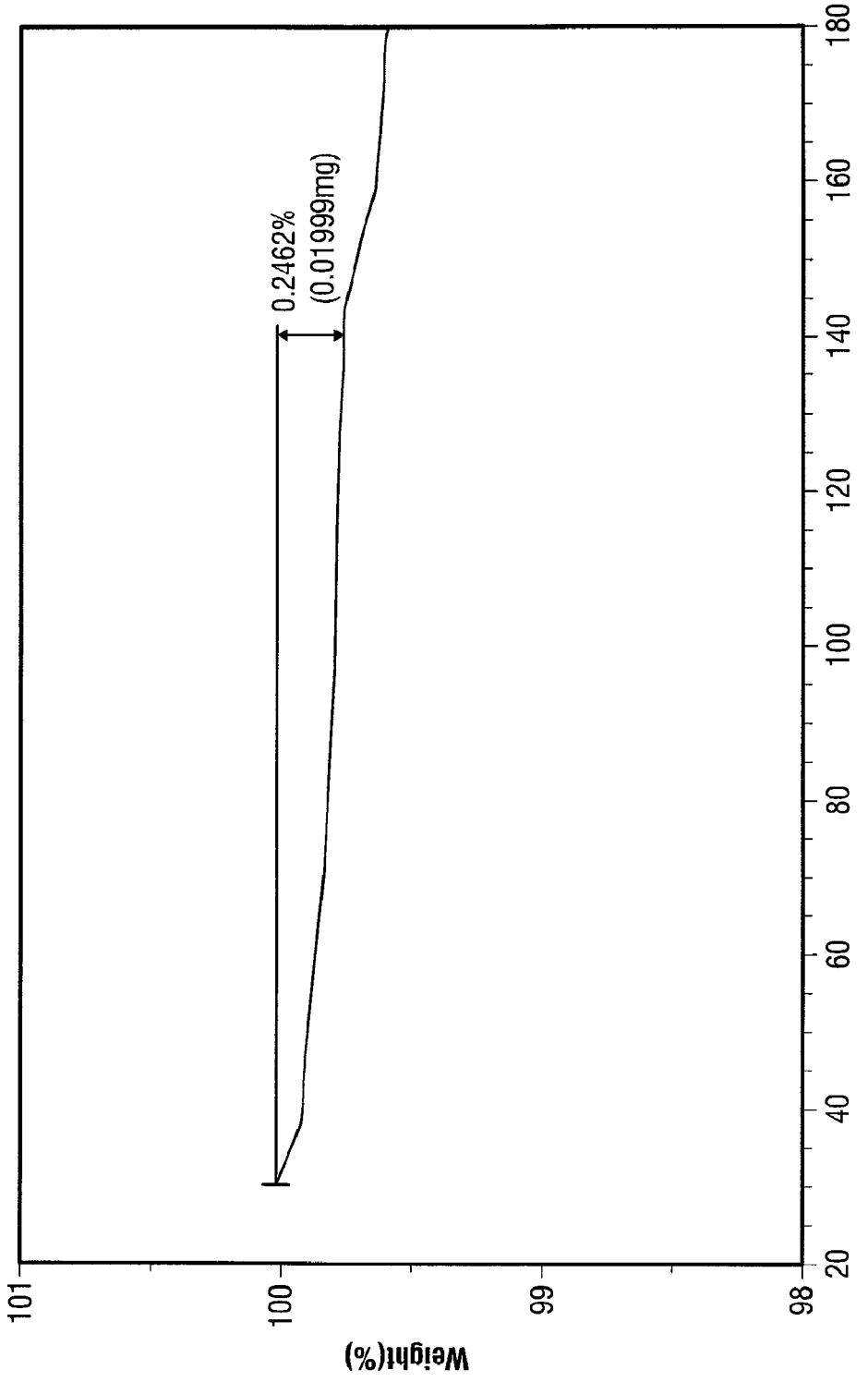

0.2462%
(0.01999mg)
Weight(%)
101
100
99
98
Temperature (°C)
20
40
60
80
100
120
140
160
180

FIG. 7

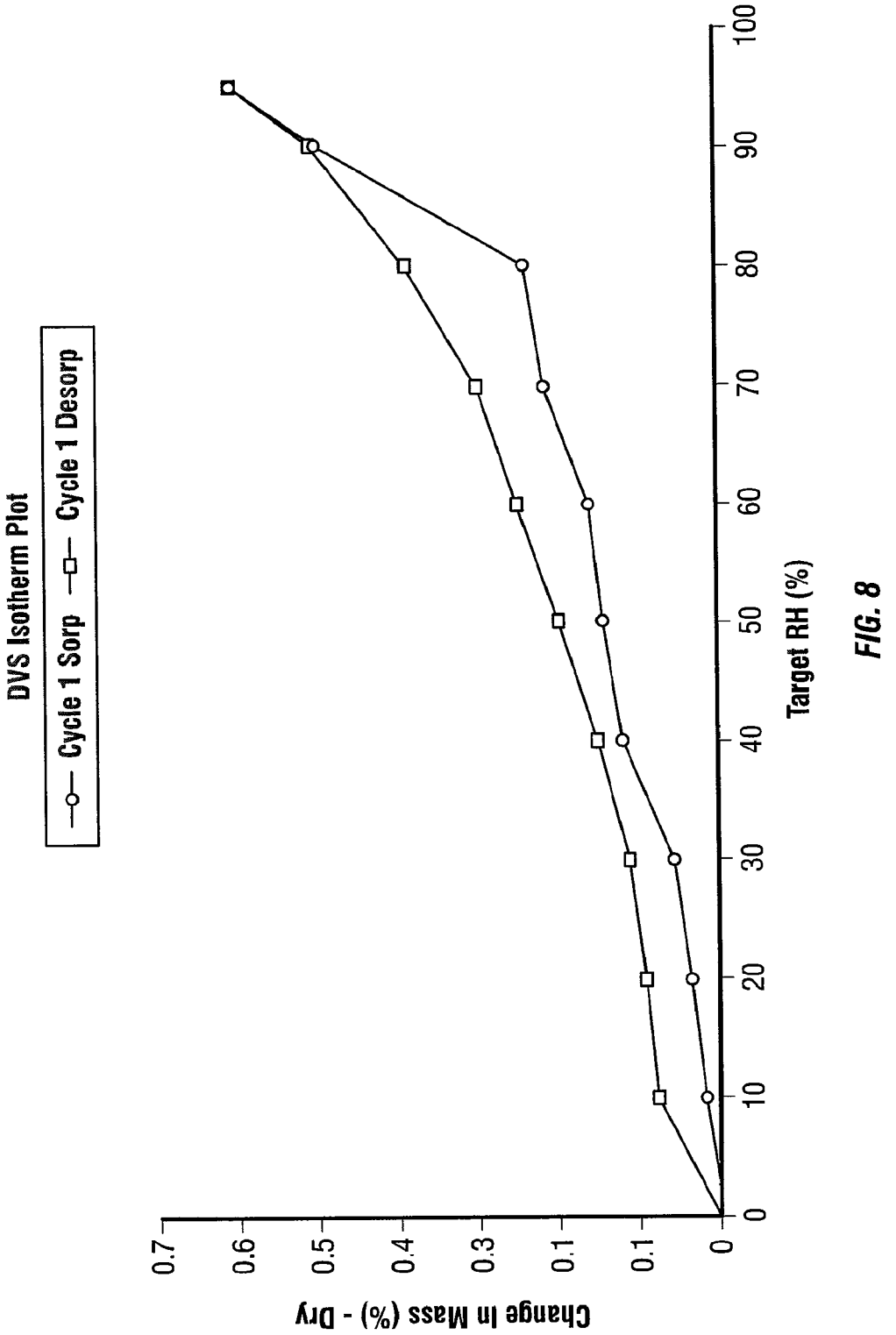

DVS Isotherm Plot
Cycle 1 Sorp
Cycle 1 Desorp
Change In Mass (%) - Dry
0.7
0.6
0.5
0.4
0.3
0.1
0.1
0
0
10
20
30
40
50
60
70
80
90
100
Target RH (%)

FIG. 8

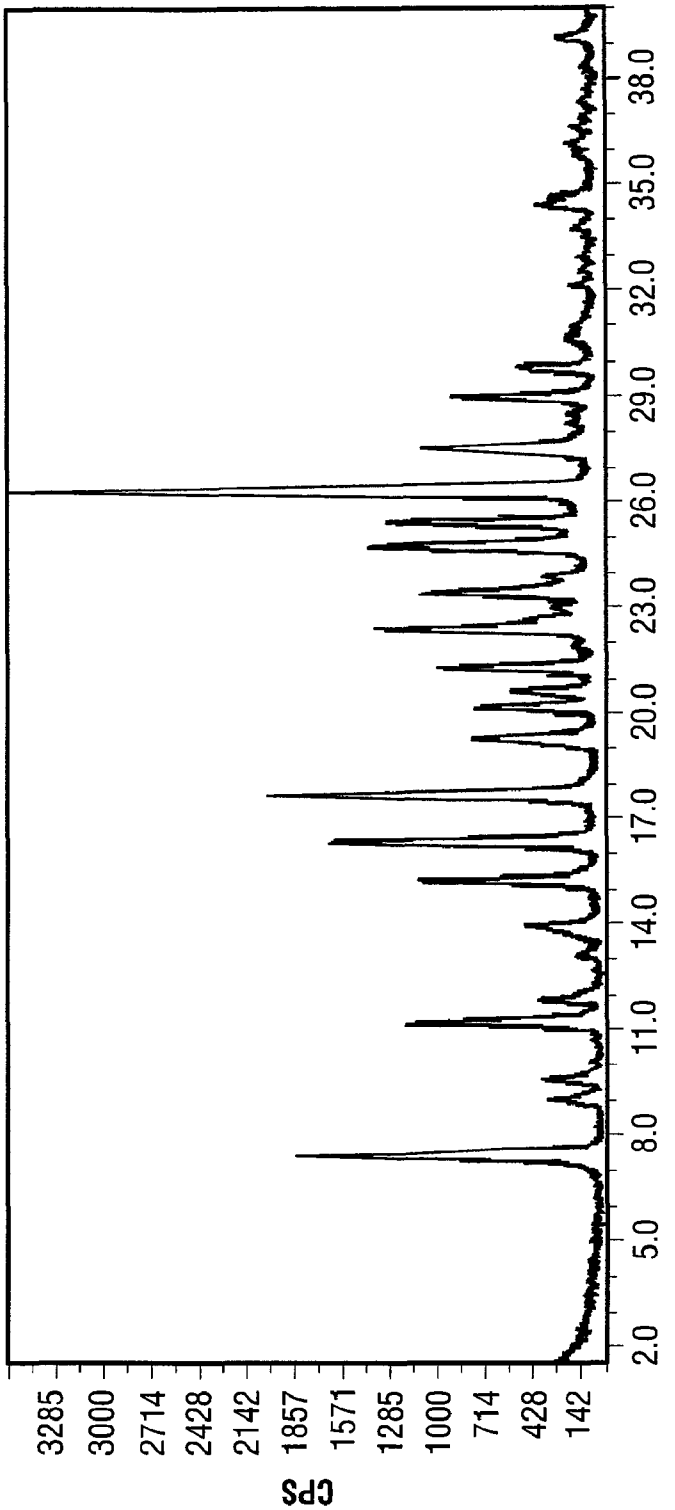

CPS
3285
3000
2714
2428
2142
1857
1571
1285
1000
714
428
142
2.0
5.0
8.0
11.0
14.0
17.0
20.0
23.0
26.0
29.0
32.0
35.0
38.0
Deg.

FIG. 9

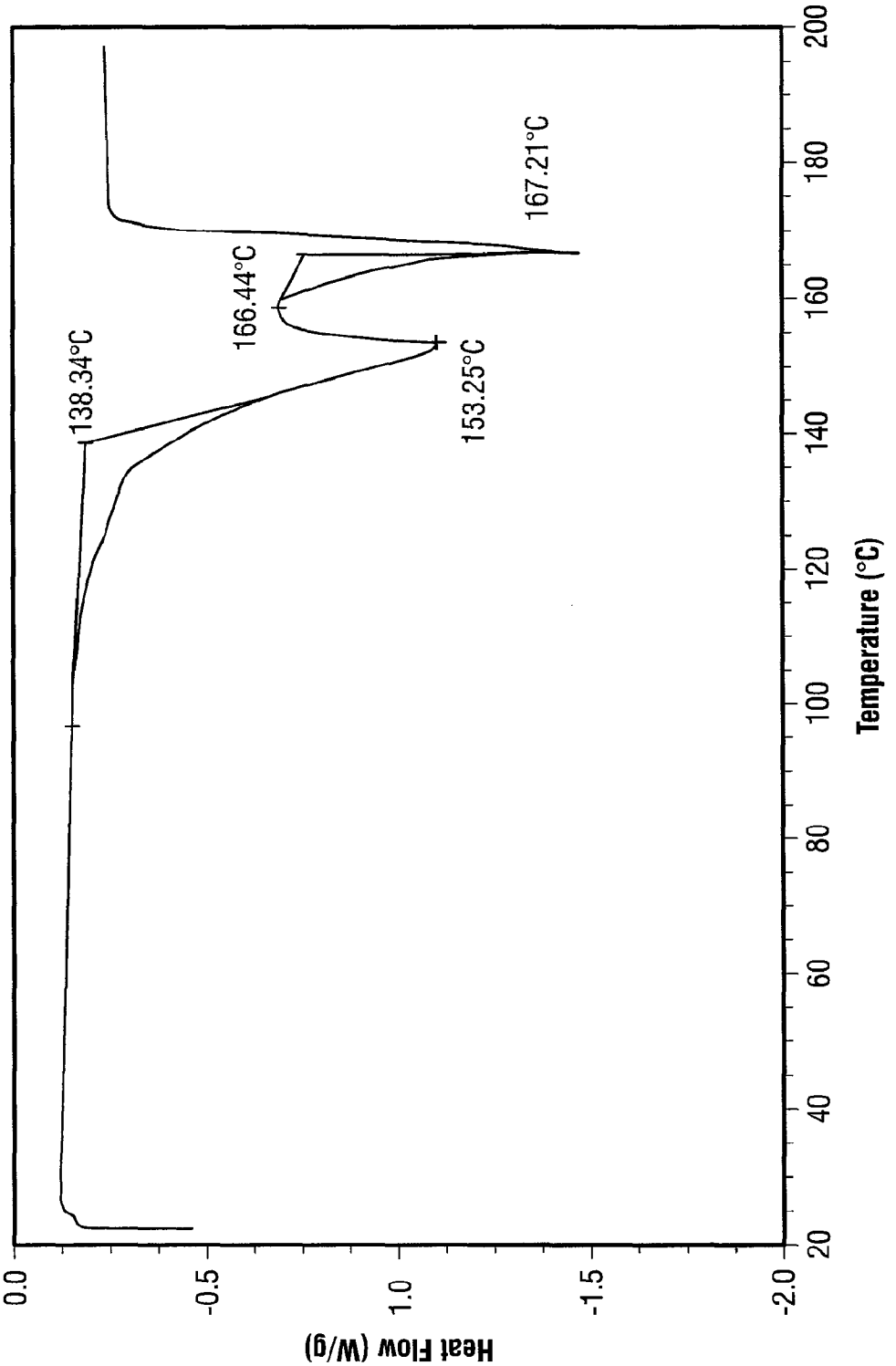

167.21°C
166.44°C
153.25°C
138.34°C
Heat Flow (W/g)
0.0
-0.5
1.0
-1.5
-2.0
Temperature (°C)
20
40
60
80
100
120
140
160
180
200

FIG. 10

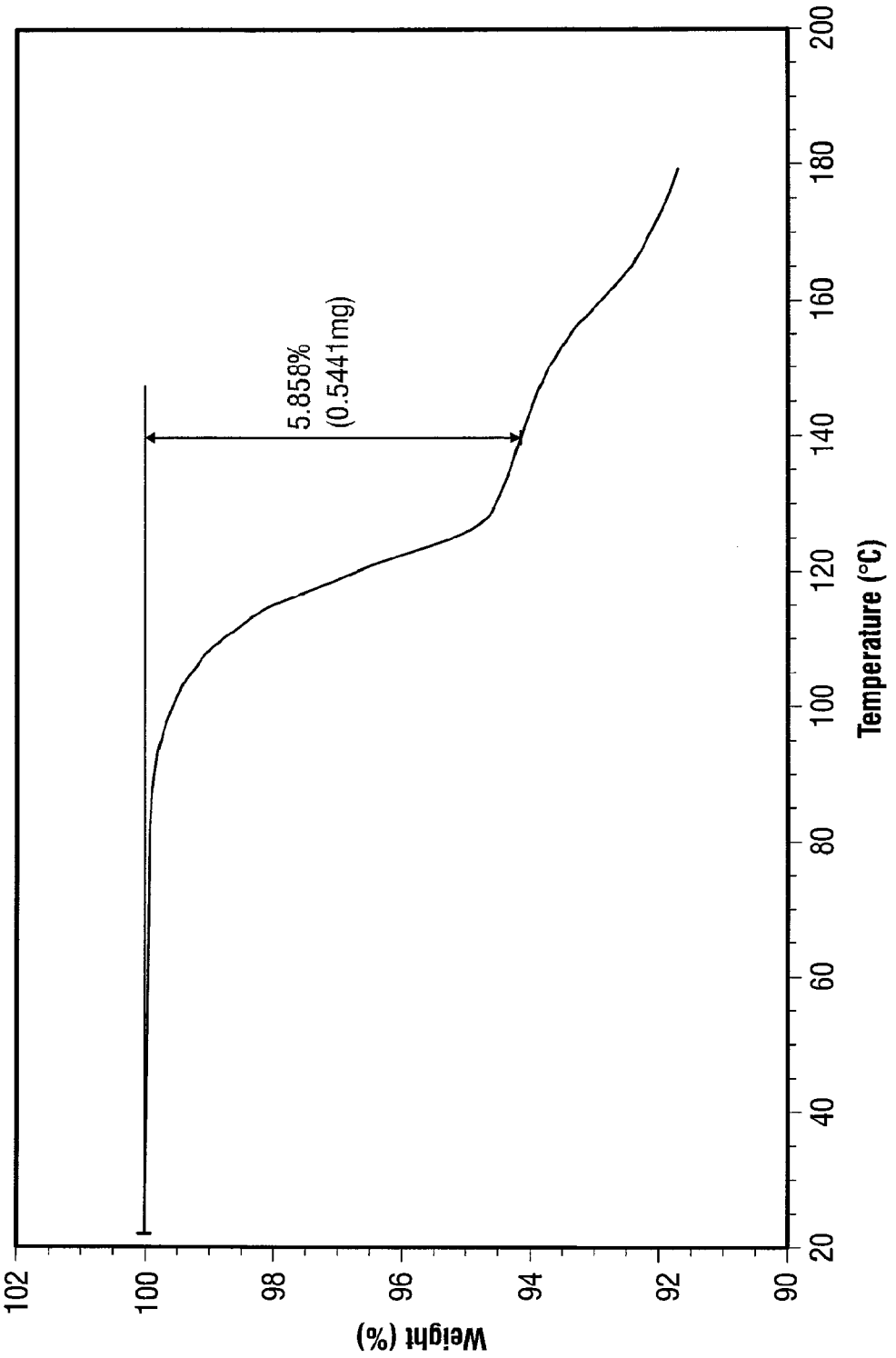

5.858%
(0.5441mg)
Weight (%)
102
100
98
96
94
92
90
Temperature (°C)
20
40
60
80
100
120
140
160
180
200

FIG. 11

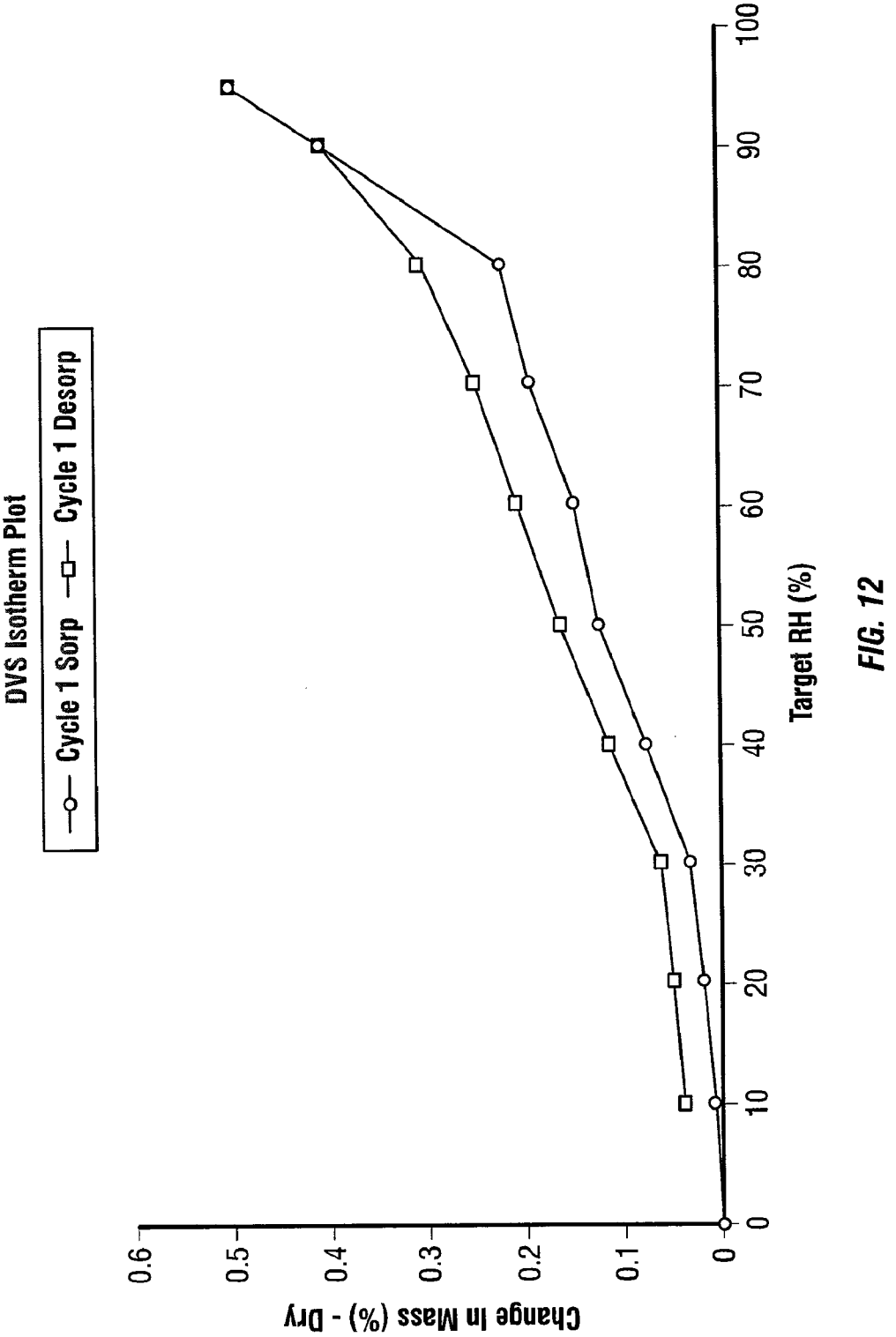

DVS Isotherm Plot
Cycle 1 Sorp
Cycle 1 Desorp
Change In Mass (%) - Dry
0.6
0.5
0.4
0.3
0.2
0.1
0
Target RH (%)
0
10
20
30
40
50
60
70
80
90
100

FIG. 12

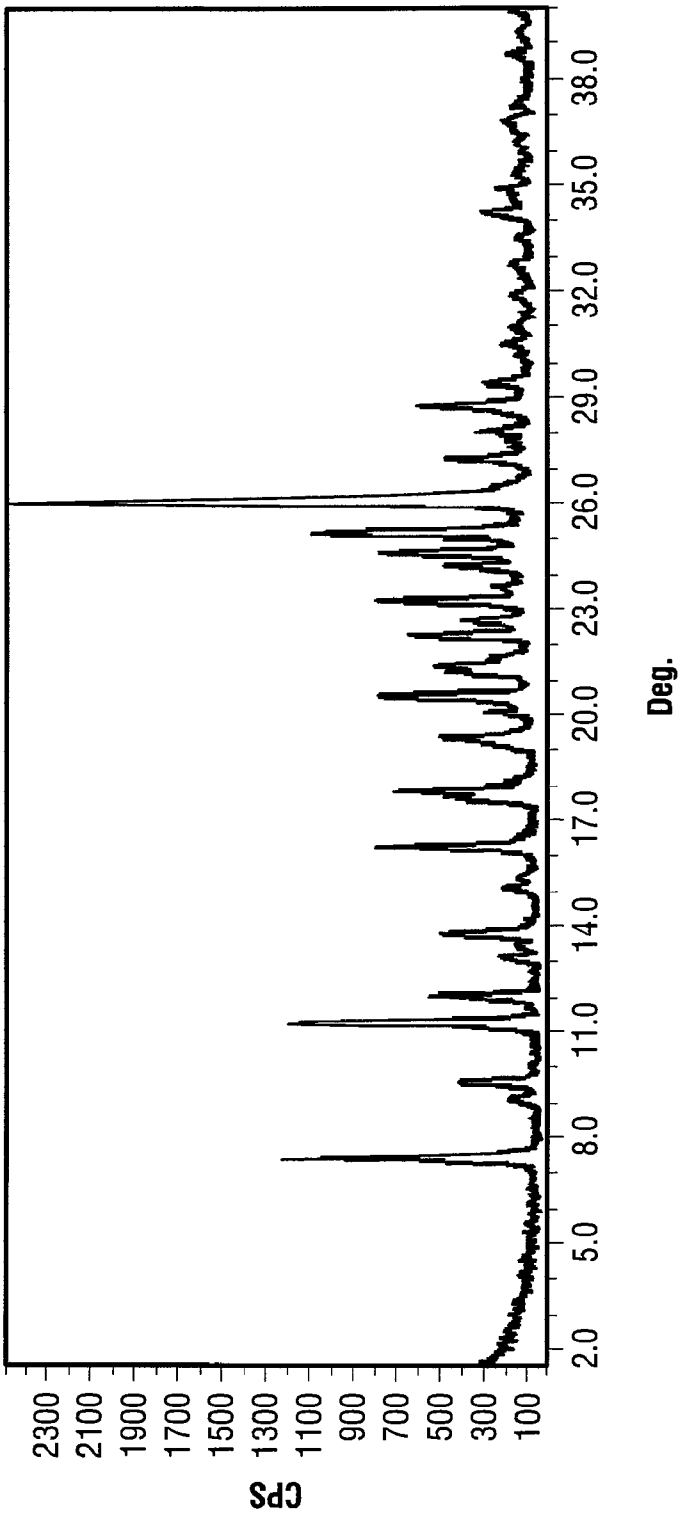

CPS
2300
2100
1900
1700
1500
1300
1100
900
700
500
300
100
2.0
5.0
8.0
11.0
14.0
17.0
20.0
23.0
26.0
29.0
32.0
35.0
38.0
Deg.

FIG. 13

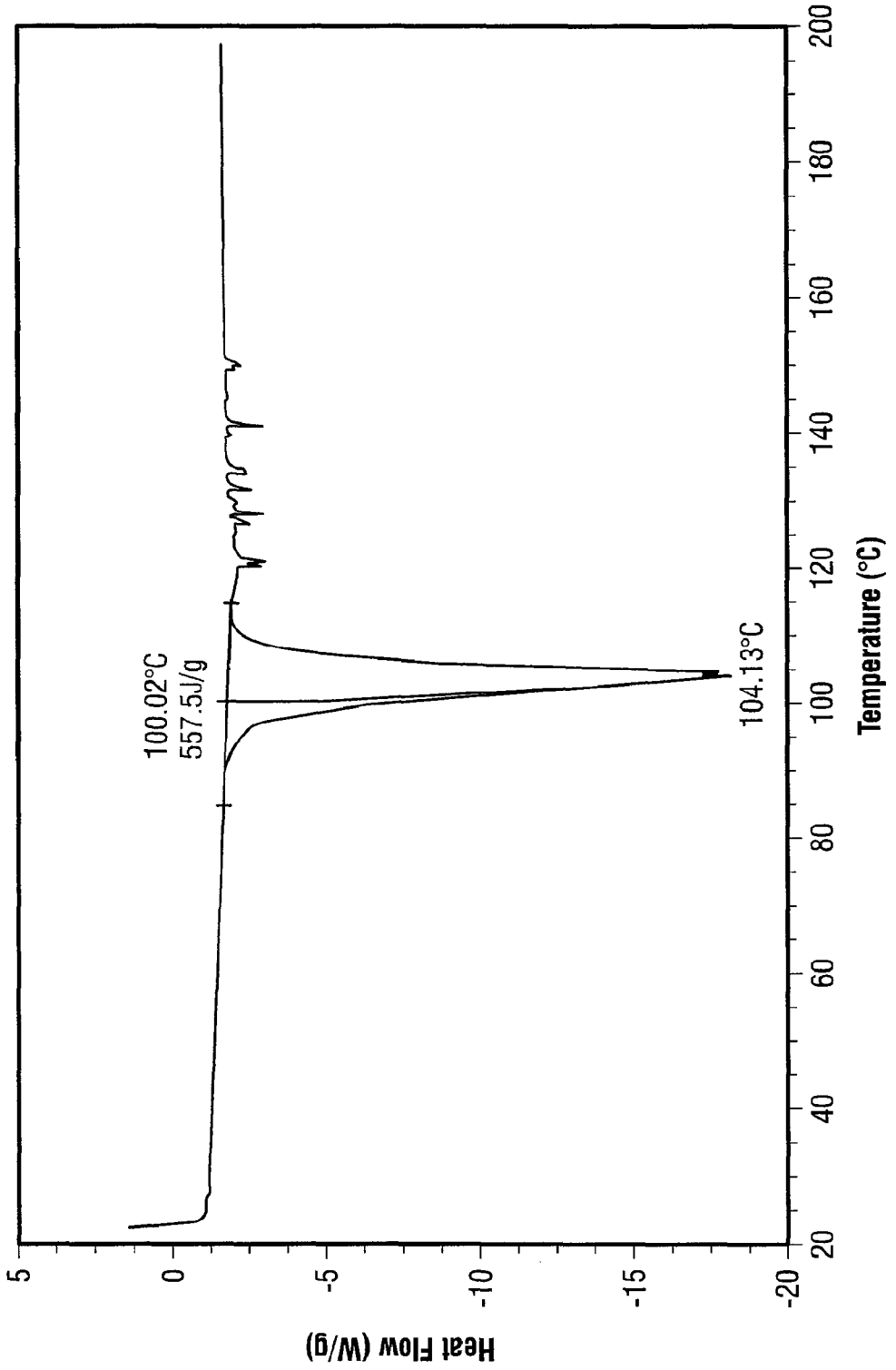

100.02°C
557.5J/g
104.13°C
Heat Flow (W/g)
5
0
-5
-10
-15
-20
20
40
60
80
100
120
140
160
180
200
Temperature (°C)

FIG. 14

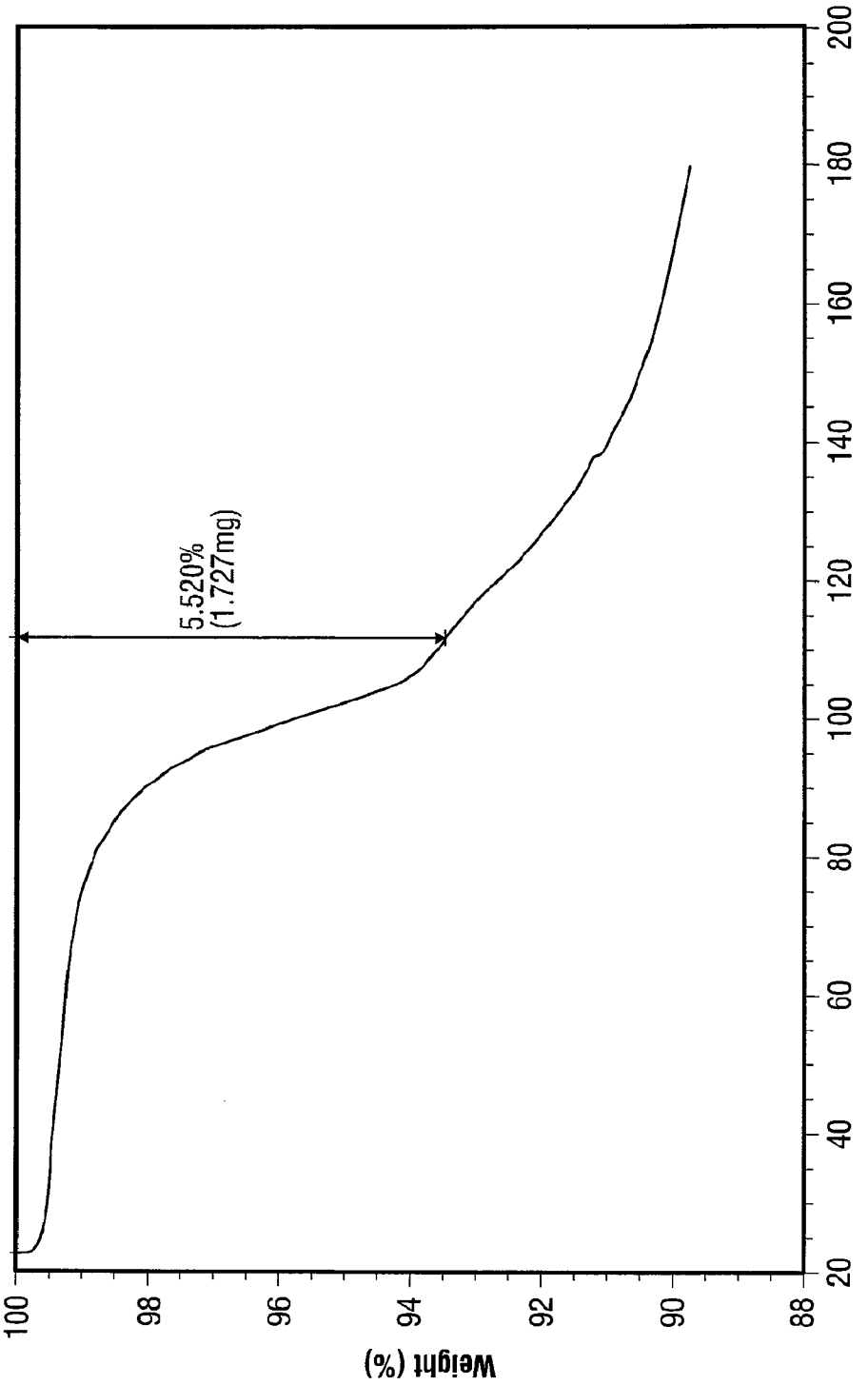

5.520%
(1.727mg)
Weight (%)
100
98
96
94
92
90
88
Temperature (°C)
20
40
60
80
100
120
140
160
180
200

FIG. 15

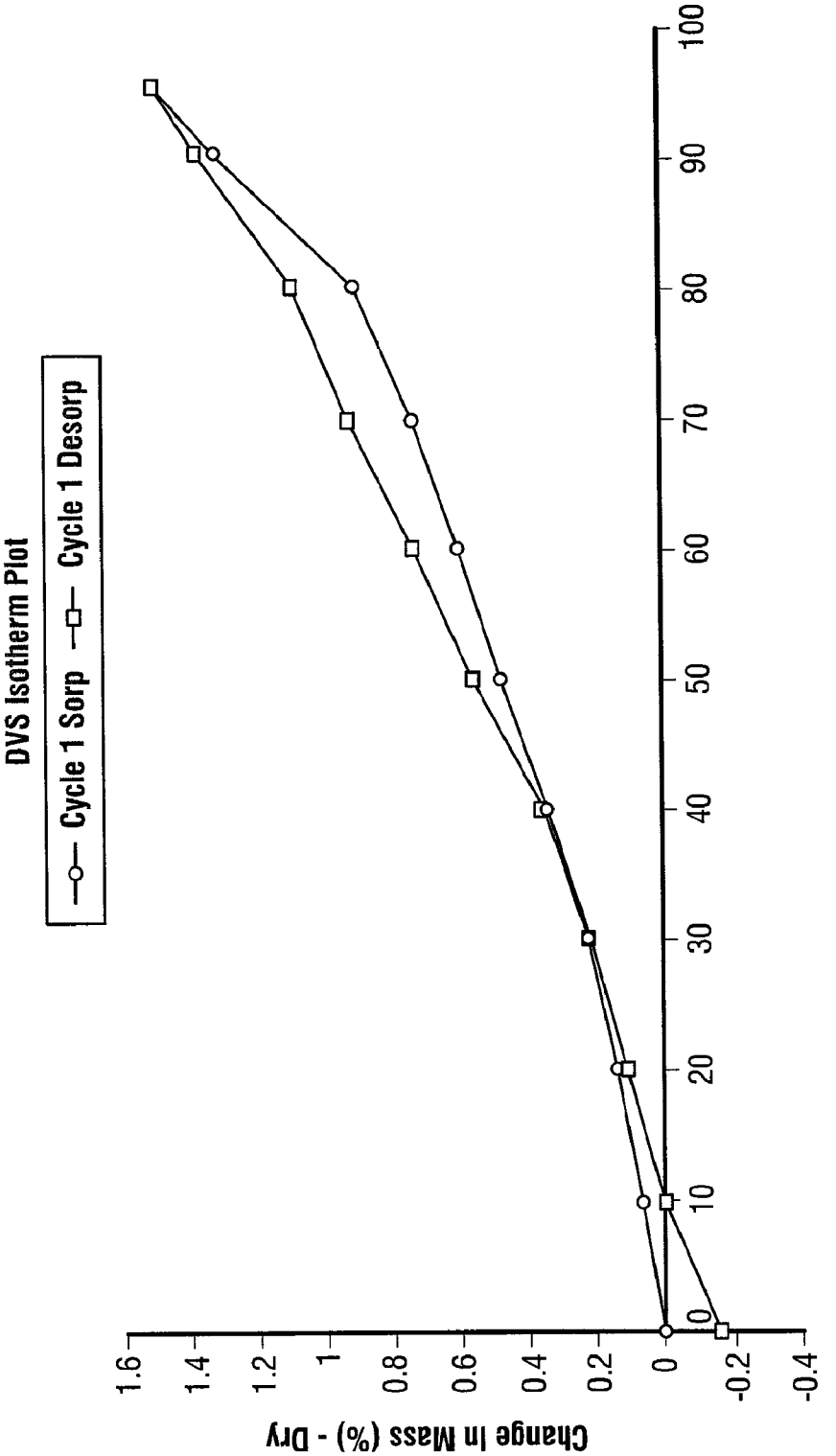

DVS Isotherm Plot
Cycle 1 Sorp
Cycle 1 Desorp
Change In Mass (%) - Dry
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
0
10
20
30
40
50
60
70
80
90
100
Target RH (%)

FIG. 16

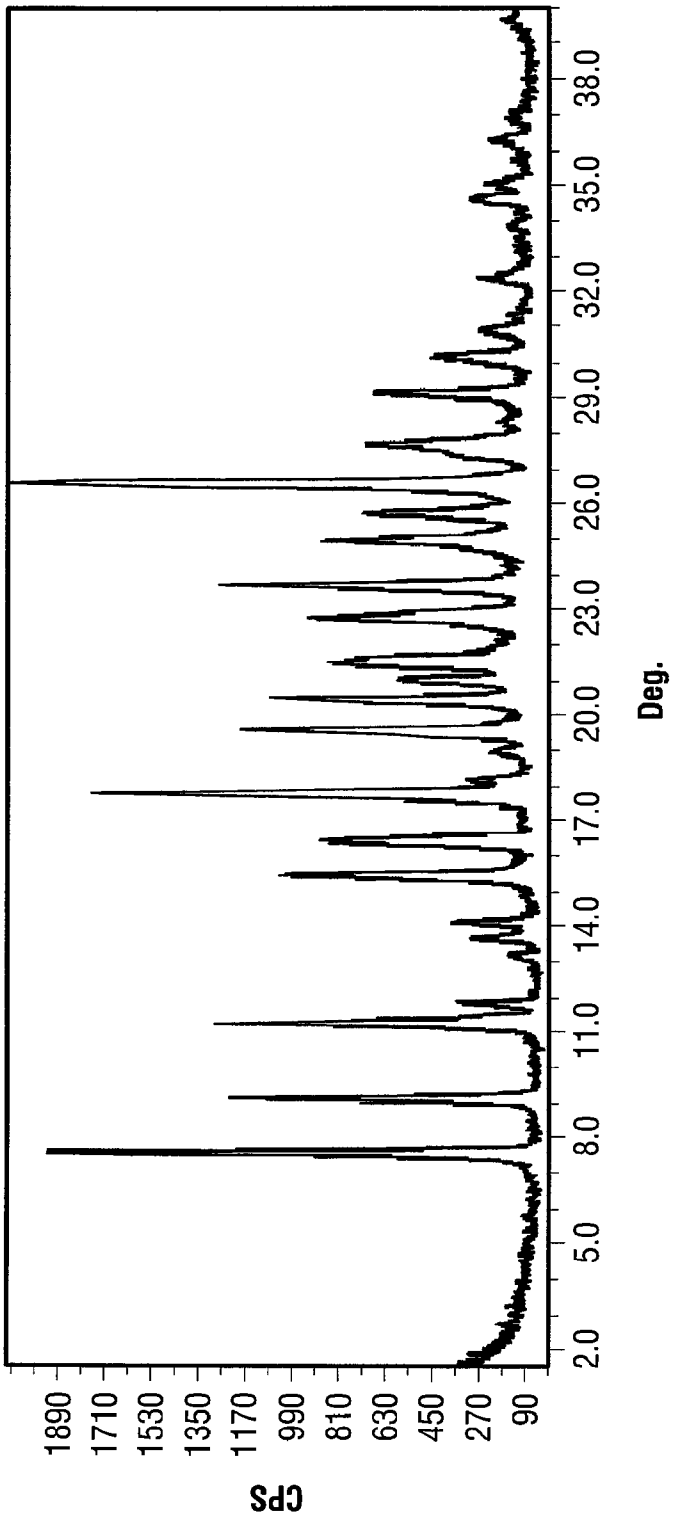

CPS
1890
1710
1530
1350
1170
990
810
630
450
270
90
2.0
5.0
8.0
11.0
14.0
17.0
20.0
23.0
26.0
29.0
32.0
35.0
38.0
Deg.

FIG. 17

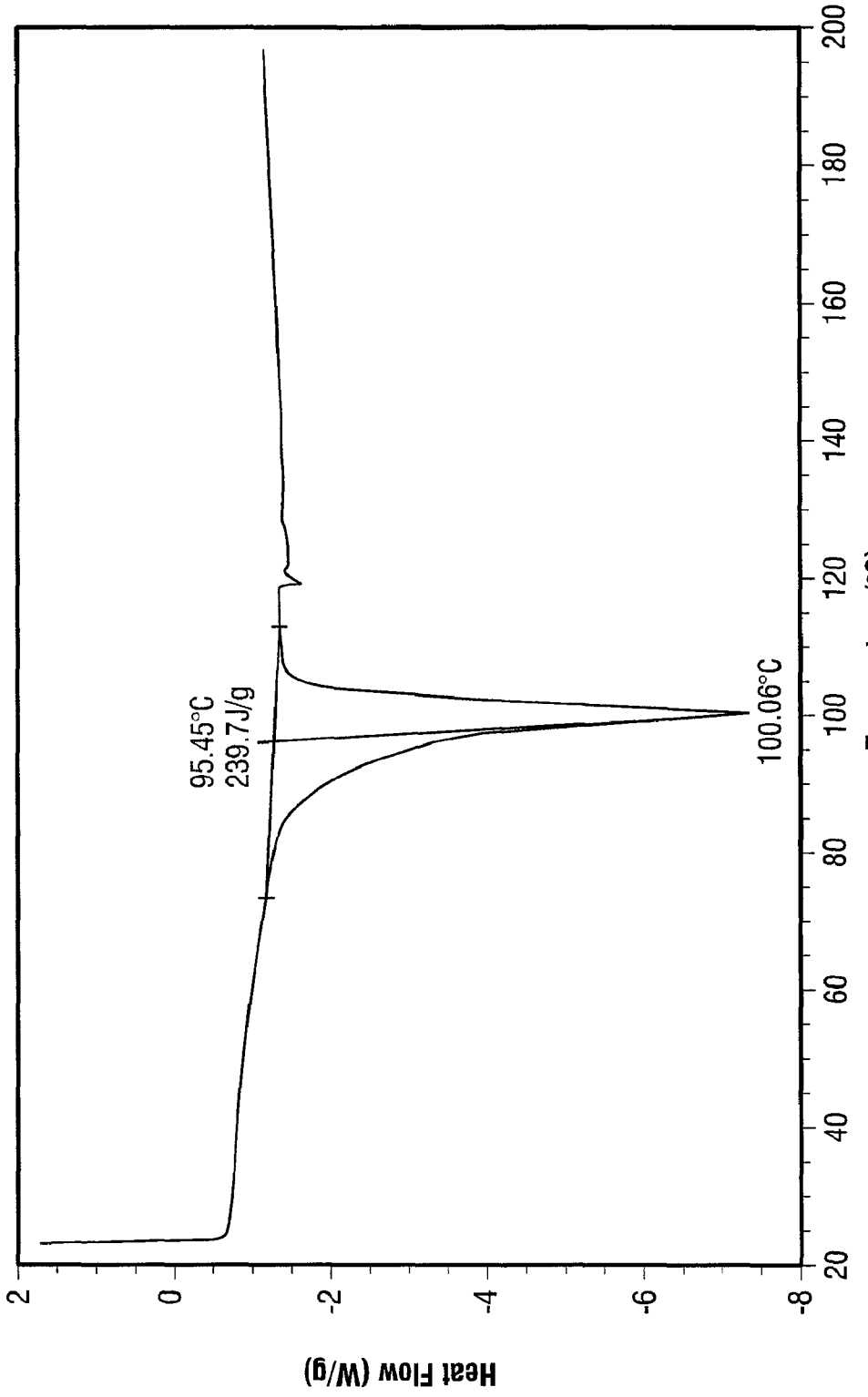

95.45°C
239.7J/g
100.06°C
Temperature (°C)
Heat Flow (W/g)
20
40
60
80
100
120
140
160
180
200
2
0
-2
-4
-6
-8

FIG. 18

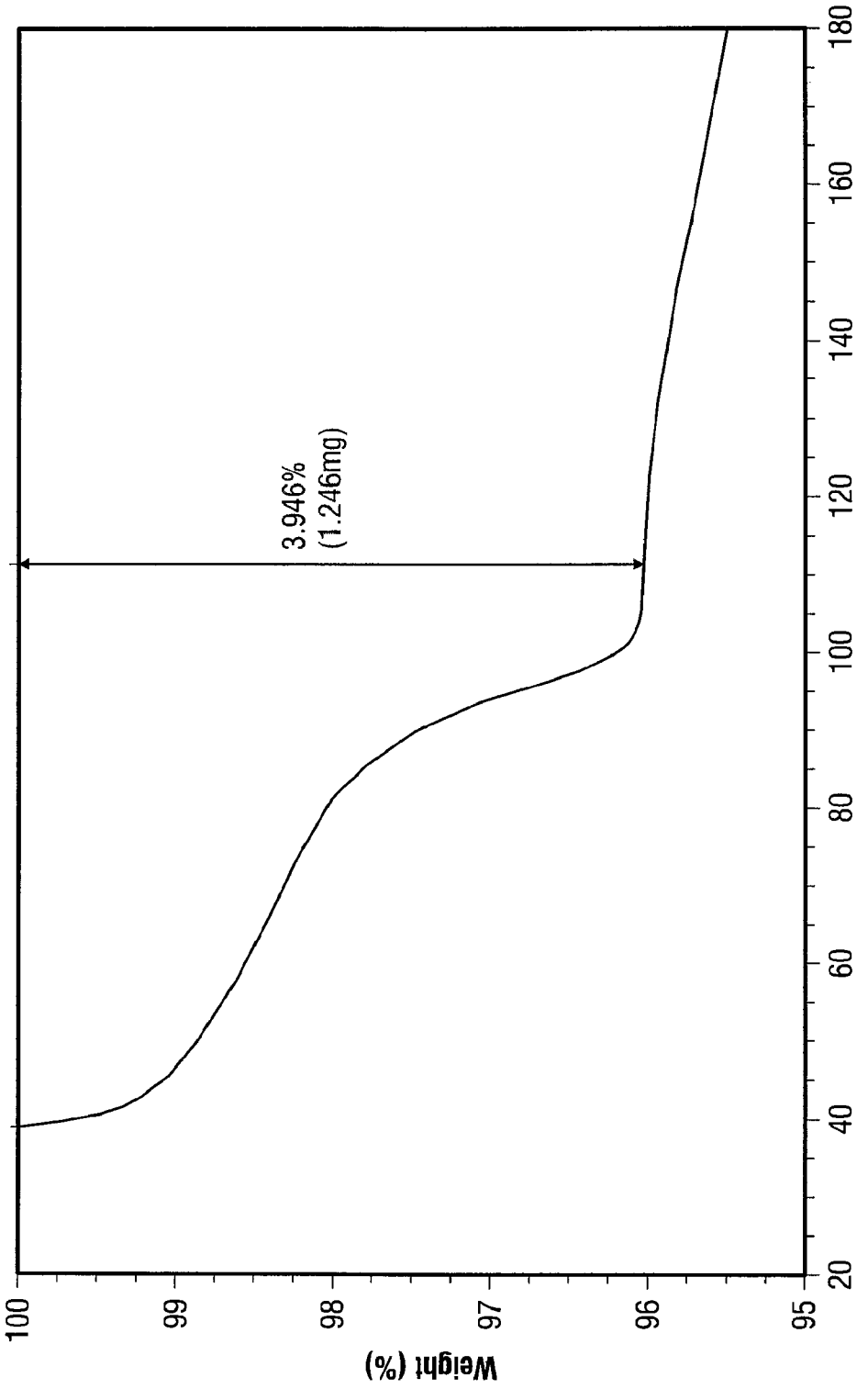

3.946%
(1.246mg)
Weight (%)
100
99
98
97
96
95
Temperature (°C)
20
40
60
80
100
120
140
160
180

FIG. 19

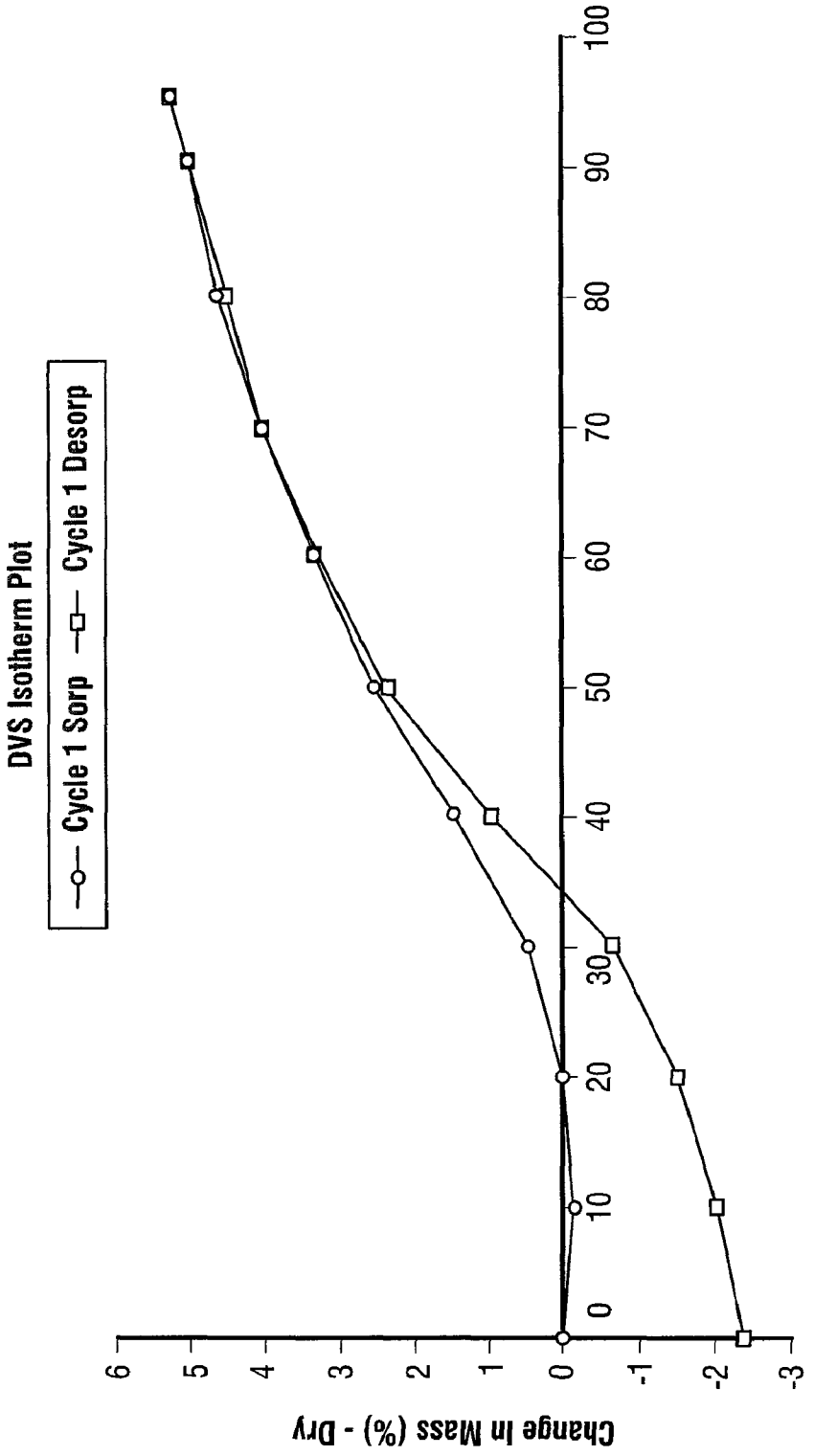

DVS Isotherm Plot
Cycle 1 Sorp
Cycle 1 Desorp
Change In Mass (%) - Dry
6
5
4
3
2
1
0
-1
-2
-3
0
10
20
30
40
50
60
70
80
90
100
Target RH (%)

FIG. 20

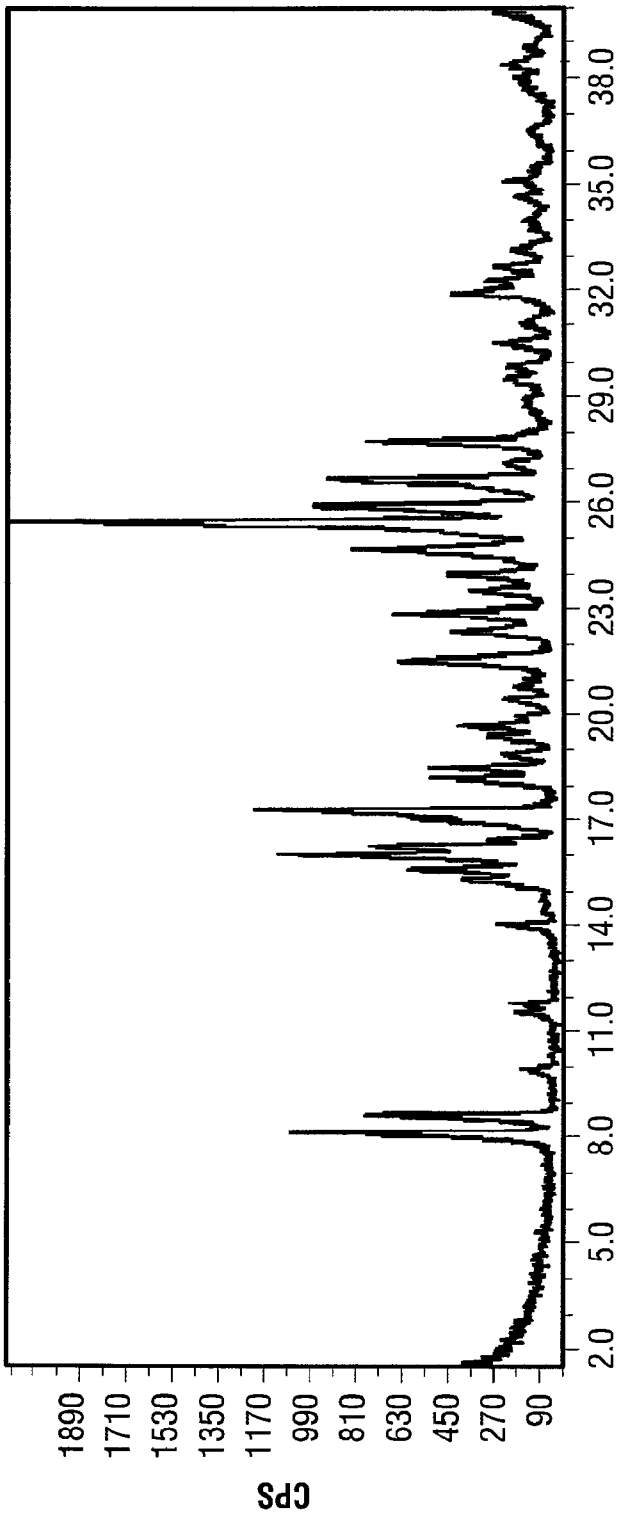

CPS
1890
1710
1530
1350
1170
990
810
630
450
270
90
2.0
5.0
8.0
11.0
14.0
17.0
20.0
23.0
26.0
29.0
32.0
35.0
38.0
Deg.

FIG. 21

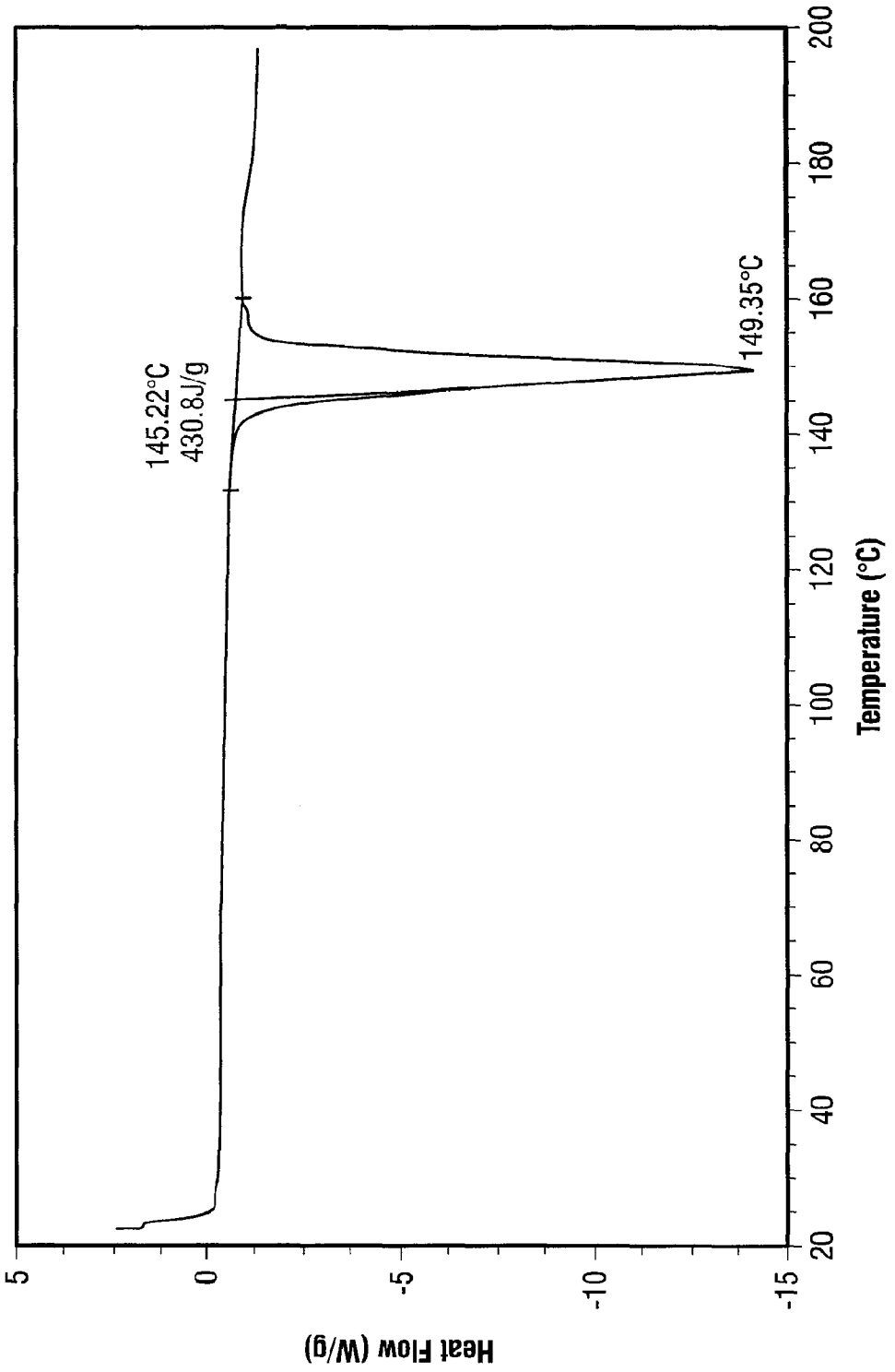

145.22°C
430.8J/g
149.35°C
Heat Flow (W/g)
5
0
-5
-10
-15
20
40
60
80
100
120
140
160
180
200
Temperature (°C)

FIG. 22

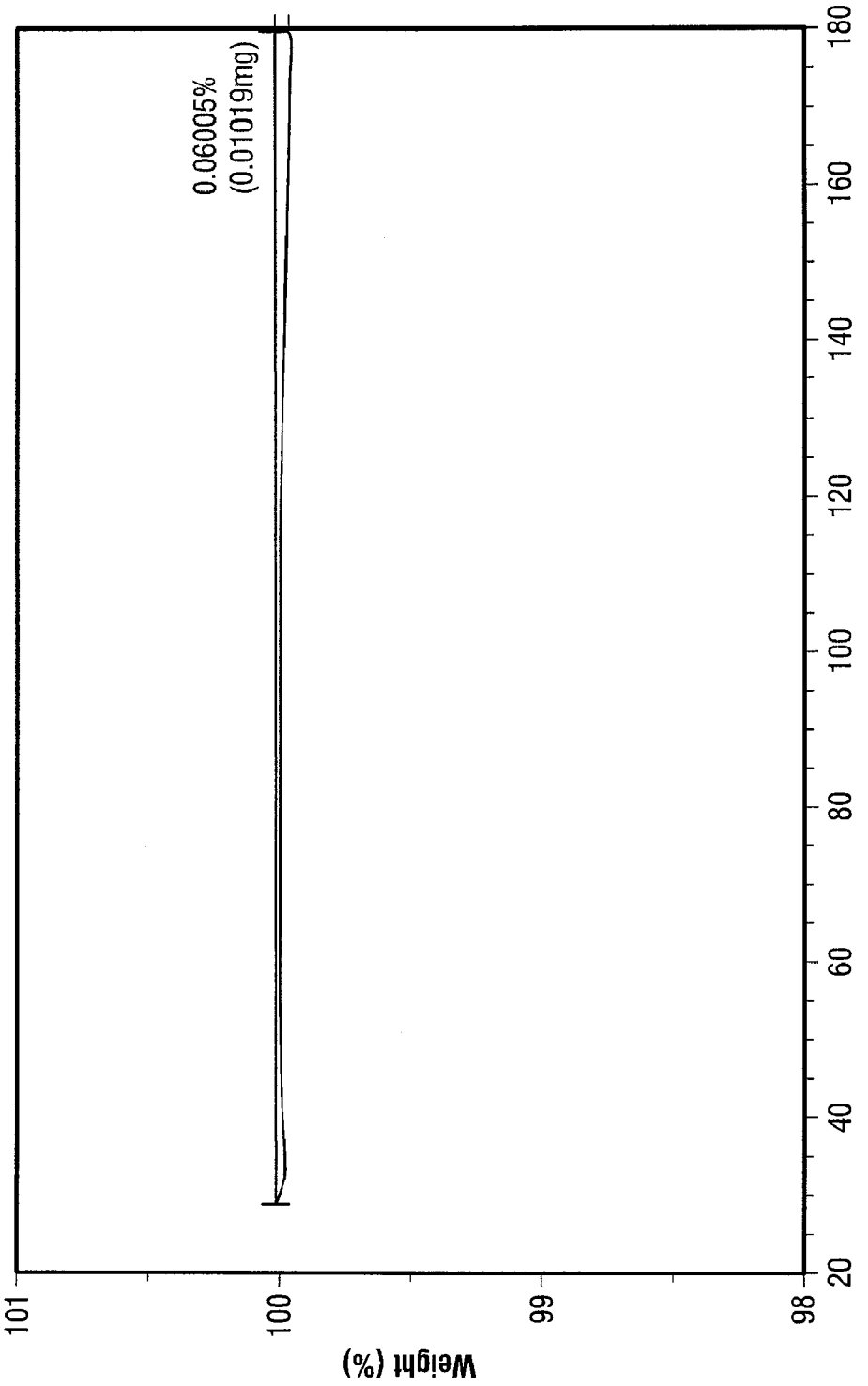

0.06005%
(0.01019mg)
Weight (%)
101
100
99
98
20
40
60
80
100
120
140
160
180
Temperature (°C)

FIG. 23

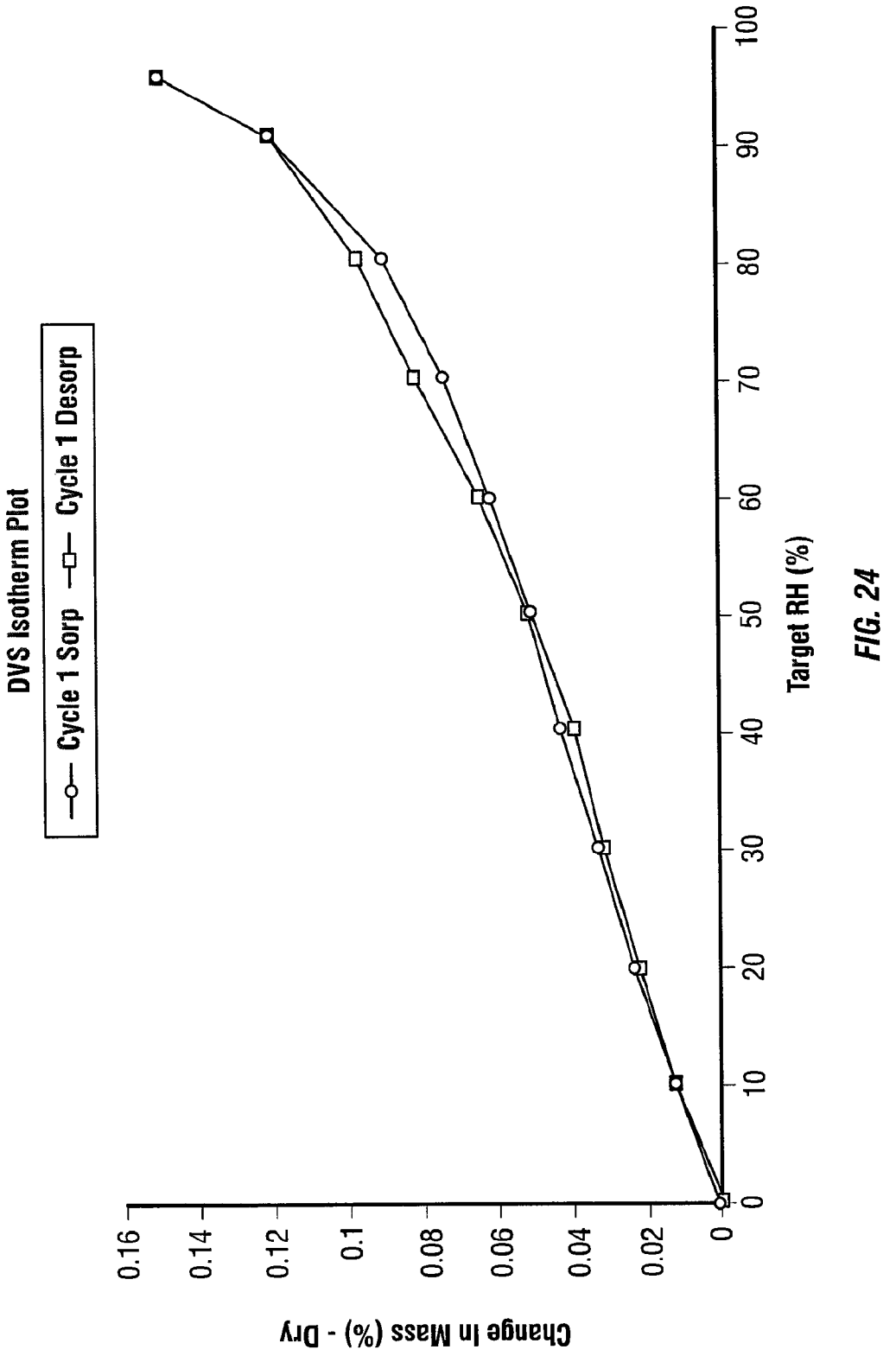

DVS Isotherm Plot
Cycle 1 Sorp
Cycle 1 Desorp
Change In Mass (%) - Dry
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
10
20
30
40
50
60
70
80
90
100
Target RH (%)

FIG. 24

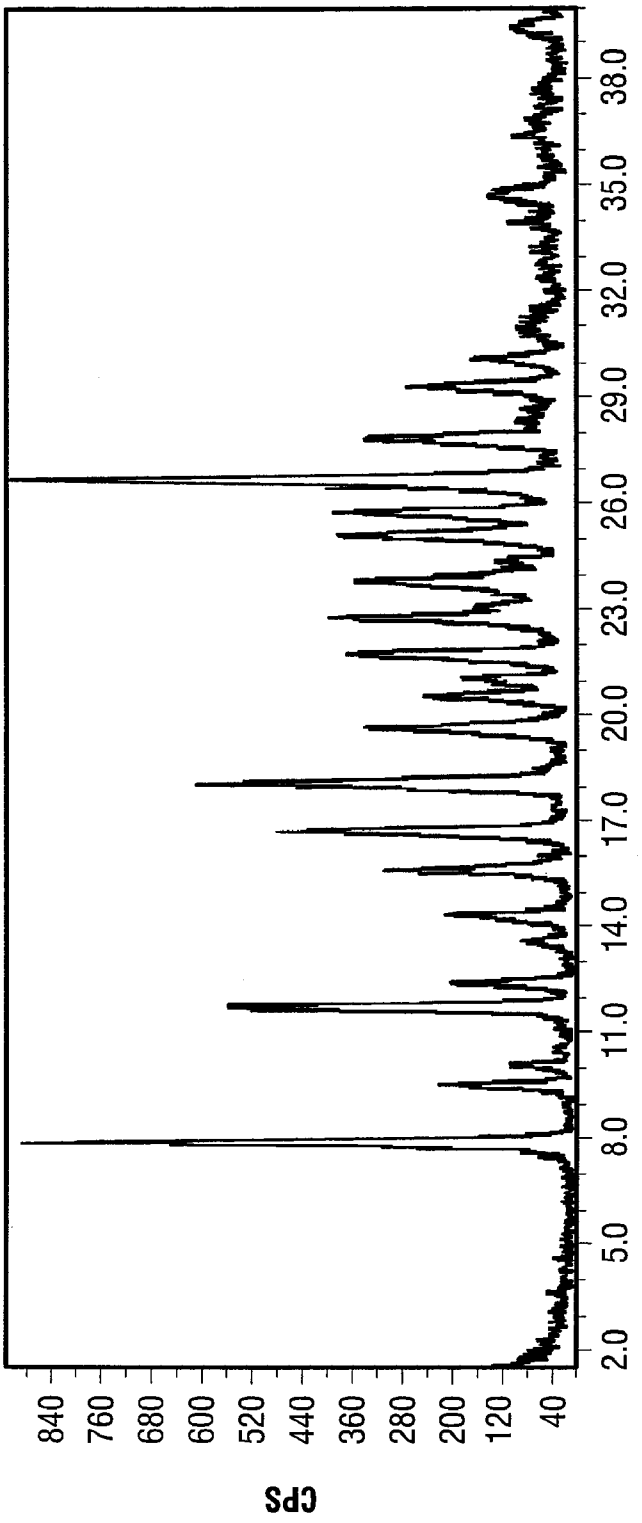

CPS
840
760
680
600
520
440
360
280
200
120
40
2.0
5.0
8.0
11.0
14.0
17.0
20.0
23.0
26.0
29.0
32.0
35.0
38.0
Deg.

FIG. 25

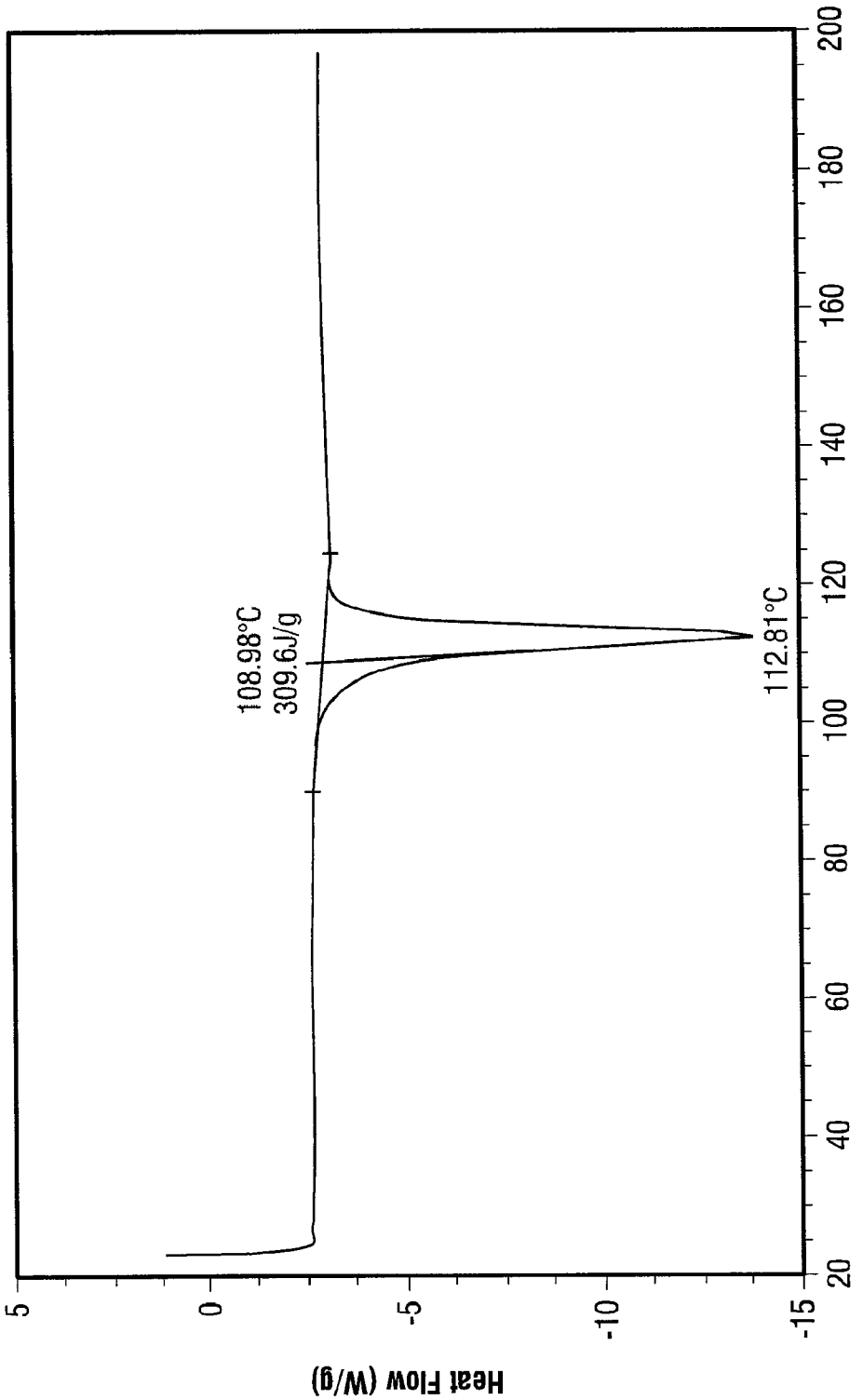

108.98°C
309.6J/g
112.81°C
Heat Flow (W/g)
5
0
-5
-10
-15
20
40
60
80
100
120
140
160
180
200
Temperature (°C)

FIG. 26

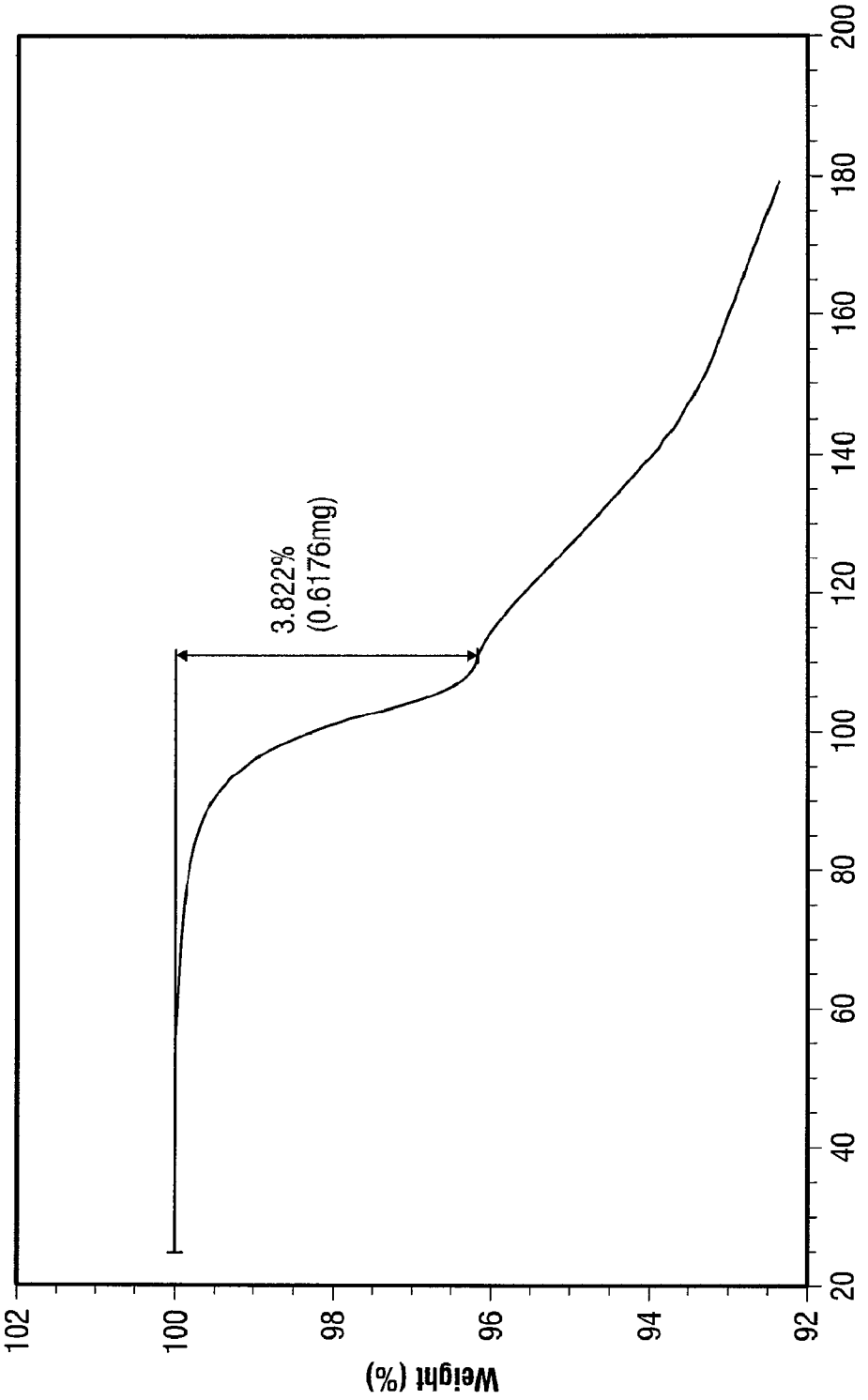

3.822%
(0.6176mg)
Weight (%)
102
100
98
96
94
92
Temperature (°C)
20
40
60
80
100
120
140
160
180
200

FIG. 27

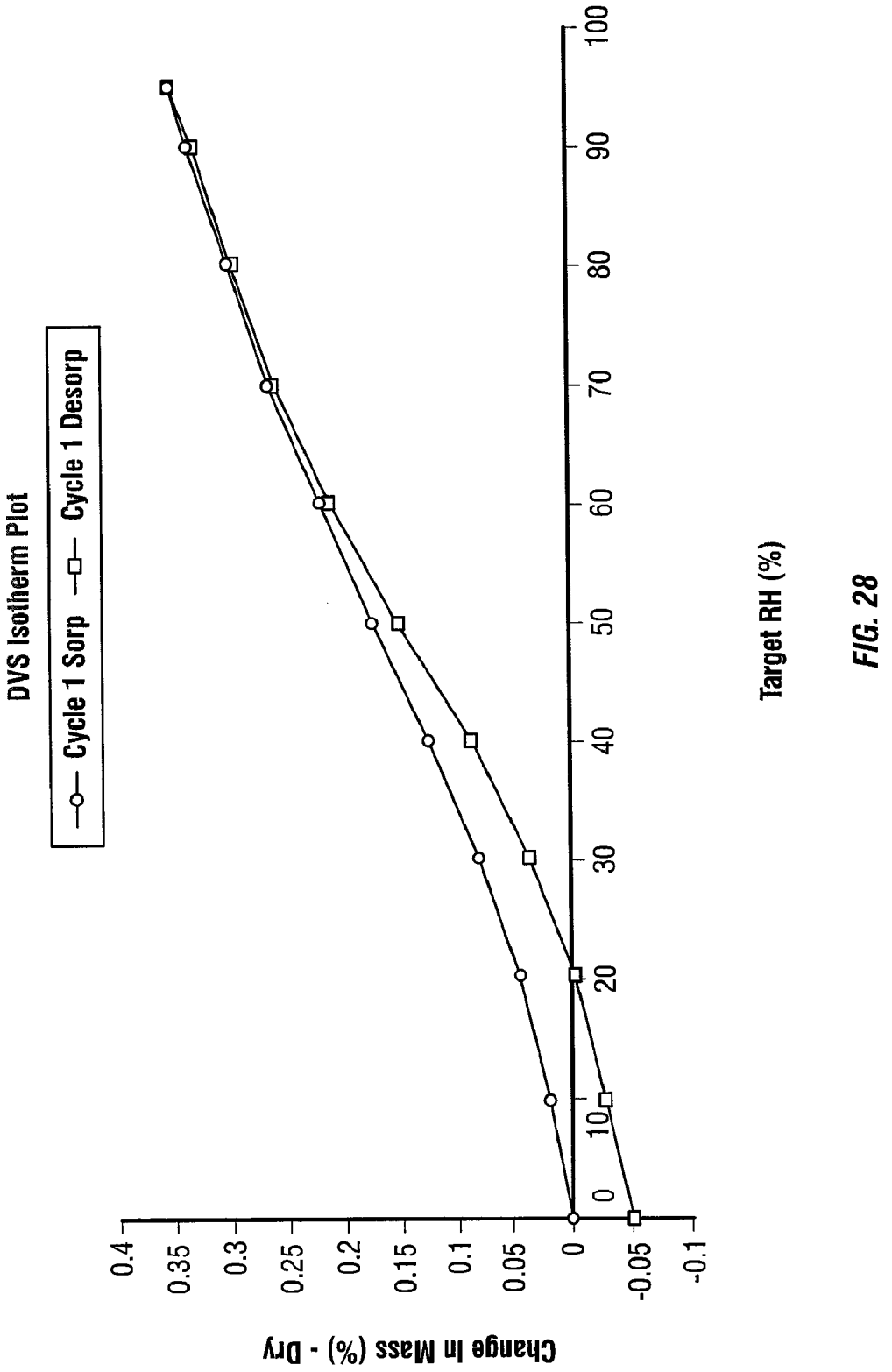
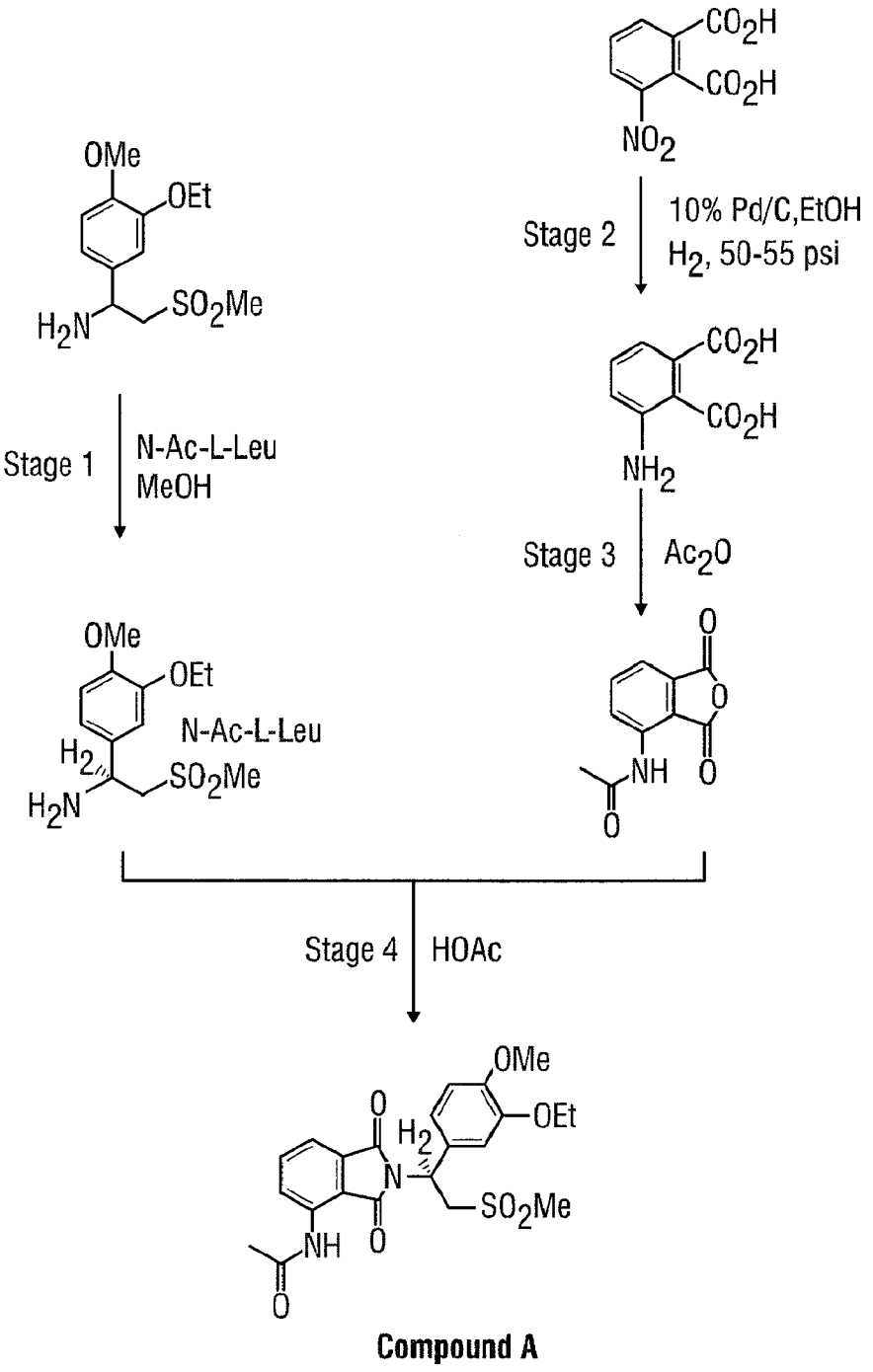

DVS Isotherm Plot
Cycle 1 Sorp
Cycle 1 Desorp
Change In Mass (%) - Dry
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
0
10
20
30
40
50
60
70
80
90
100
Target RH (%)

FIG. 28



Compound A

FIG. 29

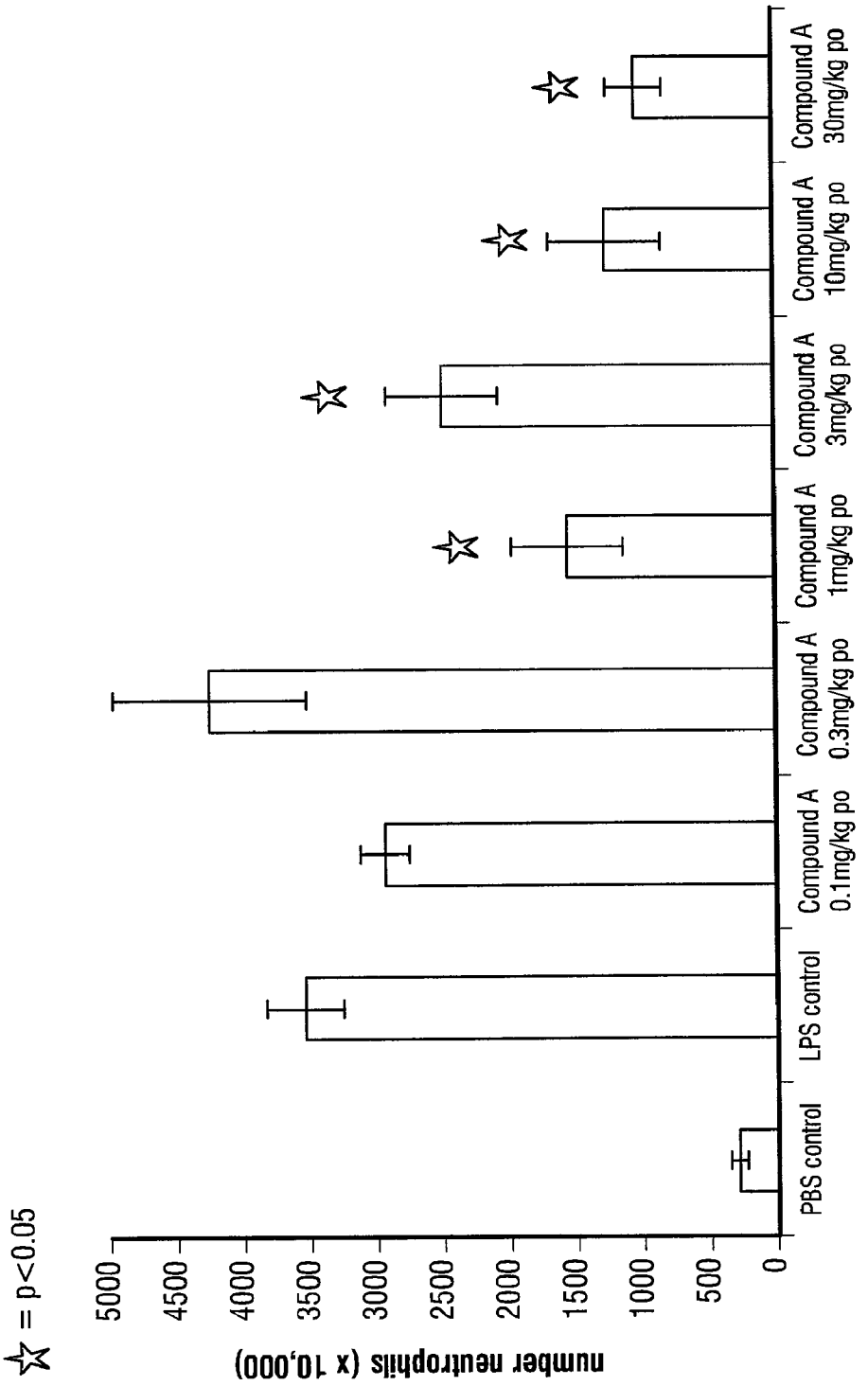

☆ = p<0.05
number neutrophils (x 10,000)
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
PBS control
LPS control
Compound A 0.1mg/kg po
Compound A 0.3mg/kg po
Compound A 1mg/kg po
Compound A 3mg/kg po
Compound A 10mg/kg po
Compound A 30mg/kg po

FIG. 30

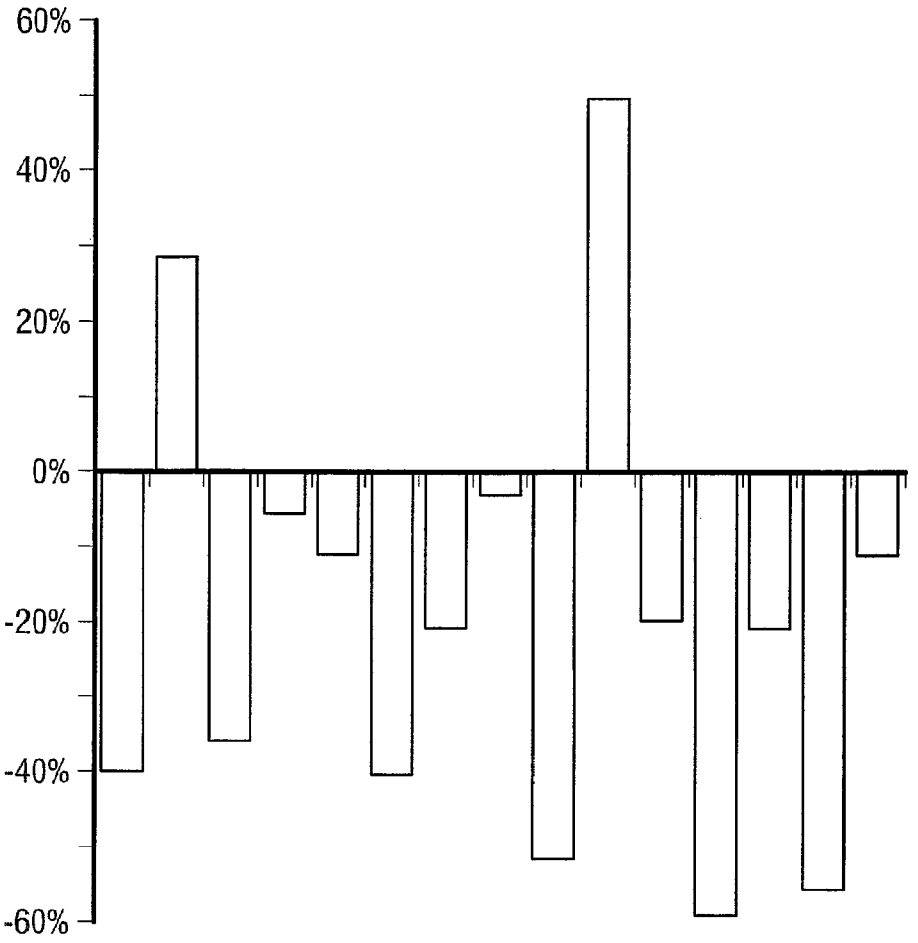


FIG. 31

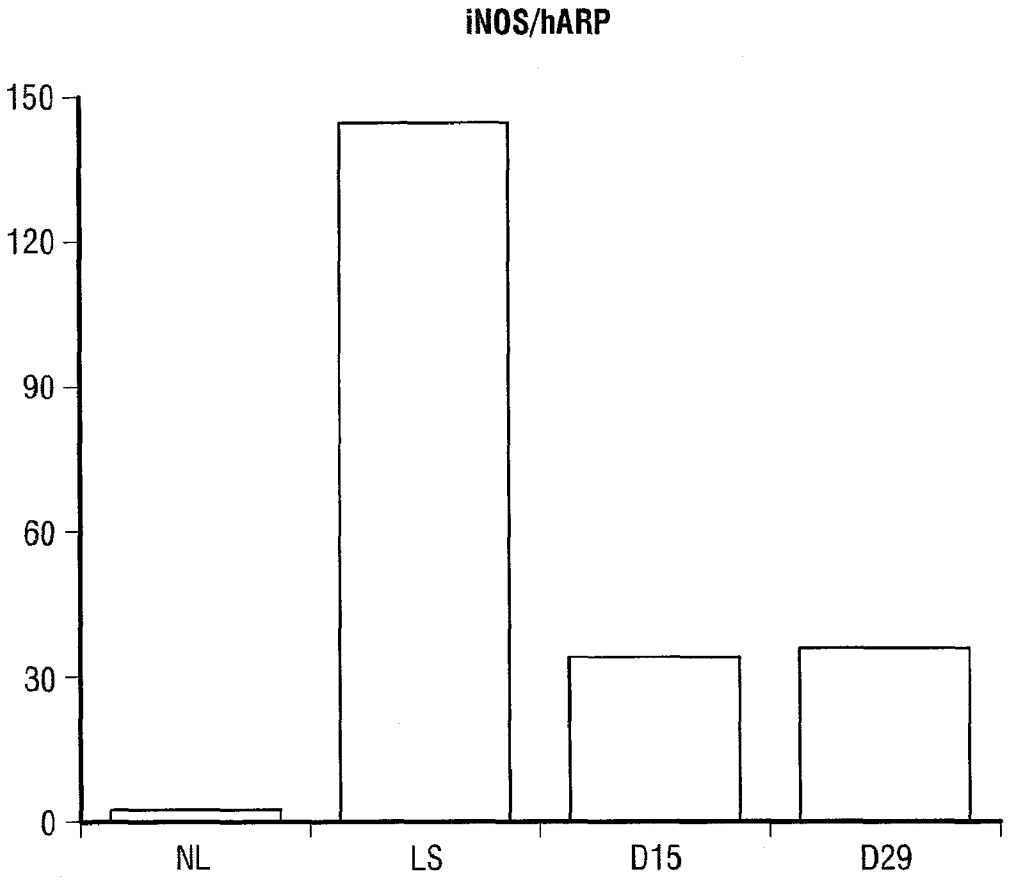


FIG. 32

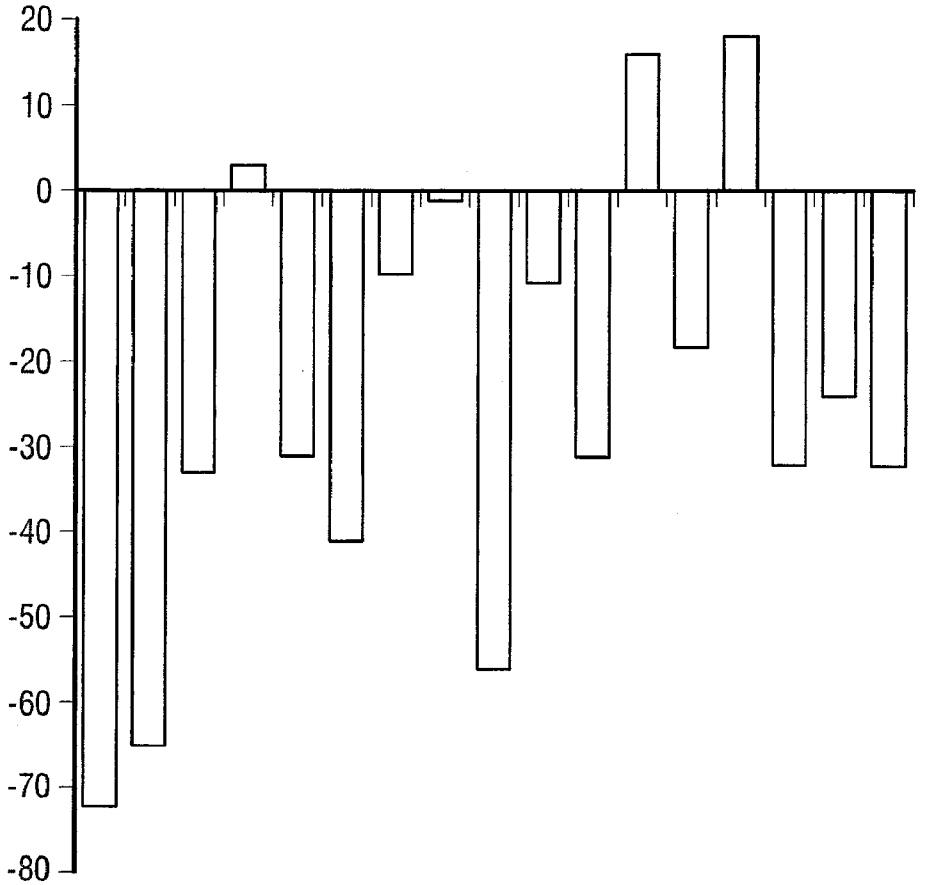


FIG. 33

SOLID FORMS COMPRISING (+)-2-[1-(3-ETHOXY-4-METHOXYPHENYL)-2-METHYLSULFONYLETHYL]-4-ACETYLAMINOISOINDOLINE-1,3-DIONE, COMPOSITIONS THEREOF, AND USES THEREOF

This application is a divisional of U.S. patent application Ser. No. 13/300,458, filed Nov. 18, 2011, which is a divisional of U.S. patent application Ser. No. 12/945,800, filed Nov. 12, 2010, which is a continuation of U.S. patent application Ser. No. 12/079,615, filed Mar. 27, 2008, which is a continuation-in-part of U.S. patent application Ser. No. 11/106,142, filed Apr. 13, 2005, which is a divisional of U.S. patent application Ser. No. 10/392,195, filed on Mar. 19, 2003, issued as U.S. Pat. No. 6,962,940, which claims the benefit of U.S. Provisional Patent Application No. 60/366,515, filed on Mar. 20, 2002, and U.S. Provisional Patent Application No. 60/438,450, filed on Jan. 7, 2003, the entireties of which are incorporated herein by reference.

1. FIELD OF INVENTION

Provided herein are solid forms comprising (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, compositions comprising the solid forms, methods of making the solid forms and methods of their use for the treatment of various diseases and/or disorders.

2. BACKGROUND OF THE INVENTION

Tumor necrosis factor alpha (TNF-α) is a cytokine that is released primarily by mononuclear phagocytes in response to immunostimulators. TNF-α is capable of enhancing most cellular processes, such as differentiation, recruitment, proliferation, and proteolytic degradation. At low levels, TNF-α confers protection against infective agents, tumors, and tissue damage. However, TNF-α also has a role in many diseases. When administered to a patient, TNF-α causes or aggravates inflammation, fever, cardiovascular effects, hemorrhage, coagulation, and acute phase responses similar to those seen during acute infections and shock states. Enhanced or unregulated TNF-α production has been implicated in a number of diseases and medical conditions, for example, cancers, such as solid tumors and blood-borne tumors; heart disease, such as congestive heart failure; and viral, genetic, inflammatory, allergic, and autoimmune diseases.

Adenosine 3',5'-cyclic monophosphate (cAMP) also plays a role in many diseases and conditions, such as, but not limited to, asthma and inflammation, and other conditions (Lowe and Cheng, *Drugs of the Future,* 17(9), 799-807, 1992). It has been shown that the elevation of cAMP in inflammatory leukocytes inhibits their activation and the subsequent release of inflammatory mediators, including TNF-α and NF-κB. Increased levels of cAMP also leads to the relaxation of airway smooth muscle.

It is believed that the primary cellular mechanism for the inactivation of cAMP is the breakdown of cAMP by a family of isoenzymes referred to as cyclic nucleotide phosphodiesterases (PDE) (Beavo and Reitsnyder, *Trends in Pharm.,* 11, 150-155, 1990). There are eleven known PDE families. It is recognized, for example, that the inhibition of PDE type IV is particularly effective in both the inhibition of inflammatory mediator release and the relaxation of airway smooth muscle (Verghese, et al., *J. Pharm. Exper. Therapeut.,* 272(3), 1313-1320, 1995). Thus, compounds that inhibit PDE4 (PDE IV) specifically, may inhibit inflammation and aid the relaxation of airway smooth muscle with a minimum of unwanted side effects, such as cardiovascular or anti-platelet effects. Currently used PDE4 inhibitors lack the selective action at acceptable therapeutic doses.

Cancer is a particularly devastating disease, and increases in blood TNF-α levels are implicated in the risk of and the spreading of cancer. Normally, in healthy subjects, cancer cells fail to survive in the circulatory system, one of the reasons being that the lining of blood vessels acts as a barrier to tumor-cell extravasation. However, increased levels of cytokines have been shown to substantially increase the adhesion of cancer cells to endothelium in vitro. One explanation is that cytokines, such as TNF-α, stimulate the biosynthesis and expression of a cell surface receptors called ELAM-1 (endothelial leukocyte adhesion molecule). ELAM-1 is a member of a family of calcium-dependent cell adhesion receptors, known as LEC-CAMs, which includes LECAM-1 and GMP-140. During an inflammatory response, ELAM-1 on endothelial cells functions as a "homing receptor" for leukocytes. Recently, ELAM-1 on endothelial cells was shown to mediate the increased adhesion of colon cancer cells to endothelium treated with cytokines (Rice et al., 1989, *Science* 246:1303-1306).

Inflammatory diseases such as arthritis, related arthritic conditions (e.g., osteoarthritis and rheumatoid arthritis), inflammatory bowel disease (e.g., Crohn's disease and ulcerative colitis), sepsis, psoriasis, atopic dermatitis, contact dermatitis, chronic obstructive pulmonary disease, and chronic inflammatory pulmonary diseases are also prevalent and problematic ailments. TNF-α plays a central role in the inflammatory response and the administration of their antagonists block chronic and acute responses in animal models of inflammatory disease.

Enhanced or unregulated TNF-α production has been implicated in viral, genetic, inflammatory, allergic, and autoimmune diseases. Examples of such diseases include but are not limited to: HIV; hepatitis; adult respiratory distress syndrome; bone-resorption diseases; chronic obstructive pulmonary diseases; chronic pulmonary inflammatory diseases; asthma; dermatitis; cystic fibrosis; septic shock; sepsis; endotoxic shock; hemodynamic shock; sepsis syndrome; post ischemic reperfusion injury; meningitis; psoriasis; fibrotic disease; cachexia; graft rejection; auto-immune disease; rheumatoid spondylitis; arthritic conditions, such as rheumatoid arthritis and osteoarthritis; osteoporosis; Crohn's disease; ulcerative colitis; inflammatory-bowel disease; multiple sclerosis; systemic lupus erythrematosus; ENL in leprosy; radiation damage; asthma; and hyperoxic alveolar injury. Tracey et al., 1987, *Nature* 330:662-664 and Hinshaw et al., 1990, *Circ. Shock* 30:279-292 (endotoxic shock); Dezube et al., 1990, *Lancet,* 335:662 (cachexia); Millar et al., 1989, *Lancet* 2:712-714 and Ferrai-Baliviera et al., 1989, *Arch. Surg.* 124:1400-1405 (adult respiratory distress syndrome); Bertolini et al., 1986, *Nature* 319:516-518, Johnson et al., 1989, *Endocrinology* 124:1424-1427, Holler et al., 1990, *Blood* 75:1011-1016, and Grau et al., 1989, *N. Engl. J. Med.* 320:1586-1591 (bone resorption diseases); Pignet et al., 1990, *Nature,* 344:245-247, Bissonnette et al., 1989, *Inflammation* 13:329-339 and Baughman et al., 1990, *J. Lab. Clin. Med.* 115:36-42 (chronic pulmonary inflammatory diseases); Elliot et al., 1995, *Int. J. Pharmac.* 17:141-145 (rheumatoid arthritis); von Dullemen et al., 1995, *Gastroenterology,* 109:129-135 (Crohn's disease); Duh et al., 1989, *Proc. Nat. Acad. Sci.* 86:5974-5978, Poll et al., 1990, *Proc. Nat. Acad. Sci.* 87:782-785, Monto et al., 1990, *Blood* 79:2670, Clouse et al., 1989, *J. Immunol.* 142, 431-438, Poll et al., 1992, *AIDS Res. Hum. Retrovirus,* 191-197, Poli et al. 1990, *Proc. Natl. Acad.*

Sci. 87:782-784, Folks et al., 1989, PNAS 86:2365-2368 (HIV and opportunistic infections resulting from HIV).

Pharmaceutical compounds that can block the activity or inhibit the production of certain cytokines, including TNF-α, may be beneficial therapeutics. Many small-molecule inhibitors have demonstrated an ability to treat or prevent inflammatory diseases implicated by TNF-α (for a review, see Lowe, 1998 *Exp. Opin. Ther. Patents* 8:1309-1332). One such class of molecules are the substituted phenethylsulfones described in U.S. Pat. No. 6,020,358.

The preparation and selection of a solid form of a pharmaceutical compound is complex, given that a change in solid form may affect a variety of physical and chemical properties, which may provide benefits or drawbacks in processing, formulation, stability and bioavailability, among other important pharmaceutical characteristics. Potential pharmaceutical solids include crystalline solids and amorphous solids. Amorphous solids are characterized by a lack of long-range structural order, whereas crystalline solids are characterized by structural periodicity. The desired class of pharmaceutical solid depends upon the specific application; amorphous solids are sometimes selected on the basis of e.g., an enhanced dissolution profile, while crystalline solids may be desirable for properties such as, e.g., physical or chemical stability (see, e.g., S. R. Vippagunta et al., *Adv. Drug. Deliv. Rev.*, (2001) 48:3-26; L. Yu, *Adv. Drug. Deliv. Rev.*, (2001) 48:27-42).

Whether crystalline or amorphous, potential solid forms of a pharmaceutical compound include single-component and multiple-component solids. Single-component solids consist essentially of the pharmaceutical compound in the absence of other compounds. Variety among single-component crystalline materials may potentially arise, e.g., from the phenomenon of polymorphism, wherein multiple three-dimensional arrangements exist for a particular pharmaceutical compound (see, e.g., S. R. Byrn et al., *Solid State Chemistry of Drugs*, (1999) SSCI, West Lafayette). The importance of studying polymorphs was underscored by the case of Ritonavir, an HIV protease inhibitor that was formulated as soft gelatin capsules. About two years after the product was launched, the unanticipated precipitation of a new, less soluble polymorph in the formulation necessitated the withdrawal of the product from the market until a more consistent formulation could be developed (see S. R. Chemburkar et al., *Org. Process Res. Dev.*, (2000) 4:413-417).

Additional diversity among the potential solid forms of a pharmaceutical compound may arise, e.g., from the possibility of multiple-component solids. Crystalline solids comprising two or more ionic species may be termed salts (see, e.g., *Handbook of Pharmaceutical Salts Properties, Selection and Use*, P. H. Stahl and C. G. Wermuth, Eds., (2002), Wiley, Weinheim). Additional types of multiple-component solids that may potentially offer other property improvements for a pharmaceutical compound or salt thereof include, e.g., hydrates, solvates, co-crystals and clathrates, among others (see, e.g., S. R. Byrn et al., *Solid State Chemistry of Drugs*, (1999) SSCI, West Lafayette). Moreover, multiple-component crystal forms may potentially be susceptible to polymorphism, wherein a given multiple-component composition may exist in more than one three-dimensional crystalline arrangement. The preparation of solid forms is of great importance in the development of a safe, effective, stable and marketable pharmaceutical compound.

Provided herein are embodiments addressing a need for solid forms of the compound chemically named (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione ("Compound A"), which was disclosed in U.S. application Ser. No. 10/392,195, filed Mar. 19, 2003 (issued as U.S. Pat. No. 6,962,940), as well as U.S. Provisional Application Ser. Nos. 60/366,515, filed Mar. 20, 2002 and 60/438,450, filed Jan. 7, 2003.

3. SUMMARY OF THE INVENTION

This invention relates to methods of treating diseases and disorders utilizing an enantiomer of a substituted phenethylsulfone compound and pharmaceutically acceptable solvates, hydrates, co-crystals, clathrates, prodrugs and polymorphs thereof and methods for reducing the level of cytokines and their precursors in mammals. The invention also relates to pharmaceutical compositions comprising the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione and a pharmaceutically acceptable carrier. The invention further relates to the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione substantially free of its (−) enantiomer.

This invention particularly relates to the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione. This compound is believed to have increased potency and other benefits as compared to its racemate, 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

The invention encompasses the use of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione for treating or preventing diseases or disorders ameliorated by the inhibition of TNF-α production in mammals. In certain embodiments, this treatment includes the reduction or avoidance of adverse effects. Such disorders include, but are not limited to, cancers, including, but not limited to cancer of the head, thyroid, neck, eye, skin, mouth, throat, esophagus, chest, bone, blood, bone marrow, lung, colon, sigmoid, rectum, stomach, prostate, breast, ovaries, kidney, liver, pancreas, brain, intestine, heart, adrenal, subcutaneous tissue, lymph nodes, heart, and combinations thereof. Specific cancers that can be treated by this method are multiple myeloma, malignant melanoma, malignant glioma, leukemia and solid tumors.

The invention also encompasses the use of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione in the treatment or prevention of heart disease, including, but not limited to congestive heart failure, cardiomyopathy, pulmonary edema, endotoxin-mediated septic shock, acute viral myocarditis, cardiac allograft rejection, and myocardial infarction.

The invention also encompasses the use of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione to treat diseases or disorders ameliorated by the inhibition of PDE4. For example, the compounds and compositions of the invention may be useful to treat or prevent viral, genetic, inflammatory, allergic, and autoimmune diseases. Examples of such diseases include, but are not limited to: HIV; hepatitis; adult respiratory distress syndrome; bone-resorption diseases; chronic obstructive pulmonary diseases; chronic pulmonary inflammatory diseases; dermatitis; inflammatory skin disease, atopic dermatitis, cystic fibrosis; septic shock; sepsis; endotoxic shock; hemodynamic shock; sepsis syndrome; post ischemic reperfusion injury; meningitis; psoriasis; fibrotic disease; cachexia; graft rejection including graft versus host disease; auto-immune disease; rheumatoid spondylitis; arthritic conditions, such as rheumatoid arthritis and osteoarthritis; osteoporosis; Crohn's disease; ulcerative colitis; inflammatory-bowel disease; multiple sclerosis; systemic

lupus erythrematosus; erythema nodosum leprosum (ENL) in leprosy; radiation damage; asthma; and hyperoxic alveolar injury.

In yet another embodiment, the stereomerically pure (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is also useful in the treatment or prevention of microbial infections or the symptoms of microbial infections including, but not limited to, bacterial infections, fungal infections, malaria, mycobacterial infection, and opportunistic infections resulting from HTV.

The invention further encompasses pharmaceutical compositions and single unit dosage forms comprising the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione and pharmaceutically acceptable polymorphs, prodrugs, hydrates, clathrates, and solvates thereof.

In a separate embodiment, the invention encompasses the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

In a further embodiment, the invention encompasses a method of producing the stereomerically pure (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione which comprises contacting 1-(3-Ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethylamine with a chiral amino acid and contacting the product of the first step with N-(1,3-Dioxo-1,3-dihydroisobenzofuran-4-yl)-acetamide. In a related embodiment the invention encompasses a chiral salt of 1-(3-Ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethylamine.

Embodiments herein provide solid forms comprising the compound chemically named (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione ("Compound A"). Compound A can be synthesized or obtained according to any method apparent to those of skill in the art based upon the teachings herein, including the methods described in the Examples below. Compound A can also be prepared according to the methods described in U.S. Pat. No. 6,962,940, issued Nov. 8, 2005, the entirety of which is incorporated by reference herein.

In certain embodiments, the solid forms are single-component crystal forms of Compound A. In certain embodiments, the solid forms are multiple-component crystal forms, including, but not limited to, co-crystals and/or solvates (including hydrates) comprising Compound A. In other embodiments, the solid forms are single-component amorphous forms of Compound A. In other embodiments, the solid forms are multiple-component amorphous forms. Without intending to be limited by any particular theory, certain novel solid forms provided herein have particular advantageous physical and/or chemical properties making them useful, e.g., for manufacturing, processing, formulation and/or storage, while also possessing particularly advantageous biological properties, such as, e.g., bioavailability and/or biological activity.

In particular embodiments, solid forms provided herein include solid forms comprising Compound A, including, but not limited to, single-component and multiple-component solid forms comprising Compound A. In certain embodiments, solid forms provided herein include polymorphs, solvates (including hydrates) and co-crystals comprising Compound A. Certain embodiments herein provide methods of making, isolating and/or characterizing the solid forms provided herein.

The solid forms provided herein are useful as active pharmaceutical ingredients for the preparation of formulations for use in patients. Thus, embodiments herein encompass the use of these solid forms as a final drug product. Certain embodiments provide solid forms useful in making final dosage forms with improved properties, e.g., powder flow properties, compaction properties, tableting properties, stability properties, and excipient compatibility properties, among others, that are needed for manufacturing, processing, formulation and/or storage of final drug products. Certain embodiments herein provide pharmaceutical compositions comprising a single-component crystal form, a multiple-component crystal form, a single-component amorphous form and/or a multiple-component amorphous form comprising Compound A and a pharmaceutically acceptable diluent, excipient or carrier. The solid forms and the final drug products provided herein are useful, for example, for the treatment, prevention or management of diseases and disorders provided herein.

Certain embodiments herein provide methods using the solid forms provided herein for treating, preventing or managing diseases or disorders ameliorated by the inhibition of TNF-α production in mammals, such as HIV; hepatitis; adult respiratory distress syndrome; bone resorption diseases; chronic obstructive pulmonary diseases; chronic pulmonary inflammatory diseases; asthma; dermatitis; cystic fibrosis; septic shock; sepsis; endotoxic shock; hemodynamic shock; sepsis syndrome; post ischemic reperfusion injury; meningitis; psoriasis; fibrotic disease; cachexia; graft rejection; auto immune disease; rheumatoid spondylitis; arthritic conditions, such as psoriatic arthritis, rheumatoid arthritis and osteoarthritis; osteoporosis; Crohn's disease; ulcerative colitis; inflammatory bowel disease; multiple sclerosis; systemic lupus erythematosus; cutaneous lupus erythematosus; pulmonary sarcoidosis; ENL in leprosy; radiation damage; asthma; and hyperoxic alveolar injury. Such disorders further include, but are not limited to, cancers, including, but not limited to cancer of the head, thyroid, neck, eye, skin, mouth, throat, esophagus, chest, bone, blood, bone marrow, lung, colon, sigmoid, rectum, stomach, prostate, breast, ovaries, kidney, liver, pancreas, brain, intestine, heart, adrenal, subcutaneous tissue, lymph nodes, heart, and combinations thereof. Specific cancers that can be treated by this method are multiple myeloma, malignant melanoma, malignant glioma, leukemia and solid tumors. In certain embodiments, methods using the solid forms provided herein include the reduction or avoidance of certain adverse effects.

Certain embodiments herein provide methods of using the solid forms provided herein in the treatment or prevention of heart disease, including, but not limited to congestive heart failure, cardiomyopathy, pulmonary edema, endotoxin-mediated septic shock, acute viral myocarditis, cardiac allograft rejection, and myocardial infarction.

Certain embodiments herein provide methods of using the solid forms provided herein to treat diseases or disorders ameliorated by the inhibition of PDE4. For example, the solid forms provided herein may be useful to treat or prevent viral, genetic, inflammatory, allergic, and autoimmune diseases. Examples of such diseases include, but are not limited to: HIV; hepatitis; adult respiratory distress syndrome; bone-resorption diseases; chronic obstructive pulmonary diseases; chronic pulmonary inflammatory diseases; dermatitis; inflammatory skin disease; atopic dermatitis; cystic fibrosis; septic shock; sepsis; endotoxic shock; hemodynamic shock; sepsis syndrome; post ischemic reperfusion injury; meningitis; psoriasis; fibrotic disease; cachexia; graft rejection including graft versus host disease; auto-immune disease; rheumatoid spondylitis; arthritic conditions, such as rheumatoid arthritis and osteoarthritis; osteoporosis; Crohn's disease; ulcerative colitis; inflammatory-bowel disease; multiple sclerosis; systemic lupus erythrematosus; erythema

nodosum leprosum (ENL) in leprosy; radiation damage; asthma; and hyperoxic alveolar injury.

Certain embodiments herein provide methods of using the solid forms provided herein in the treatment or prevention of microbial infections or the symptoms of microbial infections including, but not limited to, bacterial infections, fungal infections, malaria, mycobacterial infection, and opportunistic infections resulting from HIV.

Particular embodiments herein provide methods of using the solid forms provided herein in the treatment or prevention of diseases including: psoriasis; psoriatic arthritis; rheumatoid arthritis; chronic cutaneous sarcoid; giant cell arteritis; Parkinson's; prurigo nodularis; lichen planus; complex apthosis; Behcet's disease; lupus; hepatitis; uveitis; Sjogren's disease; depression (including major depression); interstitial cystitis; vulvodynia; prostatitis; osteoarthritis; diffuse large B cell lymphoma; polymysoitis; dermatomyositis; inclusiuon body myositis; erosive osteoarthritis; interstitial cystitis; hepatitis; endometriosis; radiculopathy; and pyoderma gangrenosum.

Certain embodiments herein provide pharmaceutical compositions and single unit dosage forms comprising one or more solid forms provided herein.

3.1. BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** provides a representative X-ray Powder Diffraction ("XRPD") pattern of Form A of Compound A.

FIG. **2** provides a representative Differential Scanning calorimetry ("DSC") plot of Form A of Compound A.

FIG. **3** provides a representative Thermal Gravimetric Analysis ("TGA") plot of Form A of Compound A.

FIG. **4** provides a representative Dynamic Vapor Sorption ("DVS") plot of Form A of Compound A.

FIG. **5** provides a representative XRPD pattern of Form B of Compound A.

FIG. **6** provides a representative DSC plot of Form B of Compound A.

FIG. **7** provides a representative TGA plot of Form B of Compound A.

FIG. **8** provides a representative DVS plot of Form B of Compound A.

FIG. **9** provides a representative XRPD pattern of Form C of Compound A.

FIG. **10** provides a representative DSC plot of Form C of Compound A.

FIG. **11** provides a representative TGA plot of Form C of Compound A.

FIG. **12** provides a representative DVS plot of Form C of Compound A.

FIG. **13** provides a representative XRPD pattern of Form D of Compound A.

FIG. **14** provides a representative DSC plot of Form D of Compound A.

FIG. **15** provides a representative TGA plot of Form D of Compound A.

FIG. **16** provides a representative DVS plot of Form D of Compound A.

FIG. **17** provides a representative XRPD pattern of Form E of Compound A.

FIG. **18** provides a representative DSC plot of Form E of Compound A.

FIG. **19** provides a representative TGA plot of Form E of Compound A.

FIG. **20** provides a representative DVS plot of Form E of Compound A.

FIG. **21** provides a representative XRPD pattern of Form F of Compound A.

FIG. **22** provides a representative DSC plot of Form F of Compound A.

FIG. **23** provides a representative TGA plot of Form F of Compound A.

FIG. **24** provides a representative DVS plot of Form F of Compound A.

FIG. **25** provides a representative XRPD of Form G of Compound A.

FIG. **26** provides a representative DSC plot of Form G of Compound A.

FIG. **27** provides a representative TGA plot of Form G of Compound A.

FIG. **28** provides a representative DVS plot of Form G of Compound A.

FIG. **29** illustrates a preparation of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

FIG. **30** illustrates the effect of Compound A on LPS-induced neutrophilia in the lungs of conscious ferrets.

FIG. **31** illustrates the percent change in epidermal thickness among all 15 subjects at Day 29 in a clinical study evaluating Compound A in patients with severe plaque-type psoriasis.

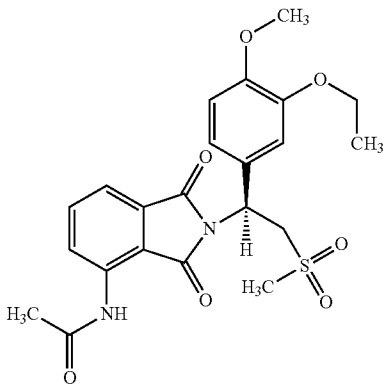
FIG. **32** illustrates the change in mean iNOS (normalized to hARP) in biopsy specimens of lesional skin at Day 29 in a clinical study evaluating Compound A in patients with severe plaque-type psoriasis.

FIG. **33** illustrates the percentage change in total Psoriasis Area and Severity Index (PASO score among evaluable patients from baseline at Day 29 in a clinical study evaluating Compound A in patients with severe plaque-type psoriasis.

3.2. DEFINITIONS

As used herein, term "Compound A" refers to enantiomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione which comes off of an HPLC column at about 25.4 minutes when that column is a 150 mm×4.6 mm Ultron Chiral ES-OVS chiral HPLC column (Agilent Technology), the eluent is 15:85 ethanol: 20 mM KH_2PO_4 at pH 3.5, and the observation wavelength is 240 nm. The ^1H NMR spectrum of Compound A is substantially as follows: δ($CDCl_3$); 1.47 (t, 3H); 2.26 (s, 3H); 2.87 (s, 3H); 3.68-3.75 (dd, 1H); 3.85 (s, 3H); 4.07-4.15 (q, 2H); 4.51-4.61 (dd, 1H); 5.84-5.90 (dd, 1H); 6.82-8.77 (m, 6H); 9.46 (s, 1H). The ^{13}C NMR spectrum of Compound A is substantially as follows: δ(DMSO-d_6); 14.66; 24.92; 41.61; 48.53; 54.46; 55.91; 64.51; 111.44; 112.40; 115.10; 118.20; 120.28; 124.94; 129.22; 131.02; 136.09; 137.60; 148.62; 149.74; 167.46; 169.14; 169.48. Compound A dissolved in methanol rotates plane polarized light in the (+) direction.

Without being limited by theory, Compound A is believed to be S-{2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione}, which has the following structure:



As used herein, the term "patient" refers to a mammal, particularly a human.

As used herein, the term "pharmaceutically acceptable salts" refer to salts prepared from pharmaceutically acceptable non-toxic acids or bases including inorganic acids and bases and organic acids and bases.

As used herein and unless otherwise indicated, the term "prodrug" means a derivative of a compound that can hydrolyze, oxidize, or otherwise react under biological conditions (in vitro or in vivo) to provide the compound. Examples of prodrugs include, but are not limited to, derivatives and metabolites of Compound A that include biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Prodrugs can typically be prepared using well-known methods, such as those described by 1 *Burger's Medicinal Chemistry and Drug Discovery,* 172-178, 949-982 (Manfred E. Wolff ed., 5th ed. 1995).

As used herein and unless otherwise indicated, the terms "biohydrolyzable amide," "biohydrolyzable ester," "biohydrolyzable carbamate," "biohydrolyzable carbonate," "biohydrolyzable ureide," "biohydrolyzable phosphate" mean an amide, ester, carbamate, carbonate, ureide, or phosphate, respectively, of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties in vivo, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted in vivo to the biologically active compound. Examples of biohydrolyzable esters include, but are not limited to, lower alkyl esters, alkoxyacyloxy esters, alkyl acylamino alkyl esters, and choline esters. Examples of biohydrolyzable amides include, but are not limited to, lower alkyl amides, α-amino acid amides, alkoxyacyl amides, and alkylaminoalkylcarbonyl amides. Examples of biohydrolyzable carbamates include, but are not limited to, lower alkylamines, substituted ethylenediamines, aminoacids, hydroxyalkylamines, heterocyclic and heteroaromatic amines, and polyether amines.

As used herein and unless otherwise indicated, the term "stereomerically pure" means a composition that comprises one stereoisomer of a compound and is substantially free of other stereoisomers of that compound. For example, a stereomerically pure composition of a compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically pure composition of a compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, more preferably greater than about 90% by weight of one stereoisomer of the compound and less than about 10% by weight of the other stereoisomers of the compound, even more preferably greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, and most preferably greater than about 97% by weight of one stercoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound.

As used herein and unless otherwise indicated, the term "enantiomerically pure" means a stereomerically pure composition of a compound having one chiral center.

As used herein, term "adverse effects" includes, but is not limited to gastrointestinal, renal and hepatic toxicities, leukopenia, increases in bleeding times due to, e.g., thrombocytopenia, and prolongation of gestation, nausea, vomiting, somnolence, asthenia, dizziness, teratogenicity, extra-pyramidal symptoms, akathisia, cardiotoxicity including cardiovascular disturbances, inflammation, male sexual dysfunction, and elevated scrum liver enzyme levels. The term "gastrointestinal toxicities" includes but is not limited to gastric and intestinal ulcerations and erosions. The term "renal toxicities" includes but is not limited to such conditions as papillary necrosis and chronic interstitial nephritis.

As used herein and unless otherwise indicated, the phrases "reduce or avoid adverse effects" and "reducing or avoiding adverse effects" mean the reduction of the severity of one or more adverse effects as defined herein.

It should be noted that if there is a discrepancy between a depicted structure and a name given that structure, the depicted structure is to be accorded more weight. In addition, if the stereochemistry of a structure or a portion of a structure is not indicated with, for example, bold or dashed lines, the structure or portion of the structure is to be interpreted as encompassing all stereoisomers of it.

As used herein and unless otherwise specified, the terms "solid form" and related terms refer to a physical form which is not predominantly in a liquid or a gaseous state. As used herein and unless otherwise specified, the term "solid form" and related terms, when used herein to refer to Compound A, refer to a physical form comprising Compound A which is not predominantly in a liquid or a gaseous state. Solid forms may be crystalline, amorphous or mixtures thereof. In particular embodiments, solid forms may be liquid crystals. A "single-component" solid form comprising Compound A consists essentially of Compound A. A "multiple-component" solid form comprising Compound A comprises a significant quantity of one or more additional species, such as ions and/or molecules, within the solid form. For example, in particular embodiments, a crystalline multiple-component solid form comprising Compound A further comprises one or more species non-covalently bonded at regular positions in the crystal lattice. Multiple-component solid forms comprising Compound A include co-crystals, solvates (e.g., hydrates), and clathrates of Compound A. In particular embodiments, the term "solid form comprising Compound A" and related terms include single-component and multiple-component solid forms comprising Compound A. In particular embodiments, "solid forms comprising Compound A" and related terms include crystal forms comprising Compound A, amorphous forms comprising Compound A, and mixtures thereof.

As used herein and unless otherwise specified, the term "crystalline" and related terms used herein, when used to describe a compound, substance, modification, material, component or product, unless otherwise specified, mean that the compound, substance, modification, material, component

or product is substantially crystalline as determined by X-ray diffraction. See, e.g., *Remington: The Science and Practice of Pharmacy,* 21st edition, Lippincott, Williams and Wilkins, Baltimore, Md. (2005); *The United States Pharmacopeia,* 23rd ed., 1843-1844 (1995).

As used herein and unless otherwise specified, the term "crystal forms," "crystalline forms" and related terms herein refer to solid forms that are crystalline. Crystal forms include single-component crystal forms and multiple-component crystal forms, and include, but are not limited to, polymorphs, solvates, hydrates, and/or other molecular complexes. In certain embodiments, a crystal form of a substance may be substantially free of amorphous forms and/or other crystal forms. In certain embodiments, a crystal form of a substance may contain less than about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45% or 50% of one or more amorphous forms and/or other crystal forms on a weight basis. In certain embodiments, a crystal form of a substance may be physically and/or chemically pure. In certain embodiments, a crystal form of a substance may be about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91% or 90% physically and/or chemically pure.

As used herein and unless otherwise specified, the terms "polymorphs," "polymorphic forms" and related terms herein, refer to two or more crystal forms that consist essentially of the same molecule, molecules, and/or ions. Like different crystal forms, different polymorphs may have different physical properties such as, e.g., melting temperature, heat of fusion, solubility, dissolution properties and/or vibrational spectra, as a result of the arrangement or conformation of the molecules and/or ions in the crystal lattice. The differences in physical properties may affect pharmaceutical parameters such as storage stability, compressibility and density (important in formulation and product manufacturing), and dissolution rate (an important factor in bioavailability). Differences in stability can result from changes in chemical reactivity (e.g., differential oxidation, such that a dosage form discolors more rapidly when comprised of one polymorph than when comprised of another polymorph) or mechanical changes (e.g., tablets crumble on storage as a kinetically favored polymorph converts to thermodynamically more stable polymorph) or both (e.g., tablets of one polymorph are more susceptible to breakdown at high humidity). As a result of solubility/dissolution differences, in the extreme case, some solid-state transitions may result in lack of potency or, at the other extreme, toxicity. In addition, the physical properties may be important in processing (e.g., one polymorph might be more likely to form solvates or might be difficult to filter and wash free of impurities, and particle shape and size distribution might be different between polymorphs).

As used herein and unless otherwise specified, the terms "solvate" and "solvated," refer to a crystal form of a substance which contains solvent. The terms "hydrate" and "hydrated" refer to a solvate wherein the solvent comprises water. "Polymorphs of solvates" refers to the existence of more than one crystal form for a particular solvate composition. Similarly, "polymorphs of hydrates" refers to the existence of more than one crystal form for a particular hydrate composition. The term "desolvated solvate," as used herein, refers to a crystal form of a substance which may be prepared by removing the solvent from a solvate.

As used herein and unless otherwise specified, the term "amorphous," "amorphous form," and related terms used herein, mean that the substance, component or product in question is not substantially crystalline as determined by X-ray diffraction. In particular, the term "amorphous form" describes a disordered solid form, i.e., a solid form lacking long range crystalline order. In certain embodiments, an amorphous form of a substance may be substantially free of other amorphous forms and/or crystal forms. In other embodiments, an amorphous form of a substance may contain less than about 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45% or 50% of one or more other amorphous forms and/or crystal forms on a weight basis. In certain embodiments, an amorphous form of a substance may be physically and/or chemically pure. In certain embodiments, an amorphous form of a substance be about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91% or 90% physically and/or chemically pure.

Techniques for characterizing crystal forms and amorphous forms include, but are not limited to, thermal gravimetric analysis (TGA), differential scanning calorimetry (DSC), X-ray powder diffractometry (XRPD), single-crystal X-ray diffractometry, vibrational spectroscopy, e.g., infrared (IR) and Raman spectroscopy, solid-state and solution nuclear magnetic resonance (NMR) spectroscopy, optical microscopy, hot stage optical microscopy, scanning electron microscopy (SEM), electron crystallography and quantitative analysis, particle size analysis (PSA), surface area analysis, solubility measurements, dissolution measurements, elemental analysis and Karl Fischer analysis. Characteristic unit cell parameters may be determined using one or more techniques such as, but not limited to, X-ray diffraction and neutron diffraction, including single-crystal diffraction and powder diffraction. Techniques useful for analyzing powder diffraction data include profile refinement, such as Rietveld refinement, which may be used, e.g., to analyze diffraction peaks associated with a single phase in a sample comprising more than one solid phase. Other methods useful for analyzing powder diffraction data include unit cell indexing, which allows one of skill in the art to determine unit cell parameters from a sample comprising crystalline powder.

As used herein and unless otherwise specified, the terms "about" and "approximately," when used in connection with a numeric value or a range of values which is provided to characterize a particular solid form, e.g., a specific temperature or temperature range, such as, e.g., that describing a DSC or TGA thermal event, including, e.g., melting, dehydration, desolvation or glass transition events; a mass change, such as, e.g., a mass change as a function of temperature or humidity; a solvent or water content, in terms of, e.g., mass or a percentage; or a peak position, such as, e.g., in analysis by IR or Raman spectroscopy or XRPD; indicate that the value or range of values may deviate to an extent deemed reasonable to one of ordinary skill in the art while still describing the particular solid form. For example, in particular embodiments, the terms "about" and "approximately," when used in this context and unless otherwise specified, indicate that the numeric value or range of values may vary within 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1.5%, 1%, 0.5%, or 0.25% of the recited value or range of values.

As used herein and unless otherwise specified, a sample comprising a particular crystal form or amorphous form that is "substantially pure," e.g., substantially free of other solid forms and/or of other chemical compounds, contains, in particular embodiments, less than about 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.75%, 0.5%, 0.25% or 0.1% percent by weight of one or more other solid forms and/or of other chemical compounds.

As used herein and unless otherwise specified, a sample or composition that is "substantially free" of one or more other solid forms and/or other chemical compounds means that the composition contains, in particular embodiments, less than about 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%,

2%, 1%, 0.75%, 0.5%, 0.25% or 0.1% percent by weight of one or more other solid forms and/or other chemical compounds.

As used herein, and unless otherwise specified, the terms "treat," "treating" and "treatment" refer to the eradication or amelioration of a disease or disorder, or of one or more symptoms associated with the disease or disorder. In certain embodiments, the terms refer to minimizing the spread or worsening of the disease or disorder resulting from the administration of one or more prophylactic or therapeutic agents to a patient with such a disease or disorder. In some embodiments, the terms refer to the administration of a compound provided herein, with or without other additional active agent, after the onset of symptoms of the particular disease.

As used herein, and unless otherwise specified, the terms "prevent," "preventing" and "prevention" refer to the prevention of the onset, recurrence or spread of a disease or disorder, or of one or more symptoms thereof. In certain embodiments, the terms refer to the treatment with or administration of a compound provided herein, with or without other additional active compound, prior to the onset of symptoms, particularly to patients at risk of diseases or disorders provided herein. The terms encompass the inhibition or reduction of a symptom of the particular disease. Patients with familial history of a disease in particular are candidates for preventive regimens in certain embodiments. In addition, patients who have a history of recurring symptoms are also potential candidates for the prevention. In this regard, the term "prevention" may be interchangeably used with the term "prophylactic treatment."

As used herein, and unless otherwise specified, the terms "manage," "managing" and "management" refer to preventing or slowing the progression, spread or worsening of a disease or disorder, or of one or more symptoms thereof. Often, the beneficial effects that a patient derives from a prophylactic and/or therapeutic agent do not result in a cure of the disease or disorder. In this regard, the term "managing" encompasses treating a patient who had suffered from the particular disease in an attempt to prevent or minimize the recurrence of the disease.

As used herein, and unless otherwise specified, a "therapeutically effective amount" of a compound is an amount sufficient to provide a therapeutic benefit in the treatment or management of a disease or disorder, or to delay or minimize one or more symptoms associated with the disease or disorder. A therapeutically effective amount of a compound means an amount of therapeutic agent, alone or in combination with other therapies, which provides a therapeutic benefit in the treatment or management of the disease or disorder. The term "therapeutically effective amount" can encompass an amount that improves overall therapy, reduces or avoids symptoms or causes of disease or disorder, or enhances the therapeutic efficacy of another therapeutic agent.

As used herein, and unless otherwise specified, a "prophylactically effective amount" of a compound is an amount sufficient to prevent a disease or disorder, or prevent its recurrence. A prophylactically effective amount of a compound means an amount of therapeutic agent, alone or in combination with other agents, which provides a prophylactic benefit in the prevention of the disease. The term "prophylactically effective amount" can encompass an amount that improves overall prophylaxis or enhances the prophylactic efficacy of another prophylactic agent.

The term "composition" as used herein is intended to encompass a product comprising the specified ingredients (and in the specified amounts, if indicated), as well as any product which results, directly or indirectly, from combination of the specified ingredients in the specified amounts. By "pharmaceutically acceptable" it is meant that the diluent, excipient or carrier must be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof

4. DETAILED DESCRIPTION OF THE INVENTION

This invention relates to stereomerically pure Compound A, which is the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, substantially free of its (−) enantiomer, as well as novel methods of using, and compositions comprising, stereomerically pure Compound A and/or solid forms comprising Compound A. For example, the present invention encompasses the in vitro and in vivo use of Compound A, and the incorporation of Compound A into pharmaceutical compositions and single unit dosage forms useful in the treatment and prevention of a variety of diseases and disorders. Diseases and disorders which are ameliorated by the reduction of levels of TNF-α or inhibition of PDE4 are well known in the art and are described herein. Specific methods of the invention reduce or avoid the adverse effects associated with compounds used as TNF-α inhibitor. Other specific methods of the invention reduce or avoid the adverse effects associated with use of racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

Specific methods of the invention include methods of treating or preventing diseases and disorders including, but not limited to, solid tumors, blood-borne tumors and inflammatory diseases.

Pharmaceutical and dosage forms of the invention, which comprise Compound A or a pharmaceutically acceptable polymorph, prodrug, clathrate, solvate or hydrate thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein) can be used in the methods of the invention.

Without being limited by theory, it is believed that Compound A, including solid forms comprising Compound A, can inhibit TNF-α production. Consequently, a first embodiment of the invention relates to a method of inhibiting TNF-α production which comprises contacting a cell exhibiting abnormal TNF-α production with an effective amount of stereomerically pure Compound A or a pharmaceutically acceptable prodrug, metabolite, polymorph, solvate, hydrate, or clathrate thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein). In a particular embodiment, the invention relates to a method of inhibiting TNF-α production which comprises contacting a mammalian cell exhibiting abnormal TNF-α production with an effective amount of stereomerically pure Compound A or a pharmaceutically acceptable prodrug, metabolite, polymorph, solvate, hydrate, or clathrate thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein).

The invention also relates to a method of treating, preventing or managing disorders ameliorated by the reduction of levels of TNF-α in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A or a pharmaceutically acceptable prodrug, metabolite, polymorph, solvate, hydrate, or clathrate thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein). In particular embodiments, diseases or disorders ameliorated by the

inhibition of TNF-α production in mammals include, but are not limited to: HIV; hepatitis; adult respiratory distress syndrome; bone resorption diseases; chronic obstructive pulmonary diseases; chronic pulmonary inflammatory diseases; asthma; dermatitis; cystic fibrosis; septic shock; sepsis; endotoxic shock; hemodynamic shock; sepsis syndrome; post ischemic reperfusion injury; meningitis; psoriasis; fibrotic disease; cachexia; graft rejection; auto immune disease; rheumatoid spondylitis; arthritic conditions, such as psoriatic arthritis, rheumatoid arthritis and osteoarthritis; osteoporosis; Crohn's disease; ulcerative colitis; inflammatory bowel disease; multiple sclerosis; systemic lupus erythematosus; cutaneous lupus erythematosus; pulmonary sarcoidosis; erythema nodosum leprosum (ENL) in leprosy; radiation damage; asthma; and hyperoxic alveolar injury. Such disorders further include, but are not limited to, cancers, including, but not limited to cancer of the head, thyroid, neck, eye, skin, mouth, throat, esophagus, chest, bone, blood, bone marrow, lung, colon, sigmoid, rectum, stomach, prostate, breast, ovaries, kidney, liver, pancreas, brain, intestine, heart, adrenal, subcutaneous tissue, lymph nodes, heart, and combinations thereof. Specific cancers that can be treated by this method are multiple myeloma, malignant melanoma, malignant glioma, leukemia and solid tumors.

A further embodiment of the invention relates to a method of treating or preventing cancer, including but not limited to, solid tumor, blood-borne tumor, leukemias, and in particular, multiple myeloma in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically effective amount of stereomerically pure Compound A or a pharmaceutically acceptable prodrug, metabolite, polymorph, solvate, hydrate, or clathrate thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein); in particular wherein the patient is a mammal.

In another embodiment, the invention relates to a method of inhibiting PDE4 which comprises contacting PDE4 in a cell (e.g. a mammalian cell) with an effective amount of stereomerically pure Compound A or a pharmaceutically acceptable prodrug, metabolite, polymorph, solvate, hydrate, or clathrate thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein).

A further embodiment of the invention relates to a method of treating or preventing diseases or disorders ameliorated by the inhibition of PDE4 in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A or a pharmaceutically acceptable prodrug, metabolite, polymorph, solvate, hydrate, or clathrate thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein). Disorders ameliorated by the inhibition of PDE4 include, but are not limited to, asthma, inflammation (e.g., inflammation due to reperfusion), chronic or acute obstructive pulmonary diseases, chronic or acute pulmonary inflammatory diseases, inflammatory bowel disease, Crohn's Disease, Behcet's Disease, or colitis.

In another embodiment, the invention relates to a method of controlling cAMP levels in a cell which comprises contacting a cell with an effective amount of stereomerically pure Compound A or a pharmaceutically acceptable prodrug, metabolite, polymorph, solvate, hydrate, or clathrate thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein). As used herein the term "controlling cAMP levels" includes preventing or reducing the rate of the breakdown of Adenosine 3',5'-cyclic monophosphatc (cAMP) in a cell or increasing the amount of Adenosine 3',5'-cyclic monophosphate present in a cell, preferably a mammalian cell, more preferably a human cell. In a particular method, the rate of cAMP breakdown is reduced by about 10, 25, 50, 100, 200, or 500 percent as compared to the rate in comparable cells which have not been contacted with a compound of the invention.

A further embodiment of the invention relates to a method of treating or preventing depression, asthma, inflammation (e.g., contact dermatitis, atopic dermatitis, psoriasis, rheumatoid arthritis, osteoarthritis, inflammatory skin disease, inflammation due to reperfusion), chronic or acute obstructive pulmonary diseases, chronic or pulmonary inflammatory diseases, inflammatory bowel disease, Crohn's Disease, Behcet's Disease or colitis in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A or a pharmaceutically acceptable prodrug, metabolite, polymorph, solvate, hydrate, or clathrate thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein); in particular wherein the patient is a mammal.

A separate embodiment of the invention encompasses methods of treating or preventing myelodysplastic syndrome (MDS) which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A or a pharmaceutically acceptable solvate, hydrate, stereoisomer, clathrate, or prodrug thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein). MDS refers to a diverse group of hematopoietic stem cell disorders. MDS is characterized by a cellular marrow with impaired morphology and maturation (dysmyelopoiesis), peripheral blood cytopenias, and a variable risk of progression to acute leukemia, resulting from ineffective blood cell production. See *The Merck Manual* 953 (17th ed. 1999) and List et al., 1990, *J. Clin. Oncol.* 8:1424.

A separate embodiment of the invention encompasses methods of treating or preventing myeloproliferative disease (MPD) which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A or a pharmaceutically acceptable solvate, hydrate, stereoisomer, clathrate, or prodrug thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein). Myeloproliferative disease (MPD) refers to a group of disorders characterized by clonal abnormalities of the hematopoietic stem cell. See e.g., *Current Medical Diagnosis & Treatment*, pp. 499 (37th ed., Tierney et al., ed., Appleton & Lange, 1998).

The invention also encompasses a method of treating, preventing or managing pain, including, but not limited to, complex regional pain syndrome, which comprises administering to a patient in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of a stereomerically pure Compound A or a pharmaceutically acceptable solvate, hydrate, stereoisomer, clathrate, or prodrug thereof (wherein particular embodiments encompass solid forms comprising Compound A as described herein). In a specific embodiment, the administration is before, during or after surgery or physical therapy directed at reducing or avoiding a symptom of complex regional pain syndrome in the patient.

In particular methods of the invention, stereomerically pure Compound A or a pharmaceutically acceptable polymorph, prodrug, solvate, hydrate, or clathrate thereof (wherein particular embodiments encompass solid forms

comprising Compound A as described herein), is adjunctively administered with at least one additional therapeutic agent. Examples of additional therapeutic agents include, but are not limited to, anti-cancer drugs, anti-inflammatories, antihistamines and decongestants.

4.1. Solid Forms Comprising Compound A

Certain embodiments herein provide solid forms comprising Compound A, which has the chemical structure shown above. Racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is readily prepared using the methods in U.S. Pat. No. 6,020,358, which is incorporated herein by reference. Compound A, which is the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, can be prepared according to any method apparent to those of skill in the art, including the methods described in U.S. Pat. No. 6,962,940, which is incorporated herein by reference.

Solid forms comprising Compound A include single-component and multiple-component forms, including crystal forms and amorphous forms, and including, but not limited to, polymorphs, solvates, hydrates, co-crystals and clathrates. Particular embodiments herein provide single-component amorphous solid forms of Compound A. Particular embodiments herein provide single-component crystalline solid forms of Compound A. Particular embodiments herein provide multiple-component amorphous forms comprising Compound A. Particular embodiments herein provide multiple-component crystalline solid forms comprising Compound A. Multiple-component solid forms provided herein include solid forms which may be described by the terms salt, co-crystal, hydrate, solvate, clathrate and/or polymorph, and include solid forms which may be described by one or more of these terms.

Solid forms comprising Compound A can be prepared by the methods described herein, including the methods described in the Examples below, or by techniques known in the art, including heating, cooling, freeze drying, lyophilization, quench cooling the melt, rapid solvent evaporation, slow solvent evaporation, solvent recrystallization, antisolvent addition, slurry recrystallization, crystallization from the melt, desolvation, recrystallization in confined spaces such as, e.g., in nanopores or capillaries, recrystallization on surfaces or templates such as, e.g., on polymers, recrystallization in the presence of additives, such as, e.g., co-crystal counter-molecules, desolvation, dehydration, rapid cooling, slow cooling, exposure to solvent and/or water, drying, including, e.g., vacuum drying, vapor diffusion, sublimation, grinding (including, e.g., cryo-grinding, solvent-drop grinding or liquid assisted grinding), microwave-induced precipitation, sonication-induced precipitation, laser-induced precipitation and precipitation from a supercritical fluid. The particle size of the resulting solid forms, which can vary, (e.g., from nanometer dimensions to millimeter dimensions), can be controlled, e.g., by varying crystallization conditions, such as, e.g., the rate of crystallization and/or the crystallization solvent system, or by particle-size reduction techniques, e.g., grinding, milling, micronizing or sonication.

While not intending to be bound by any particular theory, certain solid forms are characterized by physical properties, e.g., stability, solubility and dissolution rate, appropriate for pharmaceutical and therapeutic dosage forms. Moreover, while not wishing to be bound by any particular theory, certain solid forms are characterized by physical properties (e.g., density, compressibility, hardness, morphology, cleavage, stickiness, solubility, water uptake, electrical properties, thermal behavior, solid-state reactivity, physical stability, and chemical stability) affecting particular processes (e.g., yield, filtration, washing, drying, milling, mixing, tableting, flowability, dissolution, formulation, and lyophilization) which make certain solid forms suitable for the manufacture of a solid dosage form. Such properties can be determined using particular analytical chemical techniques, including solid-state analytical techniques (e.g., X-ray diffraction, microscopy, spectroscopy and thermal analysis), as described herein and known in the art.

Certain embodiments herein provide compositions comprising one or more of the solid forms. Certain embodiments provide compositions of one or more solid forms in combination with other active ingredients. Certain embodiments provide methods of using these compositions in the treatment, prevention or management of diseases and disorders including, but not limited to, the diseases and disorders provided herein.

In addition to solid forms comprising Compound A, provided herein are solid forms comprising prodrugs of Compound A.

Solid forms provided herein may also comprise unnatural proportions of atomic isotopes at one or more of the atoms in Compound A. For example, the compound may be radiolabeled with radioactive isotopes, such as for example tritium (^{3}H), iodine-125 (^{125}I) sulfur-35 (^{35}S), or carbon-14 (^{14}C). Radiolabeled compounds are useful as therapeutic agents, e.g., cancer therapeutic agents, research reagents, e.g., binding assay reagents, and diagnostic agents, e.g., in vivo imaging agents. All isotopic variations of Compound A, whether radioactive or not, are intended to be encompassed within the scope of the embodiments provided herein.

4.1.1. Form A of Compound A

Certain embodiments herein provide the Form A crystal form of Compound A. In certain embodiments, Form A of Compound A can be obtained from various solvents, including, but not limited to, solvent systems comprising acetone, ethanol, and mixtures thereof. In certain embodiments, Form A can be obtained using a fast cooling crystallization process.

In certain embodiments, Form A of Compound A may be characterized by X-ray powder diffraction analysis. A representative XRPD pattern of Form A of Compound A is provided in FIG. **1**. In certain embodiments, Form A of Compound A is characterized by XRPD peaks located at one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve of the following approximate positions: 8.1, 14.4, 15.2, 17.4, 18.4, 19.2, 20.5, 22.8, 23.2 23.6, 24.5, 25.1 degrees 2θ. In certain embodiments, Form A of Compound A is characterized by an XRPD pattern which matches the pattern exhibited in FIG. **1**. In certain embodiments, Form A of Compound A is characterized by an XRPD pattern having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 peaks matching peaks in the representative Form A pattern provided herein.

In certain embodiments, Form A of Compound A may be characterized by thermal analysis. A representative DSC plot for Form A of Compound A is shown in FIG. **2**. In certain embodiments, Form A is characterized by a DSC plot comprising an endothermic event with an onset temperature of about 145° C. In certain embodiments, Form A is characterized by a DSC plot further comprising an endothermic event with an onset temperature of about 155° C. A representative TGA plot for Form A of Compound A is shown in FIG. **3**. In certain embodiments, Form A is characterized by a TGA plot comprising a mass loss of less than about 1%, e.g., about 0.05%, of the total mass of the sample upon heating from about 25° C. to about 140° C. In certain embodiments, Form A of Compound A does not contain substantial amounts of

either water or other solvent in the crystal lattice. In certain embodiments, Form A is unsolvated. In certain embodiments, Form A is anhydrous.

In certain embodiments, Form A of Compound A may be characterized by moisture sorption analysis. A representative moisture sorption isotherm plot is shown in FIG. **4**. In certain embodiments, when the relative humidity ("RH") is increased from about 0% to about 95% RH, Form A exhibits a mass change of less than about 1%, e.g., about 0.4%, of the starting mass of the sample. In certain embodiments, mass gained upon adsorption is lost when the RH is decreased back to about 0% RH. Accordingly, in certain embodiments, Form A is substantially nonhygroscopic. In certain embodiments, the XRPD pattern of the Form A material is substantially unchanged following the adsorption/desorption analysis. In certain embodiments, Form A is stable with respect to humidity.

In certain embodiments, Form A of Compound A may be characterized by its stability profile. In certain embodiments, Form A material is stable, e.g., its XRPD pattern remains substantially unchanged, upon exposure to elevated temperature, upon exposure to elevated humidity, upon exposure to one or more solvents, and/or upon compression. In certain embodiments, for example, Form A is stable following exposure to an environment of about 40° C. and about 75% RH environment for about four weeks. In certain embodiments, Form A is stable following exposure to one or more solvent systems comprising, e.g., ethanol, water and/or heptane, at about 40° C. for at least about four weeks. In certain embodiments, Form A converts to Form C of Compound A upon exposure to a solvent including, but not limited to, toluene for four weeks. In certain embodiments, Form A is stable upon compression at about 2000 psi pressure for about one minute.

In certain embodiments, Form A of Compound A may be characterized by particle analysis. In certain embodiments, Form A is characterized as a white powder. In certain embodiments, a sample of Form A comprises particles having a plate-like morphology. In certain embodiments, a sample of Form A comprises particles with a D_{90} of less than about 18 (As used herein, the D_{90} value represents the 90th percentile of the particle size distribution as measured by length; i.e., 90% of the particles have a length of this value or less).

Certain embodiments herein provide Form A of Compound A which is substantially pure. Certain embodiments herein provide Form A of Compound A which is substantially free of other solid forms comprising Compound A including, e.g., Forms B, C, D, E, F, G and/or an amorphous solid form comprising Compound A as provided herein. Certain embodiments herein provide Form A as a mixture of solid forms comprising Compound A, including, e.g., a mixture comprising one or more of the following: Forms B, C, D, E, F, G and an amorphous solid form comprising Compound A as provided herein.

4.1.2. Form B of Compound A

Certain embodiments herein provide the Form B crystal form of Compound A. In certain embodiments, Form B of Compound A can be obtained from various solvents, including, but not limited to, solvent systems comprising 2-propanol, acetone, acetonitrile, ethanol, ethyl acetate, heptane, methanol, methyl ethyl ketone, methyl t-butyl ether, methylene chloride, n-butanol, n-butyl acetate, tetrahydrofuran, toluene, water and mixtures comprising two or more thereof. For example, in certain embodiments, Form B can be obtained by crystallization from a solvent system comprising 1:1 ethanol:water, e.g., by a process comprising evaporation of the 1:1 ethanol:water solvent system at about 25° C., followed by isolation of Form B. For example, in certain embodiments, Form B can be obtained by crystallization from a solvent system comprising 1:1 acetone:ethanol, e.g., by a process comprising slurrying a solid form comprising Compound A in 1:1 acetone:ethanol at about 25° C. for about 2 days, followed by isolation of Form B.

In certain embodiments, Form B of Compound A may be characterized by X-ray powder diffraction analysis. A representative XRPD pattern of Form B of Compound A is provided in FIG. **5**. In certain embodiments, Form B of Compound A is characterized by XRPD peaks located at one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve of the following approximate positions: 10.1, 12.4, 13.5, 15.7, 16.3, 18.1, 20.7, 22.5, 24.7, 26.2, 26.9, 29.1 degrees 2θ. In certain embodiments, Form B of Compound A is characterized by an XRPD pattern which matches the pattern exhibited in FIG. **5**. In certain embodiments, Form B of Compound A is characterized by an XRPD pattern having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 peaks matching peaks in the representative Form B pattern provided herein.

In certain embodiments, Form B of Compound A may be characterized by thermal analysis. A representative DSC plot for Form B of Compound A is shown in FIG. **6**. In certain embodiments, Form B is characterized by a DSC plot comprising an endothermic event with an onset temperature of about 154° C. A representative TGA plot for Form B of Compound A is shown in FIG. **7**. In certain embodiments, Form B is characterized by a TGA plot comprising a mass loss of less than about 1%, e.g., about 0.25%, of the total mass of the sample upon heating from about 25° C. to about 140° C. In certain embodiments, Form B of Compound A does not contain substantial amounts of either water or other solvent in the crystal lattice. In certain embodiments, Form B is anhydrous. In certain embodiments, Form B is unsolvated.

In certain embodiments, Form B of Compound A may be characterized by moisture sorption analysis. A representative moisture sorption isotherm plot is shown in FIG. **8**. In certain embodiments, when the RH is increased from about 0% to about 95% RH, Form B exhibits a mass change of less than about 1%, e.g., about 0.6%, of the starting mass of the sample. In certain embodiments, mass gained upon adsorption is lost when the RH is decreased back to about 0% RH. In certain embodiments, Form B is substantially nonhygroscopic. In certain embodiments, the XRPD pattern of Form B material is substantially unchanged following the adsorption/desorption analysis. In certain embodiments, Form B is stable with respect to humidity.

In certain embodiments, Form B of Compound A may be characterized by its stability profile. In certain embodiments, Form B material is stable, e.g., its XRPD pattern remains substantially unchanged, upon exposure to elevated temperature, upon exposure to elevated humidity, upon exposure to one or more solvents, and/or upon compression. In certain embodiments, for example, Form B is stable following exposure to an environment of about 40° C. and about 75% RH environment for about four weeks. In certain embodiments, Form B is stable following exposure to a solvent system comprising, e.g., ethanol, water or heptane, at about 40° C. for at least about four weeks. In certain embodiments, Form B converts to Form C of Compound A upon exposure to a solvent system comprising, e.g., toluene for about four weeks. In certain embodiments, Form B is stable following compression at about 2000 psi pressure for about one minute.

In certain embodiments, Form B of Compound A may be characterized by particle analysis. In certain embodiments, Form B is characterized as a white powder. In certain embodiments, a sample of Form B comprises particles having a

flake-like morphology. In certain embodiments, a sample of Form B comprises particles with a D_{90} of less than about 12 μm.

Certain embodiments herein provide Form B of Compound A which is substantially pure. Certain embodiments herein provide Form B of Compound A which is substantially free of other solid forms comprising Compound A including, e.g., Forms A, C, D, E, F, G and/or an amorphous solid form comprising Compound A as provided herein. Certain embodiments herein provide Form B as a mixture of solid forms comprising Compound A, including, e.g., a mixture comprising one or more of the following: Forms A, C, D, E, F, G and an amorphous solid form comprising Compound A as provided herein.

4.1.3. Form C of Compound A

Certain embodiments herein provide the Form C crystal form of Compound A. In certain embodiments, Form C of Compound A can be obtained from various solvent systems, including, but not limited to, solvent systems comprising acetone, acetonitrile, ethanol, heptane, methanol, methyl ethyl ketone, tetrahydrofuran, toluene, water, and mixtures comprising two or more thereof. For example, in certain embodiments, Form C can be obtained by crystallization from a solvent system comprising toluene, e.g., by a process comprising the use of toluene as an anti-solvent, followed by isolation of Form C.

In certain embodiments, Form C of Compound A may be characterized by X-ray powder diffraction analysis. A representative XRPD pattern of Form C of Compound A is provided in FIG. **9**. In certain embodiments, Form C of Compound A is characterized by XRPD peaks located at one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve of the following approximate positions: 7.5, 11.3, 15.3, 16.4, 17.8, 21.4, 22.6, 23.5, 24.8, 25.5, 26.4, 27.6 degrees 2θ. In certain embodiments, Form C of Compound A is characterized by an XRPD pattern which matches the pattern exhibited in FIG. **9**. In certain embodiments, Form C of Compound A is characterized by an XRPD pattern having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 peaks matching peaks in the representative Form C pattern provided herein.

In certain embodiments, Form C of Compound A may be characterized by thermal analysis. A representative DSC plot for Form C of Compound A is shown in FIG. **10**. In certain embodiments, Form C is characterized by a DSC plot comprising an endothermic event with an onset temperature of about 138° C. In certain embodiments, a characteristic Form C DSC plot further comprises one or more additional events, such as, e.g., an endothermic event with an onset temperature of about 166° C. A representative TGA plot for Form C of Compound A is shown in FIG. **11**. In certain embodiments, Form C is characterized by a TGA plot comprising a mass loss of less than about 10%, e.g., about 5.9%, of the total mass of the sample upon heating from about 25° C. to about 140° C. In certain embodiments, the TGA mass loss event comprises the loss of the solvent toluene, as indicated, e.g., by TG-IR analysis. In certain embodiments, Form C of Compound A is solvated. In certain embodiments, Form C is a toluene solvate. In certain embodiments, the crystal lattice of Form C comprises about three molar equivalents of toluene per mole of Compound A.

In certain embodiments, Form C of Compound A may be characterized by moisture sorption analysis. A representative moisture sorption isotherm plot is shown in FIG. **12**. In certain embodiments, when the RH is increased from about 0% to about 95% RH, Form C exhibits a mass change of less than about 1%, e.g., about 0.5%, of the starting mass of the sample. In certain embodiments, mass gained upon adsorption is lost when the RH is decreased back to about 0% RH. In certain embodiments, Form C is substantially nonhygroscopic. In certain embodiments, the XRPD pattern of Form C material is substantially unchanged following the adsorption/desorption analysis. In certain embodiments, Form C is stable with respect to humidity.

In certain embodiments, Form C of Compound A may be characterized by its stability profile. In certain embodiments, Form C material is stable, e.g., its XRPD pattern remains substantially unchanged, upon exposure to elevated temperature, upon exposure to elevated humidity, upon exposure to one or more solvents, and/or upon compression. In certain embodiments, for example, Form C is stable following exposure to an environment of about 40° C. and about 75% RH environment for about four weeks. In certain embodiments, Form C is stable following exposure to a solvent system comprising, e.g., ethanol, water, heptane or toluene, at about 40° C. for at least about four weeks. In certain embodiments, Form C is stable following compression at about 2000 psi pressure for about one minute.

In certain embodiments, Form C of Compound A may be characterized by particle analysis. In certain embodiments, Form C is characterized as a white powder. In certain embodiments, a sample of Form C comprises particles having a plate-like morphology. In certain embodiments, a sample of Form C comprises particles with a D_{90} of less than about 12 μm.

Certain embodiments herein provide Form C of Compound A which is substantially pure. Certain embodiments herein provide Form C of Compound A which is substantially free of other solid forms comprising Compound A including, e.g., Forms A, B, D, E, F, G and/or an amorphous solid form comprising Compound A as provided herein. Certain embodiments herein provide Form C as a mixture of solid forms comprising Compound A, including, e.g., a mixture comprising one or more of the following: Forms A, B, D, E, F, G and an amorphous solid form comprising Compound A as provided herein.

4.1.4. Form D of Compound A

Certain embodiments herein provide the Form D crystal form of Compound A. In certain embodiments, Form D of Compound A can be obtained from various solvents, including, but not limited to, solvent systems comprising methylene chloride. For example, in certain embodiments, Form D can be obtained by crystallization from a solvent system comprising methylene chloride, e.g., by a process comprising the evaporation of methylene chloride, followed by isolation of Form D.

In certain embodiments, Form D of Compound A may be characterized by X-ray powder diffraction analysis. A representative XRPD pattern of Form D of Compound A is provided in FIG. **13**. In certain embodiments, Form D of Compound A is characterized by XRPD peaks located at one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve of the following approximate positions: 7.5, 9.6, 11.3, 13.9, 16.3, 17.7, 20.5, 23.2, 24.6, 25.2, 26.0, 28.8 degrees 2θ. In certain embodiments, Form D of Compound A is characterized by an XRPD pattern which matches the pattern exhibited in FIG. **13**. In certain embodiments, Form D of Compound A is characterized by an XRPD pattern having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 peaks matching peaks in the representative Form D pattern provided herein.

In certain embodiments, Form D of Compound A may be characterized by thermal analysis. A representative DSC plot for Form D of Compound A is shown in FIG. **14**. In certain

embodiments, Form D is characterized by a DSC plot comprising an endothermic event with an onset temperature of about 100° C. A representative TGA plot for Form D of Compound A is shown in FIG. **15**. In certain embodiments, Form D is characterized by a TGA plot comprising a mass loss of less than about 10%, e.g., about 6.5%, of the total mass of the sample upon heating from about 25° C. to about 110° C. In certain embodiments, the TGA mass loss event comprises the loss of the solvent methylene chloride (i.e. dichloromethane), as indicated, e.g., by TG-IR analysis. In certain embodiments, Form D of Compound A is solvated. In certain embodiments, Form D is a methylene chloride solvate. In certain embodiments, the crystal lattice of Form D comprises about 2.5 molar equivalents of methylene chloride per mole of Compound A.

In certain embodiments, Form D of Compound A may be characterized by moisture sorption analysis. A representative moisture sorption isotherm plot is shown in FIG. **16**. In certain embodiments, when the RH is increased from about 0% to about 95% RH, Form D exhibits a mass change of less than about 3%, e.g., about 1.5%, of the starting mass of the sample. In certain embodiments, mass gained upon adsorption is lost when the RH is decreased back to about 0% RH. Accordingly, in certain embodiments, Form D is slightly hygroscopic. In certain embodiments, the XRPD pattern of Form D material is substantially unchanged following the adsorption/desorption analysis. In certain embodiments, Form D is stable with respect to humidity.

In certain embodiments, Form D of Compound A may be characterized by its stability profile. In certain embodiments, Form D material is stable, e.g., its XRPD pattern remains substantially unchanged, upon compression. For example, in certain embodiments, Form D is stable following compression at about 2000 psi pressure for about one minute. In certain embodiments, Form D is stable following exposure to an environment of about 40° C. and about 75% RH environment for about four weeks, although, in certain embodiments, the resulting peak intensity of the Form D XRPD pattern is reduced. In certain embodiments, this reduction in XRPD peak intensity results from the formation of amorphous material comprising Compound A. In certain embodiments, Form D converts to Form B of Compound A upon exposure to a solvent system comprising, e.g., heptane, ethanol and/or water at about 40° C. for about four weeks. In certain embodiments, Form D converts to Form C of Compound A upon exposure to a solvent system comprising toluene at about 40° C. for about four weeks.

In certain embodiments, Form D of Compound A may be characterized by particle analysis. In certain embodiments, Form D is characterized as a white powder. In certain embodiments, a sample of Form D comprises particles having a flake-like morphology. In certain embodiments, a sample of Form D comprises particles with a D_{90} of less than about 18 μm.

Certain embodiments herein provide Form D of Compound A which is substantially pure. Certain embodiments herein provide Form D of Compound A which is substantially free of other solid forms comprising Compound A including, e.g., Forms A, B, C, E, F, G and/or an amorphous solid form comprising Compound A as provided herein. Certain embodiments herein provide Form D as a mixture of solid forms comprising Compound A, including, e.g., a mixture comprising one or more of the following: Forms A, B, C, E, F, G and an amorphous solid form comprising Compound A as provided herein.

4.1.5. Form E of Compound A

Certain embodiments herein provide the Form E crystal form of Compound A. In certain embodiments, Form E of Compound A can be obtained from various solvents, including, but not limited to, solvent systems comprising acetone, acetonitrile, heptane, methylene chloride, and mixtures comprising two or more thereof. For example, in certain embodiments, Form E can be obtained by crystallization from a solvent system comprising acetonitrile, e.g., by a process comprising the evaporation of acetonitrile, followed by isolation of Form E.

In certain embodiments, Form E of Compound A may be characterized by X-ray powder diffraction analysis. A representative XRPD pattern of Form E of Compound A is provided in FIG. **17**. In certain embodiments, Form E of Compound A is characterized by XRPD peaks located at one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve of the following approximate positions: 7.6, 9.2, 11.4, 15.5, 16.5, 17.9, 19.6, 20.5, 21.6, 22.8, 23.8, 26.6 degrees 2θ. In certain embodiments, Form E of Compound A is characterized by an XRPD pattern which matches the pattern exhibited in FIG. **17**. In certain embodiments, Form E of Compound A is characterized by an XRPD pattern having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 peaks matching peaks in the representative Form E pattern provided herein.

In certain embodiments, Form E of Compound A may be characterized by thermal analysis. A representative DSC plot for Form E of Compound A is shown in FIG. **18**. In certain embodiments, Form E is characterized by a DSC plot comprising an endothermic event with an onset temperature of about 95° C. A representative TGA plot for Form E of Compound A is shown in FIG. **19**. In certain embodiments, Form E is characterized by a TGA plot comprising a mass loss of less than about 8%, e.g., about 4.0%, of the total mass of the sample upon heating from about 25° C. to about 110° C. In certain embodiments, the TGA mass loss event comprises the loss of the solvent acetonitrile, as indicated, e.g., by TG-IR analysis. In certain embodiments, Form E of Compound A is solvated. In certain embodiments, Form E is an acetonitrile solvate. In certain embodiments, the crystal lattice of Form E comprises about 2.5 molar equivalents of acetonitrile per mole of Compound A.

In certain embodiments, Form E of Compound A may be characterized by moisture sorption analysis. A representative moisture sorption isotherm plot is shown in FIG. **20**. In certain embodiments, when the RH is increased from about 0% to about 95% RH, Form E exhibits a mass change of less than about 10%, e.g., about 5.1%, of the starting mass of the sample. In certain embodiments, mass gained upon adsorption is lost when the RH is decreased back to about 0% RH. In certain embodiments, Form E is hygroscopic. In certain embodiments, the XRPD pattern of Form E material is substantially unchanged following the adsorption/desorption analysis. In certain embodiments, Form E is stable with respect to humidity.

In certain embodiments, Form E of Compound A may be characterized by its stability profile. In certain embodiments, Form E material is stable, e.g., its XRPD pattern remains substantially unchanged, upon compression. For example, in certain embodiments, Form E is stable following compression at about 2000 psi pressure for about one minute.

In certain embodiments, Form E of Compound A may be characterized by particle analysis. In certain embodiments, Form E is characterized as a white powder. In certain embodiments, a sample of Form E comprises particles having a

flake-like morphology. In certain embodiments, a sample of Form E comprises particles with a D_{90} of less than about 18 μm.

Certain embodiments herein provide Form E of Compound A which is substantially pure. Certain embodiments herein provide Form E of Compound A which is substantially free of other solid forms comprising Compound A including, e.g., Forms A, B, C, D, F, G and/or an amorphous solid form comprising Compound A as provided herein. Certain embodiments herein provide Form E as a mixture of solid forms comprising Compound A, including, e.g., a mixture comprising one or more of the following: Forms A, B, C, D, F, G and an amorphous solid form comprising Compound A as provided herein.

4.1.6. Form F of Compound A

Certain embodiments herein provide the Form F crystal form of Compound A. In certain embodiments, Form F of Compound A can be obtained from various solvents, including, but not limited to, solvent systems comprising acetone, ethanol, water, and mixtures comprising two or more thereof. For example, in certain embodiments, Form F can be obtained by crystallization from a solvent system comprising ethanol and/or water, e.g., by a process comprising contacting a solid form comprising Compound A with a solvent system comprising ethanol and/or water, followed by isolation of Form F.

In certain embodiments, Form F of Compound A may be characterized by X-ray powder diffraction analysis. A representative XRPD pattern of Form F of Compound A is provided in FIG. **21**. In certain embodiments, Form F of Compound A is characterized by XRPD peaks located at one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve of the following approximate positions: 8.1, 8.6, 15.6, 17.3, 19.3, 21.4, 22.8, 24.6, 25.4, 25.9, 26.6, 27.7 degrees 2θ. In certain embodiments, Form F of Compound A is characterized by an XRPD pattern which matches the pattern exhibited in FIG. **21**. In certain embodiments, Form F of Compound A is characterized by an XRPD pattern having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 peaks matching peaks in the representative Form F pattern provided herein.

In certain embodiments, Form F of Compound A may be characterized by thermal analysis. A representative DSC plot for Form F of Compound A is shown in FIG. **22**. In certain embodiments, Form F is characterized by a DSC plot comprising an endothermic event with an onset temperature of about 145° C. A representative TGA plot for Form F of Compound A is shown in FIG. **23**. In certain embodiments, Form F is characterized by a TGA plot comprising a mass loss of less than about 1%, e.g., about 0.1%, of the total mass of the sample upon heating from about 25° C. to about 180° C. In certain embodiments, Form F of Compound A does not contain substantial amounts of either water or other solvent in the crystal lattice. In certain embodiments, Form F is unsolvated. In certain embodiments, Form F is anhydrous.

In certain embodiments, Form F of Compound A may be characterized by moisture sorption analysis. A representative moisture sorption isotherm plot is shown in FIG. **24**. In certain embodiments, when the RH is increased from about 0% to about 95% RH, Form F exhibits a mass change of less than about 1%, e.g., about 0.2%, of the starting mass of the sample. In certain embodiments, mass gained upon adsorption is lost when the RH is decreased back to about 0% RH. In certain embodiments, Form F is substantially nonhygroscopic. In certain embodiments, the XRPD pattern of Form F material is substantially unchanged following the adsorption/desorption analysis. In certain embodiments, Form F is stable with respect to humidity.

In certain embodiments, Form F of Compound A may be characterized by its stability profile. In certain embodiments, Form F material is stable, e.g., its XRPD pattern remains substantially unchanged, upon compression. For example, in certain embodiments, Form F is stable following compression at about 2000 psi pressure for about one minute. In certain embodiments, Form F is stable following exposure to a solvent system comprising, e.g., ethanol, acetone or mixtures thereof, for about two days at about 25° C.

In certain embodiments, Form F of Compound A may be characterized by particle analysis. In certain embodiments, Form F is characterized as a white powder. In certain embodiments, a sample of Form F comprises particles having a flake-like morphology. In certain embodiments, a sample of Form F comprises particles with a D_{90} of less than about 18 μm.

Certain embodiments herein provide Form F of Compound A which is substantially pure. Certain embodiments herein provide Form F of Compound A which is substantially free of other solid forms comprising Compound A including, e.g., Forms A, B, C, D, E, G and/or an amorphous solid form comprising Compound A as provided herein. Certain embodiments herein provide Form F as a mixture of solid forms comprising Compound A, including, e.g., a mixture comprising one or more of the following: Forms A, B, C, D, E, G and an amorphous solid form comprising Compound A as provided herein.

4.1.7. Form G of Compound A

Certain embodiments herein provide the Form G crystal form of Compound A. In certain embodiments, Form G of Compound A can be obtained from various solvents, including, but not limited to, solvent systems comprising ethyl acetate. For example, in certain embodiments, Form G can be obtained by crystallization from a solvent system comprising ethyl acetate, e.g., by a process comprising contacting a solid form comprising Compound A with a solvent system comprising ethyl acetate, followed by isolation of Form G.

In certain embodiments, Form G of Compound A may be characterized by X-ray powder diffraction analysis. A representative XRPD pattern of Form G of Compound A is provided in FIG. **25**. In certain embodiments, Form G of Compound A is characterized by XRPD peaks located at one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve of the following approximate positions: 7.9, 9.5, 11.7, 15.7, 16.8, 18.1, 19.7, 21.8, 22.8, 25.1, 25.8, 26.7 degrees 2θ. In certain embodiments, Form G of Compound A is characterized by an XRPD pattern which matches the pattern exhibited in FIG. **25**. In certain embodiments, Form G of Compound A is characterized by an XRPD pattern having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 peaks matching peaks in the representative Form G pattern provided herein.

In certain embodiments, Form G of Compound A may be characterized by thermal analysis. A representative DSC plot for Form G of Compound A is shown in FIG. **26**. In certain embodiments, Form G is characterized by a DSC plot comprising an endothermic event with an onset temperature of about 109° C. A representative TGA plot for Form G of Compound A is shown in FIG. **27**. In certain embodiments, Form G is characterized by a TGA plot comprising a mass loss of less than about 8%, e.g., about 3.8%, of the total mass of the sample upon heating from about 25° C. to about 110° C. In certain embodiments, the TGA mass loss event comprises the loss of the solvent ethyl acetate, as indicated, e.g., by TG-IR analysis. In certain embodiments, Form G of Compound A is solvated. In certain embodiments, Form G is an ethyl acetate solvate. In certain embodiments, the crystal

lattice of Form G comprises about three molar equivalents of ethyl acetate per mole of Compound A.

In certain embodiments, Form G of Compound A may be characterized by moisture sorption analysis. A representative moisture sorption isotherm plot is shown in FIG. **28**. In certain embodiments, when the RH is increased from about 0% to about 95% RH, Form G exhibits a mass change of less than about 1%, e.g., about 0.4%, of the starting mass of the sample. In certain embodiments, mass gained upon adsorption is lost when the RH is decreased back to about 0% RH. In certain embodiments, Form G is substantially nonhygroscopic. In certain embodiments, the XRPD pattern of Form G material is substantially unchanged following the adsorption/desorption analysis. In certain embodiments, Form G is stable with respect to humidity.

In certain embodiments, Form G of Compound A may be characterized by its stability profile. In certain embodiments, Form G material is stable, e.g., its XRPD pattern remains substantially unchanged, upon compression. For example, in certain embodiments, Form F is stable following compression at about 2000 psi pressure for about one minute. In certain embodiments, Form G converts to Form B upon exposure to a solvent system comprising, e.g., ethanol, acetone or mixtures thereof, for about two days at about 25° C.

In certain embodiments, Form G of Compound A may be characterized by particle analysis. In certain embodiments, Form G is characterized as a white powder. In certain embodiments, a sample of Form G comprises particles having a flake-like morphology. In certain embodiments, a sample of Form G comprises particles with a D_{90} of less than about 18 μm.

Certain embodiments herein provide Form G of Compound A which is substantially pure. Certain embodiments herein provide Form G of Compound A which is substantially free of other solid forms comprising Compound A including, e.g., Forms A, B, C, D, E, F and/or an amorphous solid form comprising Compound A as provided herein. Certain embodiments herein provide Form G as a mixture of solid forms comprising Compound A, including, e.g., a mixture comprising one or more of the following: Forms A, B, C, D, E, F and an amorphous solid form comprising Compound A as provided herein.

4.2. Methods of Treatment

The invention encompasses methods of treating, preventing and managing diseases or disorders ameliorated by the reduction of levels of TNF-α in a patient which comprise administering to a patient in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of one or more solid forms comprising Compound A, such as, e.g., Form A of Compound A, Form B of Compound A, Form C of Compound A, Form D of Compound A, Form E of Compound A, Form F of Compound A, Form G of Compound A, or an amorphous form of Compound A, as provided herein.

Disorders ameliorated by the inhibition of TNF-α include, but are not limited to: heart disease, such as congestive heart failure, cardiomyopathy, pulmonary edema, endotoxin-mediated septic shock, acute viral myocarditis, cardiac allograft rejection, and myocardial infarction; solid tumors, including but not limited to, sarcoma, carcinomas, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, Kaposi's sarcoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma; and blood-borne tumors including but not limited to, acute lymphoblastic leukemia "ALL", acute lymphoblastic B-cell leukemia, acute lymphoblastic T-cell leukemia, acute myeloblastic leukemia "AML", acute promyelocytic leukemia "APL", acute monoblastic leukemia, acute erythroleukemic leukemia, acute megakaryoblastic leukemia, acute myelomonocytic leukemia, acute nonlymphocyctic leukemia, acute undifferentiated leukemia, chronic myelocytic leukemia "CML", chronic lymphocytic leukemia "CLL", hairy cell leukemia, multiple myeloma and acute and chronic leukemias, for example, lymphoblastic, myelogenous, lymphocytic, and myelocytic leukemias.

Specific methods of the invention further comprise the administration of an additional therapeutic agent (i.e., a therapeutic agent other than Compound A). Examples of additional therapeutic agents include, but are not limited to, anticancer drugs such as, but are not limited to: alkylating agents, nitrogen mustards, ethylenimines, methylmelamines, alkyl sulfonates, nitrosoureas, triazenes, folic acid analogs, pyrimidine analogs, purine analogs, vinca alkaloids, epipodophyllotoxins, antibiotics, topoisomerase inhibitors and anticancer vaccines.

Specific additional therapeutic agents include, but are not limited to: acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cisplatin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; dactinomycin; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; docetaxel; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; fluorocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; ilmofosine; interleukin II (including recombinant interleukin II, or rIL2), interferon alfa-2a; interferon alfa-2b; interferon alfa-n1; interferon alfa-n3; interferon beta-I a; interferon gamma-I b; iproplatin; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozolc hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; meto-

prine; meturedepa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; paclitaxel; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; rogletimide; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; triciribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin hydrochloride. Other anti-cancer drugs include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; antidorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-aminotriazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl spiromustine; docetaxel; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal

peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazaminc; titanocene bichloride; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

Embodiments herein further encompass a method of treating or preventing diseases or disorders ameliorated by the inhibition of PDE4 in a patient which comprise administering to a patient in need of such treatment or prevention one or more solid forms comprising Compound A. Disorders ameliorated by the inhibition of PDE4 include, but are not limited to, asthma, inflammation, chronic or acute obstructive pulmonary disease, chronic or acute pulmonary inflammatory disease, inflammatory bowel disease, Crohn's Disease, Behcet's Disease, colitis, ulcerative colitis and arthritis or inflammation due to reperfusion. In a preferred embodiment, the disease or disorder to be treated or prevented is chronic obstructive pulmonary disease.

Specific methods of the invention can comprise the administration of an additional therapeutic agent such as, but not limited to, anti-inflammatory drugs, antihistamines and decongestants. Examples of such additional therapeutic agents include, but are not limited to: antihistamines including, but not limited to, ethanolamines, ethylenediamines, piperazines, and phenothiazines; antinflammatory drugs; NSAIDS, including, but not limited to, aspirin, salicylates, acetominophen, indomethacin, sulindac, etodolac, fenamates, tolmetin, ketorolac, diclofenac, ibuprofen, naproxen, fenoprofen, ketoprofen, flurbiprofen, oxaprozin, piroxicam, meloxicam, pyrazolon derivatives; and steroids including, but not limited to, cortical steroids and adrenocortical steroids.

Specific methods of the invention avoid or reduce drug-drug interactions and other adverse effects associated with agents used in the treatment of such disorders, including racemic substituted phenylethylsulfones. Without being limited by any theory, certain solid forms comprising Compound A may further provide an overall improved therapeutic effectiveness, or therapeutic index, over racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, including solid forms thereof.

As stated above, certain solid forms comprising Compound A may be used in the treatment or prevention of a wide range of diseases and conditions. The magnitude of a prophylactic or therapeutic dose of a particular active ingredient of the invention in the acute or chronic management of a disease or condition may vary with the nature and severity of the disease or condition and the route by which the active ingredient is administered. The dose, and perhaps the dose frequency, will also vary according to the age, body weight, and response of the individual patient. Suitable dosing regimens can be readily selected by those skilled in the art with due consideration of such factors. In general, the recommended daily dose range for the conditions described herein lie within the range of from about 1 mg to about 1,000 mg per day, given as a single once-a-day dose preferably as divided doses throughout a day. More specifically, the daily dose is administered twice daily in equally divided doses. Specifically, a daily dose range may be from about 5 mg to about 500 mg per day, more specifically, between about 10 mg and about 200 mg per day. Specifically, the daily dose may be administered in 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 50 mg, or 100 mg dosage forms. In managing the patient, the therapy should be initiated at a lower dose, perhaps about 1 mg to about 25 mg, and increased if necessary up to about 200 mg to about 1,000 mg per day as either a single dose or divided doses, depending on the patient's global response. Alternatively, the daily dose is from 0.01 mg/kg to 100 mg/kg.

It may be necessary to use dosages of the active ingredient outside the ranges disclosed herein in some cases, as will be apparent to those of ordinary skill in the art. Furthermore, it is noted that the clinician or treating physician will know how and when to interrupt, adjust, or terminate therapy in conjunction with individual patient response.

The phrases "therapeutically effective amount", "prophylactically effective amount" and "therapeutically or prophylactically effective amount," as used herein encompass the above described dosage amounts and dose frequency schedules. Different therapeutically effective amounts may be applicable for different diseases and conditions, as will be readily known by those of ordinary skill in the art. Similarly, amounts sufficient to treat or prevent such disorders, but insufficient to cause, or sufficient to reduce, adverse effects associated with racemic 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione are also encompassed by the above described dosage amounts and dose frequency schedules.

4.3. Pharmaceutical Compositions

Pharmaceutical compositions and single unit dosage forms comprising one or more solid forms comprising Compound A are provided herein. Also provided herein are methods for preparing pharmaceutical compositions and single unit dosage forms comprising one or more solid forms comprising Compound A. For example, in certain embodiments, individual dosage forms comprising a solid form provided herein or prepared using solid form provided herein may be suitable for oral, mucosal (including rectal, nasal, or vaginal), parenteral (including subcutaneous, intramuscular, bolus injection, intraarterial, or intravenous), sublingual, transdermal, buccal, or topical administration.

In certain embodiments, pharmaceutical compositions and dosage forms provided herein comprise one or more solid forms comprising Compound A. Certain embodiments herein provide pharmaceutical compositions and dosage forms comprising a solid form comprising Compound A, such as, e.g., Forms A, B, C, D, E, F, G or an amorphous solid form comprising Compound A as provided herein, wherein the solid form comprising Compound A substantially pure. Certain embodiments herein provide pharmaceutical compositions and dosage forms comprising a solid form comprising Compound A, such as, e.g., Forms A, B, C, D, E, F, G or an amorphous solid form comprising Compound A as provided herein, which is substantially free of other solid forms comprising Compound A including, e.g., Forms A, B, C, D, E, F, G and/or an amorphous solid form comprising Compound A as provided herein. Certain embodiments herein provide pharmaceutical compositions and dosage forms comprising a mixture of solid forms comprising Compound A, including, e.g., a mixture comprising one or more of the following: Forms A, B, C, D, E, F and an amorphous solid form comprising Compound A as provided herein. Pharmaceutical

compositions and dosage forms provided herein typically also comprise one or more pharmaceutically acceptable excipient, diluent or carrier.

A particular pharmaceutical composition encompassed by this embodiment comprises one or more solid forms comprising Compound A and at least one additional therapeutic agent. Examples of additional therapeutic agents include, but are not limited to: anti-cancer drugs and anti-inflammation therapies including, but not limited to, those provided herein.

Single unit dosage forms of the invention are suitable for oral, mucosal (e.g., nasal, sublingual, vaginal, buccal, or rectal), parenteral (e.g., subcutaneous, intravenous, bolus injection, intramuscular, or intraarterial), or transdermal administration to a patient. Examples of dosage forms include, but are not limited to: tablets; caplets; capsules, such as soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suppositories; ointments; cataplasms (poultices); pastes; powders; dressings; creams; plasters; solutions; patches; aerosols (e.g., nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a patient, including suspensions (e.g., aqueous or non-aqueous liquid suspensions, oil-in-water emulsions, or a water-in-oil liquid emulsions), solutions, and elixirs; liquid dosage forms suitable for parenteral administration to a patient; and sterile solids (e.g., crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms suitable for parenteral administration to a patient.

The composition, shape, and type of dosage forms of the invention will typically vary depending on their use. For example, a dosage form used in the acute treatment of inflammation or a related disorder may contain larger amounts of one or more of the active ingredients it comprises than a dosage form used in the chronic treatment of the same disease. Similarly, a parenteral dosage form may contain smaller amounts of one or more of the active ingredients it comprises than an oral dosage form used to treat the same disease or disorder. These and other ways in which specific dosage forms encompassed by this invention will vary from one another will be readily apparent to those skilled in the art. See, e.g., *Remington's Pharmaceutical Sciences,* 18th ed., Mack Publishing, Easton Pa. (1990).

Typical pharmaceutical compositions and dosage forms comprise one or more excipients. Suitable excipients are well known to those skilled in the art of pharmacy, and non-limiting examples of suitable excipients are provided herein. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a patient. For example, oral dosage forms such as tablets may contain excipients not suited for use in parenteral dosage forms. The suitability of a particular excipient may also depend on the specific active ingredients in the dosage form.

Lactose-free compositions of the invention can comprise excipients that are well known in the art and are listed, for example, in the U.S. Pharmocopia (USP) SP (XXI)/NF (XVI). In general, lactose-free compositions comprise an active ingredient, a binder/filler, and a lubricant in pharmaceutically compatible and pharmaceutically acceptable amounts. Preferred lactose-free dosage forms comprise an active ingredient, microcrystalline cellulose, pre-gelatinized starch, and magnesium stearate.

This invention further encompasses anhydrous pharmaceutical compositions and dosage forms comprising active ingredients, since water can facilitate the degradation of some compounds. For example, the addition of water (e.g., 5%) is widely accepted in the pharmaceutical arts as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. See, e.g., Jens T. Carstensen, *Drug Stability: Principles & Practice,* 2d. Ed., Marcel Dekker, NY, N.Y., 1995, pp. 379-80. In effect, water and heat accelerate the decomposition of some compounds. Thus, the effect of water on a formulation can be of great significance since moisture and/or humidity are commonly encountered during manufacture, handling, packaging, storage, shipment, and use of formulations.

Anhydrous pharmaceutical compositions and dosage forms of the invention can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms that comprise lactose and at least one active ingredient that comprises a primary or secondary amine are preferably anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected.

An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions are preferably packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples of suitable packaging include, but are not limited to, hermetically sealed foils, plastics, unit dose containers (e.g., vials), blister packs, and strip packs.

The invention further encompasses pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will decompose. Such compounds, which are referred to herein as "stabilizers," include, but are not limited to, antioxidants such as ascorbic acid, pH buffers, or salt buffers.

Like the amounts and types of excipients, the amounts and specific types of active ingredients in a dosage form may differ depending on factors such as, but not limited to, the route by which it is to be administered to patients. However, typical dosage forms provided herein lie within the range of from about 1 mg to about 1,000 mg per day, given as a single once-a-day dose in the morning but preferably as divided doses throughout the day. More specifically, the daily dose is administered twice daily in equally divided doses. Specifically, a daily dose range may be from about 5 mg to about 500 mg per day, more specifically, between about 10 mg and about 200 mg per day. In managing the patient, the therapy may be initiated at a lower dose, perhaps about 1 mg to about 25 mg, and increased if necessary up to about 200 mg to about 1,000 mg per day as either a single dose or divided doses, depending on the patient's global response.

4.3.1. Oral Dosage Forms

Pharmaceutical compositions of the invention that are suitable for oral administration can be presented as discrete dosage forms, such as, but are not limited to, tablets (e.g., chewable tablets), caplets, capsules, and liquids (e.g., flavored syrups). Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of pharmacy well known to those skilled in the art. See generally *Remington's Pharmaceutical Sciences,* 18th ed., Mack Publishing, Easton Pa. (1990).

Typical oral dosage forms of the invention are prepared by combining the active ingredient(s) in an intimate admixture with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of preparation desired for administration. For example, excipients suitable for use in oral liquid or aerosol dosage forms include, but are not limited

to, water, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents. Examples of excipients suitable for use in solid oral dosage forms (e.g., powders, tablets, capsules, and caplets) include, but are not limited to, starches, sugars, micro-crystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid excipients are employed. If desired, tablets can be coated by standard aqueous or nonaqueous techniques. Such dosage forms can be prepared by any of the methods of pharmacy. In general, pharmaceutical compositions and dosage forms are prepared by uniformly and intimately admixing the active ingredients with liquid carriers, finely divided solid carriers, or both, and then shaping the product into the desired presentation if necessary.

For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as powder or granules, optionally mixed with an excipient. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

Examples of excipients that can be used in oral dosage forms of the invention include, but are not limited to, binders, fillers, disintegrants, and lubricants. Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gelatin, natural and synthetic gums such as acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre-gelatinized starch, hydroxypropyl methyl cellulose, (e.g., Nos. 2208, 2906, 2910), microcrystalline cellulose, and mixtures thereof.

Examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pregelatinized starch, and mixtures thereof. The binder or filler in pharmaceutical compositions of the invention is typically present in from about 50 to about 99 weight percent of the pharmaceutical composition or dosage form.

Suitable forms of microcrystalline cellulose include, but are not limited to, the materials sold as AVICEL-PH-101™, AVICEL-PH-103™, AVICEL RC-581™, AVICEL-PH-105™ (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, Pa.), and mixtures thereof. A specific binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC-581™. Suitable anhydrous or low moisture excipients or additives include AVICEL-PH-103™ and Starch 1500 LM™.

Disintegrants are used in the compositions of the invention to provide tablets that disintegrate when exposed to an aqueous environment. Tablets that contain too much disintegrant may disintegrate in storage, while those that contain too little may not disintegrate at a desired rate or under the desired conditions. Thus, a sufficient amount of disintegrant that is neither too much nor too little to detrimentally alter the release of the active ingredients should be used to form solid oral dosage forms of the invention. The amount of disintegrant used varies based upon the type of formulation, and is readily discernible to those of ordinary skill in the art. Typical pharmaceutical compositions comprise from about 0.5 to about 15 weight percent of disintegrant, specifically from about 1 to about 5 weight percent of disintegrant.

Disintegrants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, agar-agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrilin potassium, sodium starch glycolate, potato or tapioca starch, pre-gelatinized starch, other starches, clays, other algins, other celluloses, gums, and mixtures thereof.

Lubricants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, and mixtures thereof. Additional lubricants include, for example, a syloid silica gel (AEROSIL 200™, manufactured by W.R. Grace Co. of Baltimore, Md.), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Plano, Tex.), CAB-O-SIL™ (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, Mass.), and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about one weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated.

4.3.2. Delayed Release Dosage Forms

Solid forms comprising Compound A as provided herein can be administered by controlled release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Pat. Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; and 4,008,719, 5,674,533, 5,059,595, 5,591,767, 5,120,548, 5,073,543, 5,639,476, 5,354,556, and 5,733,566, each of which is incorporated herein by reference. Such dosage forms can be used to provide slow or controlled-release of one or more active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the active ingredients of the invention. The invention thus encompasses single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled-release.

All controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition, controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the drug, and can thus affect the occurrence of side (e.g., adverse) effects.

Most controlled-release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant

level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled-release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, temperature, enzymes, water, or other physiological conditions or compounds.

4.3.3. Parenteral Dosage Forms

Parenteral dosage forms can be administered to patients by various routes including, but not limited to, subcutaneous, intravenous (including bolus injection), intramuscular, and intraarterial. Because their administration typically bypasses patients' natural defenses against contaminants, parenteral dosage forms are preferably sterile or capable of being sterilized prior to administration to a patient. Examples of parenteral dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions.

Suitable vehicles that can be used to provide parenteral dosage forms of the invention are well known to those skilled in the art. Examples include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

Compounds that increase the solubility of one or more of the active ingredients disclosed herein can also be incorporated into the parenteral dosage forms of the invention.

4.3.4. Transdermal, Topical, and Mucosal Dosage Forms

Transdermal, topical, and mucosal dosage forms of the invention include, but are not limited to, ophthalmic solutions, sprays, aerosols, creams, lotions, ointments, gels, solutions, emulsions, suspensions, or other forms known to one of skill in the art. See, e.g., *Remington's Pharmaceutical Sciences,* 16th and 18th eds., Mack Publishing, Easton Pa. (1980 & 1990); and *Introduction to Pharmaceutical Dosage Forms,* 4th ed., Lea & Febiger, Philadelphia (1985). Dosage forms suitable for treating mucosal tissues within the oral cavity can be formulated as mouthwashes or as oral gels. Further, transdermal dosage forms include "reservoir type" or "matrix type" patches, which can be applied to the skin and worn for a specific period of time to permit the penetration of a desired amount of active ingredients.

Suitable excipients (e.g., carriers and diluents) and other materials that can be used to provide transdermal, topical, and mucosal dosage forms encompassed by this invention are well known to those skilled in the pharmaceutical arts, and depend on the particular tissue to which a given pharmaceutical composition or dosage form will be applied. With that fact in mind, typical excipients include, but are not limited to, water, acetone, ethanol, ethylene glycol, propylene glycol, butane-1,3-diol, isopropyl myristate, isopropyl palmitate, mineral oil, and mixtures thereof to form lotions, tinctures, creams, emulsions, gels or ointments, which are non-toxic and pharmaceutically acceptable. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well known in the art. See, e.g., *Remington's Pharmaceutical Sciences,* 16th and 18th eds., Mack Publishing, Easton Pa. (1980 & 1990).

Depending on the specific tissue to be treated, additional components may be used prior to, in conjunction with, or subsequent to treatment with active ingredients of the invention. For example, penetration enhancers can be used to assist in delivering the active ingredients to the tissue. Suitable penetration enhancers include, but are not limited to: acetone; various alcohols such as ethanol, olcyl, and tetrahydrofuryl; alkyl sulfoxides such as dimethyl sulfoxide; dimethyl acetamide; dimethyl formamide; polyethylene glycol; pyrrolidones such as polyvinylpyrrolidone; Kollidon grades (Povidone, Polyvidone); urea; and various water-soluble or insoluble sugar esters such as Tween 80™ (polysorbate 80) and Span 60™ (sorbitan monostearate).

The pH of a pharmaceutical composition or dosage form, or of the tissue to which the pharmaceutical composition or dosage form is applied, may also be adjusted to improve delivery of one or more active ingredients. Similarly, the polarity of a solvent carrier, its ionic strength, or tonicity can be adjusted to improve delivery. Compounds such as stearates can also be added to pharmaceutical compositions or dosage forms to advantageously alter the hydrophilicity or lipophilicity of one or more active ingredients so as to improve delivery. In this regard, stearates can serve as a lipid vehicle for the formulation, as an emulsifying agent or surfactant, and as a delivery-enhancing or penetration-enhancing agent. Different solid forms comprising the active ingredients can be used to further adjust the properties of the resulting composition.

4.3.5. Kits

This invention encompasses kits which, when used by the medical practitioner, can simplify the administration of appropriate amounts of active ingredients to a patient.

A typical kit of the invention comprises a unit dosage form of compound A, or a pharmaceutically acceptable solid form or prodrug thereof, and a unit dosage form of a second active ingredient. Examples of second active ingredients include, but are not limited to, those listed herein.

Kits of the invention can further comprise devices that are used to administer the active ingredient(s). Examples of such devices include, but are not limited to, syringes, drip bags, patches, and inhalers.

Kits of the invention can further comprise pharmaceutically acceptable vehicles that can be used to administer one or more active ingredients. For example, if an active ingredient is provided in a solid form that must be reconstituted for parenteral administration, the kit can comprise a sealed container of a suitable vehicle in which the active ingredient can be dissolved to form a particulate-free sterile solution that is suitable for parenteral administration. Examples of pharmaceutically acceptable vehicles include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

5. EXAMPLES

The present application incorporates by reference the entirety of U.S. Pat. No. 6,962,940 (issued Nov. 8, 2005), including the Examples provided therein.

5.1. Example 1

Synthesis of 2-[1-(3-Ethoxy-4-Methoxyphenyl)-2-Methylsulfonylethyl]-4-Acetylaminoisoindoline-1,3-Dione

A stirred solution of 1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethylamine (1.0 g, 3.7 mmol) and 3-acetami-

dophthalic anhydride (751 mg, 3.66 mmol) in acetic acid (20 mL) was heated at reflux for 15 h. The solvent was removed in vacuo to yield an oil. Chromatography of the resulting oil yielded the product as a yellow solid (1.0 g, 59% yield): mp, 144° C.; ^{1}H NMR ($CDCl_3$) δ: 1.47 (t, J=7.0 Hz, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.88 (s, 3H, CH_3), 3.75 (dd, J=4.4, 14.3 Hz, 1H, CH), 3.85 (s, 3H, CH3), 4.11 (q, J=7 Hz, 2H, CH_2), 5.87 (dd, J=4.3, 10.5 Hz, 1H, NCH), 6.82-6.86 (m, 1H, Ar), 7.09-7.11 (m, 2H, Ar), 7.47 (d, J=7 Hz, 1H, Ar), 7.64 (t, J=8 Hz, 1H, Ar), 8.74 (d, J=8 Hz, 1H, Ar), 9.49 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ: 14.61, 24.85, 41.54, 48.44, 54.34, 55.85, 64.43, 111.37, 112.34, 115.04, 118.11, 120.21, 124.85, 129.17, 130.96, 136.01, 137.52, 148.54, 149.65, 167.38, 169.09, 169.40; Anal Calc'd. for $C_{22}H_{24}NO_7S$: C, 57.38; H, 5.25; N, 6.08. Found: C, 57.31; H, 5.34; N, 5.83.

5.2. Example 2

Synthesis of (+)2-[1-(3-Ethoxy-4-Methoxyphenyl)-2-Methylsulfonylethyl]-4-Acetylaminoisoindoline-1,3-Dione

Preparation of 3-aminopthalic acid

10% Pd/C (2.5 g), 3-nitrophthalic acid (75.0 g, 355 mmol) and ethanol (1.5 L) were charged to a 2.5 L Parr hydrogenator under a nitrogen atmosphere. Hydrogen was charged to the reaction vessel for up to 55 psi. The mixture was shaken for 13 hours, maintaining hydrogen pressure between 50 and 55 psi. Hydrogen was released and the mixture was purged with nitrogen 3 times. The suspension was filtered through a celite bed and rinsed with methanol. The filtrate was concentrated in vacuo. The resulting solid was reslurried in ether and isolated by vacuum filtration. The solid was dried in vacuo to a constant weight, affording 54 g (84% yield) of 3-aminopthalic acid as a yellow product. ^{1}H-NMR (DMSO-d6) δ: 3.17 (s, 2H), 6.67 (d, 1H), 6.82 (d, 1H), 7.17 (t, 1H), 8-10 (br, s, 2H); ^{13}C-NMR (DMSO-d6) δ: 112.00, 115.32, 118.20, 131.28, 135.86, 148.82, 169.15, 170.09.

Preparation of 3-acetamidophthalic anhydride

A 1 L 3-necked round bottom flask was equipped with a mechanical stirrer, thermometer, and condenser and charged with 3-aminophthalic acid (108 g, 596 mmol) and acetic anhydride (550 mL). The reaction mixture was heated to reflux for 3 hours and cooled to about 25° C. and further to 0-5° C. for another 1 hour. The crystalline solid was collected by vacuum filtration and washed with ether. The solid product was dried in vacuo at ambient temperature to a constant weight, giving 75 g (61% yield) of 3-acetamidopthalic anhydride as a white product. ^{1}H-NMR ($CDCl_3$) δ: 2.21 (s, 3H), 7.76 (d, 1H), 7.94 (t, 1H), 8.42 (d, 1H), 9.84 (s, 1H).

Resolution of 2-(3-ethoxy-4-methoxyphenyl-1-(methylsulphonyl)-eth-2-ylamine

A 3 L 3-necked round bottom flask was equipped with a mechanical stirrer, thermometer, and condenser and charged with 2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine (137.0 g, 500 mmol), N-acetyl-L-leucine (52 g, 300 mmol), and methanol (1.0 L). The stirred slurry was heated to reflux for 1 hour. The stirred mixture was allowed to cool to ambient temperature and stirring was continued for another 3 hours at ambient temperature. The slurry was filtered and washed with methanol (250 L). The solid was air-dried and then dried in vacuo at ambient temperature to a constant weight, giving 109.5 g (98% yield) of the crude product (85.8% ee). The crude solid (55.0 g) and methanol (440 mL) were brought to reflux for 1 hour, cooled to room temperature and stirred for an additional 3 hours at ambient temperature. The slurry was filtered and the filter cake was washed with methanol (200 mL). The solid was air-dried and then dried in vacuo at 30° C. to a constant weight, yielding 49.6 g (90% recovery) of (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine-N-acetyl-L-leucine salt (98.4% ee). Chiral HPLC (1/99 EtOH/20 mM KH_2PO_4 @ pH 7.0, Ultron Chiral ES-OVS from Agilent Technologies, 150 mm×4.6 mm, 0.5 mL/min., @ 240 nm): 18.4 min (S-isomer, 99.2%), 25.5 min (R-isomer, 0.8%).

Preparation of Compound A

A 500 mL 3-necked round bottom flask was equipped with a mechanical stirrer, thermometer, and condenser. The reaction vessel was charged with (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-yl amine N-acetyl-L-leucine salt (25 g, 56 mmol, 98% ee), 3-acetamidophthalic anhydride (12.1 g, 58.8 mmol), and glacial acetic acid (250 mL). The mixture was refluxed over night and then cooled to <50° C. The solvent was removed in vacuo, and the residue was dissolved in ethyl acetate. The resulting solution was washed with water (250 mL×2), saturated aqueous $NaHCO_3$ (250 mL×2), brine (250 mL×2), and dried over sodium sulphate. The solvent was evaporated in vacuo, and the residue recrystallized from a binary solvent containing ethanol (150 mL) and acetone (75 mL). The solid was isolated by vacuum filtration and washed with ethanol (100 mL×2). The product was dried in vacuo at 60° C. to a constant weight, affording 19.4 g (75% yield) of S-{2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetamidoisoindoline-1,3-dione} with 98% ee. Chiral HPLC (15/85 EtOH/20 mM KH_2PO_4@ pH 5, Ultron Chiral ES-OVS from Agilent Technology, 150 mm×4.6 mm, 0.4 mL/min, @ 240 nm): 25.4 min (S-isomer, 98.7%), 29.5 min (R-isomer, 1.2%). ^{1}H-NMR ($CDCl_3$) δ: 1.47 (t, 3H), 2.26 (s, 3H), 2.87 (s, 3H), 3.68-3.75 (dd, 1H), 3.85 (s, 3H), 4.07-4.15 (q, 2H), 4.51-4.61 (dd, 1H), 5.84-5.90 (dd, 1H), 6.82-8.77 (m, 6H), 9.46 (s, 1H); ^{13}C-NMR (DMSO-d6) δ: 14.66, 24.92, 41.61, 48.53, 54.46, 55.91, 64.51, 111.44, 112.40, 115.10, 118.20, 120.28, 124.94, 129.22, 131.02, 136.09, 137.60, 148.62, 149.74, 167.46, 169.14, 169.48.

A reaction scheme illustrating a preparation of the (+) enantiomer of Compound A is provided as FIG. **29**.

5.3. Example 3

TNF-α Inhibition

Human Whole Blood LPS-induced TNF-α Assay

The ability of compounds to inhibit LPS-induced TNF-α production by human whole blood was measured essentially as described below for the LPS-induced TNF-α assay in human PBMC, except that freshly drawn whole blood was used instead of PBMC. (Muller et al., 1999, *Bioorg. & Med. Chem. Lett.*, 9:1625-1630.) Human whole blood LPS-induced TNF-α IC_{50}=294 nM for Compound A.

Mouse LPS-induced Serum TNF-α Inhibition

Compounds were tested in this animal model according to previously described methods (Corral et al., 1996, *Mol. Med.*, 2:506-515). Mouse LPS-induced scrum TNF-α inhibition (ED_{50}, mg/kg, p.o.)=0.05 for Compound A.

LPS-induced TNF-α Production

Lipopolysaccharide (LPS) is an endotoxin produced by gram-negative bacteria such as *E. coli* which induces production of many pro-inflammatory cytokines, including TNF-α. In peripheral blood mononuclear cells (PBMC), the TNF-α produced in response to LPS is derived from monocytes, which comprise approximately 5-20% of the total PBMC. Compounds were tested for the ability to inhibit LPS-induced TNF-α production from human PBMC as previously described (Muller et al., 1996, *J. Med. Chem.*, 39:3238).

PBMC from normal donors were obtained by Ficoll Hypaque (Pharmacia, Piscataway, N.J., USA) density centrifugation. Cells were cultured in RPMI (Life Technologies, Grand Island, N.Y., USA) supplemented with 10% AB± human serum (Gemini Bio-products, Woodland, Calif., USA), 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Life Technologies).

PBMC (2×10^5 cells) were plated in 96-well flat-bottom Costar tissue culture plates (Corning, N.Y., USA) in triplicate. Cells were stimulated with LPS (Sigma, St. Louis, Mo., USA) at 100 ng/ml in the absence or presence of compounds. Compounds (Celgene Corp., Warren, N.J., USA) were dissolved in DMSO (Sigma) and further dilutions were done in culture medium immediately before use. The final DMSO concentration in all samples was 0.25%. Compounds were added to cells one hour before LPS stimulation. Cells were incubated for 18-20 hours at 37° C. in 5% CO_2 and supernatants were then collected, diluted with culture medium and assayed for TNF-α levels by ELISA (Endogen, Boston, Mass., USA). LPS-induced TNF-α IC_{50}=77 nM for Compound A.

IL-1β-induced TNF-α Production

During the course of inflammatory diseases, TNF-α production is often stimulated by the cytokine 1L-1β, rather than by bacterially derived LPS. Compounds were tested for the ability to inhibit IL-1β-induced TNF-α production from human PBMC as described above for LPS-induced TNF-α production, except that the PBMC were isolated from source leukocyte units (Sera-Tec Biologicals, North Brunswick, N.J., USA) by centrifugation on Ficoll-Paque Plus (Amersham Pharmacia, Piscataway, N.J., USA), plated in 96-well tissue culture plates at 3×10^5 cells/well in RPMI-1640 medium (BioWhittaker, Walkersville, Md., USA) containing 10% heat-inactivated fetal bovine serum (Hyclone), 2 mM L-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin (complete medium), pretreated with compounds at 10, 2, 0.4, 0.08, 0.016, 0.0032, 0.00064, and 0 μM in duplicate at a final DMSO concentration of 0.1% at 37° C. in a humidified incubator at 5% CO_2 for one hour, then stimulated with 50 ng/ml recombinant human IL-1β (Endogen) for 18 hours. IL-β-induced TNF-α IC_{50}=83 nM for Compound A.

5.4. Example 4

PDE Selectivity

PDE1, 2, 3, 5, and 6 Enzyme Assays

The specificity of compounds for PDE4 was assessed by testing at a single concentration (10 μM) against bovine PDE1, human PDE2, PDE3, and PDE5 from human platelets (Hidaka and Asano, 1976, *Biochem. Biophys. Acta,* 429:485, and Nicholsen et al., 1991, *Trends Pharmaco. Sci.,* 12:19), and PDE6 from bovine retinal rod outer segments (Baehr et al., 1979, *J. Biol. Chem.,* 254:11669, and Gillespie et al. 1989, *Mol. Pharm.,* 36:773). Results are listed in Table 1.

PDE7 Enzyme Assay

PDE7 is a cAMP-selective PDE expressed mainly in T cells and in skeletal muscle. T cell-derived cytokines such as IL-2 and IFN-γ are potentially regulatable via PDE7 inhibition. PDE7 was purified from Hut78 human T cells by anion exchange chromatography as previously described (Bloom and Beavo, 1996, *Proc. Natl. Acad. Sci. USA,* 93:14188-14192). Compounds were tested against the PDE7 preparation in the presence of 10 nM cAMP as described for PDE4 in Table 1.

5.5. Example 5

PDE4 Inhibition

PDE4 (U937 Cell-derived) Enzyme Assay

PDE4 enzyme was purified from U937 human monocytic cells by gel filtration chromatography as previously described (Muller et al., 1998, *Bioorg. & Med. Chem. Lett.* 8:2669-2674). Phosphodiesterase reactions were carried out in 50 mM Tris HCl pH 7.5, 5 mM $MgCl_2$, 1 μM cAMP, 10 nM [^{3}H]-cAMP for 30 min at 30° C., terminated by boiling, treated with 1 mg/ml snake venom, and separated using AG-1XS ion exchange resin (BioRad) as described (Muller et al., 1998, *Bioorg. & Med. Chem. Lett.* 8:2669-2674). Reactions consumed less than 15% of available substrate. Results are listed in Table 1.

TABLE 1

	Racemic Compound	Compound A	Compound B*
PDE Specificity			
PDE Inhibition			
PDE4 IC_{50} (from U937 cells) (nM)	81.8	73.5	611
PDE1 (% inhib at 10 μM)	9%	23%	27%
PDE2(% inhib at 10 μM)	19%	6%	10%
PDE3 (% inhib at 10 μM)	21%	20%	31%
PDE5 (% inhib at μM)	3%	3%	−9%
PDE6 (% inhib at 10 μM)	ND	−6%	10%
PDE7 IC_{50} (nM)	22110	20500	ND
PDE Specificity Ratios from above data (*fold)			
PDE4/PDE1	>2700	>500	>50
PDE4/PDE2	>800	>10000	>260
PDE4/PDE3	>670	>1200	>45
PDE4/PDE5	>12000	>30000	>39000
PDE4/PDE6	ND	>40000	>250
PDE7 IC_{50}/PDE4 IC_{50}	270	279	ND

*Compound B is the ( ) enantiomer of Compound A.

5.6. Example 6

Human T Cell Assays

SEB-induced IL-2 and IFN-γ Production

Staphylococcal Enterotoxin B (SEB) is a superantigen derived from gram-positive bacteria *Staphylococcus aureus*. SEB provides a convenient physiological stimulus specific for T cells expressing particular T cell receptor Vβ chains. Human PBMC (consisting of approximately 50% T cells) were isolated from source leukocyte units as described above and plated in 96-well tissue culture plates at 3×10^5 cells/well in complete medium, pretreated with compounds at 10, 2, 0.4, 0.08, 0.016, 0.0032, 0.00064, and 0 μM in duplicate at a final DMSO concentration of 0.1% at 37° C. in a humidified incubator at 5% CO_2 for 1 hour, then stimulated with 100 ng/ml SEB (Sigma Chemical Co., St. Louis, Mo., USA) for 18 hours. IL-2 and IFN-γ levels were measured by ELISA (R&D Systems, Minneapolis, Minn., USA). IL-2 IC_{50}=291 nM for Compound A. IFN-γ IC_{50}=46 nM for Compound A.

5.7. Example 7

Camp Elevation Assays

PGE_2-induced cAMP Elevation

Prostaglandin E_2 (PGE_2) binds to prostanoid receptors on monocytes, T cells and other leukocytes and consequently elevates intracellular cAMP levels, resulting in inhibition of cellular responses. The combination of PGE_2 and a PDE4 inhibitor synergistically elevates cAMP levels in these cell types, and the elevation of cAMP in PBMC caused by PDE4 inhibitors in the presence of PGE_2 is proportional to the inhibitory activity of that PDE4 inhibitor. Intracellular cAMP was measured in human PBMC as follows. PBMC were isolated as described above and plated in 96-well plates at 1×10^6 cells per well in RPMI-1640. The cells were pretreated with compounds at 100, 10, 1, 0.1, 0.01, and 0 μM in a final concentration of 2% DMSO in duplicate at 37° C. in a humidified incubator at 5% CO_2 for one hour. The cells were then stimulated with PGE_2 (10 μM) (Sigma) for 1 h. The cells were lysed with HCl, 0.1 N final concentration to inhibit phosphodiesterase activity and the plates were frozen at −20° C. The cAMP produced was measured using cAMP (low pH) Immunoassay kit (R&D Systems). PBMC cAMP EC_{50} for racemate is 3.09 μM. PBMC cAMP EC_{50} for Compound A is 1.58 μM.

Elevation of cAMP in human neutrophils was measured as follows. PBMC were removed from source leukocytes (SeraTec Biologicals) by centrifugation on Ficoll-Paque Plus (Amersham Pharmacia). The resulting erythrocyte/polymorphonuclear cell (PMN) pellet was resuspended in Hank's Balanced Salt Solution (BioWhittaker) and mixed with an equal volume of 3% Dextran T-500 (Amersham Pharmacia) in 0.9% saline. Erythrocytes were allowed to sediment for 20 minutes, and the PMN were removed and centrifuged at 120 rpm for 8 minutes at 4° C. The remaining erythrocytes were lysed in cold 0.2% saline for 30 seconds, and the cells restored to isotonicity by the addition of an equal volume of 1.6% saline. The PMN were centrifuged at 1200 rpm for 8 minutes at 4° C., then resuspended in RPMI-1640 and assayed for cAMP elevation as described for PBMC above. PMN were found to be approximately 74% $CD18/CD11b^+$, 71% $CD16^+$ $CD9^+$ neutrophils by flow cytometzy on a FACSCalibur (Becton Dickinson, San Jose, Calif., USA). Results are shown in Table 2.

fMLF-induced LTB4 Production

N-formyl-methionine-leucine-phenylalanine (fMLF) is a bacterially derived peptide that activates neutrophils to rapidly degranulate, migrate, adhere to endothelial cells, and release leukotriene LTB4, a product of arachidonic acid metabolism and itself a neutrophil chemoattractant. Compounds were tested for the ability to block fMLF-induced neutrophil LTB4 production as previously described (Hatzelmann and Schudt, 2001, *J. Pharm. Exp. Ther.*, 297:267-279), with the following modifications. Neutrophils were isolated as described above and resuspended in phosphate-buffered saline without calcium or magnesium (BioWhittaker) containing 10 mM HEPES pH 7.2 and plated in 96-well tissue culture plates at a concentration of 1.7×10^6 cells/well. Cells were treated with 50 μM thimerosal (Sigma)/1 mM $CaCl_2$/1 mM $MgCl_2$ for 15 minutes at 37° C. 5% CO_2, then treated with compounds at 1000, 200, 40, 8, 1.6, 0.32, 0.064, and 0 nM in a final DMSO concentration of 0.01% in duplicate for 10 minutes. Neutrophils were stimulated with 1 μM fMLF for 30 minutes, then lysed by the addition of methanol (20% final concentration) and frozen in a dry ice/isopropanol bath for 10 minutes. Lysates were stored at −70° C. until the LTB4 content was measured by competitive LTB4 ELISA (R&D Systems). Results are shown in Table 2.

Zymosan-induced IL-8 Production

Zymosan A, or the heat-killed yeast *Saccharomyces cerevisiae*, binds to the adhesion molecule Mac-1 on the neutrophil surface and triggers phagocytosis, cell activation and IL-8 production. Zymosan-induced IL-8 production was measured as previously described (Au et al., 1998, *Brit. J. Pharm.*, 123:1260-1266) with the following modifications. Human neutrophils were purified as described above, plated in 96-well tissue culture plates at 3×10^5 cells/well in complete medium, treated with compounds at 10, 2, 0.4, 0.08, 0.016, 0.0032, 0.00064, and 0 μM in duplicate in a final DMSO concentration of 0.1% for 1 hour at 37° C. 5% CO_2. Neutrophils were then stimulated with unopsonized, boiled Zymosan A (Sigma) at 2.5×10^5 particles/well for 18 hours. Supernatants were harvested and tested for IL-8 by ELISA (R&D Systems). Results are shown in Table 2.

fMLF-induced CDI8/CD11b Expression

CD18/CD11b (Mac-1) expression on neutrophils was measured as previously described (Derian et al., 1995, *J. Immunol.*, 154:308-3 17) with the following modifications. Neutrophils were isolated as described above, then resuspended in complete medium at 1×10^6 cells/ml, pretreated with compounds at 10, 1, 0.1, 0.01, and 0 μM in duplicate at a final DMSO concentration of 0.1% for 10 minutes at 37° C. 5% CO_2. Cells were then stimulated with 30 nM fMLF for 30 minutes and then chilled to 4° C. Cells were treated with rabbit IgG (Jackson ImmunoResearch Labs, West Grove, Pa., USA) (10 μg/1×10^6 cells) to block Fc receptors, stained with CD18-FITC and CD11b-PE (Becton Dickinson), and analyzed by flow cytometry on a FACSCalibur. CD18/CD11b expression (mean fluorescence) in the absence of stimulation was subtracted from all samples to obtain inhibition curves and calculate IC_{50} values. Results are shown in Table 2.

fMLF-induced Adhesion to HUVEC

Human umbilical vein endothelial cells (HUVEC) were used as a substrate for neutrophil adhesion as previously described (Derian et al., 1995, *J. Immunol.*, 154:308-317) with the following modifications. HUVEC cells were obtained from Anthrogenesis (Cedar Knolls, N.J., USA), and neutrophils were not treated with cytochalasin B. Cells were treated with compounds at 10, 1, 0.1, 0.01, 0.001, and 0 μM in a final DMSO concentration of 0.1% in duplicate for 10 minutes, stimulated with 500 nM fMLF for 30 minutes, and washed twice with PBS before measuring fluorescence on an FLX800 plate reader (Bio-Tek Instruments, Winooski, Vt., USA). Results are shown in Table 2.

TABLE 2

Assay results

Human Neutrophil Assays (all values in nM)	Racemic Compound	Compound A
PGE_2-induced cAMP EC_{50}	12589	4570
fMLF-induced LTB4 IC_{50}	20.1	2.48
Zymosan-induced IL-8 IC_{50}	ND	94
fMLF-induced CD18 expression IC_{50}	ND	390
fMLF-induced CD11b expression IC_{50}	ND	74
fMLF-induced adhesion to HUVEC IC_{50}	ND	150

5.8. Example 8

Aqueous Solubility

Equilibrium solubilities were measured in pH 7.4 aqueous buffer. The pH 7.4 buffer was prepared by adjusting the pH of a 0.07 M NaH_2PO_4 solution to 7.4 with 10 N NaOH. The ionic strength of the solution was 0.15. At least 1 mg of powder was combined with 1 ml of buffer to make >1 mg/ml mixture.

These samples were shaken for >2 hours and left to stand overnight at room temperature. The samples were then filtered through a 0.45-1 μm Nylon syringe filter that was first saturated with the sample. The filtrate was sampled twice, consecutively. The filtrate was assayed by HPLC against standards prepared in 50% methanol. Compound A has 3.5-fold greater aqueous solubility than the racemic mixture. Measured solubility Compound A=0.012 mg/mL; racemic mixture=0.0034 mg/mL.

5.9. Example 9

LPS-Induced Lung Neutrophilia Ferret Model

The conscious ferret model has been used to investigate anti-inflammatory, emetic and behavioral effects of PDE4 inhibitors when administered by the oral (p.o.) route. From these experiments, a therapeutic index (TI) for each PDE4 inhibitor may be determined. The TI has been calculated by dividing the threshold dose for causing emetic episodes and behavioral changes by the anti-inflammatory dose (dose that causes 50% inhibition of the LPS-induced neutrophilia).

Animal Husbandry

Male ferrets (Mustela Pulorius Euro, weighing 1-2 kg). Ferrets were supplied either by Bury Green Farm or Misay Consultancy. Following transport, the animals were allowed to acclimatize in the holding rooms for a period of not less than seven days. The diet comprised SDS diet C pelleted food given ad lib with Whiskers™ cat food given three times per week. Water was pasteurized animal grade drinking water and was changed daily.

Dosing with PDE4 Inhibitor

PDE4 inhibitors were administered orally (p.o.), at doses initially of 1-10 g/kg, but subsequently up to 30 mg/kg in order to establish whether the TI was 10 or higher, and/or at lower doses to establish the minimum dose to cause 50% inhibition of neutrophilia. Ferrets were fasted overnight but allowed free access to water. The animals were orally dosed with vehicle or PDE4 inhibitor using a 15 cm dosing needle that was passed down the back of the throat into the oesophagus. After dosing, the animals were returned to holding cages fitted with Perspex doors to allow observation, and given free access to water. After dosing, the animals were constantly observed and any emesis or behavioral changes were recorded. The animals were allowed access to food 60 to 90 minutes after p.o. dosing.

Exposure to LPS

Thirty minutes after p.o. dosing with compound or vehicle control, the ferrets were placed into sealed Perspex containers and exposed to an aerosol of LPS (100 μg/ml) for 10 minutes. Aerosols of LPS were generated by a nebulizer (DeVilbiss, USA) and this was directed into the Perspex exposure chamber. Following a 10 minute exposure period, the animals were returned to the holding cages and allowed free access to water, and at a later stage, food. Observation continued for a period of at least 2.5 hours post p.o. dosing and emetic episodes and behavioral changes were recorded.

Bronchoalveolar Lavage

Six hours after LPS exposure the animals were killed by overdose of sodium pentobarbitone administered intraperitoneally. The trachea was then cannulated with polypropylene tubing and the lungs lavaged twice with 20 ml heparinized (10 units/ml) phosphate buffered saline (PBS).

Blood Sampling/Tissue Removal

A terminal blood sample (10 ml) was removed by transthoracic cardiac puncture. The blood was spun at 2,500 rpm for 15 minutes and the plasma was removed and stored at −20° C. The brain also removed and frozen at −20° C. for analysis of compound content.

Cell Counts

The bronchoalveolar lavage (BAL) samples were centrifuged at 1,500 rpm for 5 minutes. The supernatant was removed and the resulting cell pellet re-suspended in 1 ml PBS. A cell smear of the re-suspended fluid was prepared and stained with Leishmans stain to allow differential cell counting. A total cell count was made using the remaining re-suspended sample. From this, the total number of neutrophils in the BAL was determined.

Parameters Measured

1. % Inhibition of LPS-induced pulmonary neutrophilia.

2. Emetic episodes—the number of vomits and retches were counted.

3. Behavioral changes—the following behavioral effects were noted: salivation, panting, mouth clawing, flattened posture, ataxia, arched back and backward walking. Any behavioral changes were semi-quantified by applying a severity rating (mild, moderate or severe).

4. The TI was calculated as the highest dose found to not cause emetic episodes divided by the lowest dose found to inhibit pulmonary neutrophilia by 50% or more.

The effect of Compound A on LPS-induced neutrophilia in the lungs of conscious ferrets is demonstrated in FIG. **30**.

Emesis and Behavioral Changes

Following p.o. dosing of the PDE4, the ferrets were observed for at least two hours and emetic episodes (vomits and retches) and behavioral changes were recorded.

No emetic episodes (retching or vomiting) were observed in the ferrets pre-treated p.o. with the relevant vehicle (acetone/cremophor/distilled water). In a small proportion of the control-treated animals (7/22), mild behavioral changes (lip licking and backward walking) were seen.

Compound A (0.1-3 mg/kg, p.o.), caused no emetic episodes (retching and vomiting). Some behavioral changes (flattened posture, lip licking and backward walking) were observed and classified as mild. At 10 mg/kg in 2/6 ferrets, some retching but no frank emesis was observed along with salivation and behavioral changes (scored as mild or moderate). At the highest dose tested (30 mg/kg) moderate to marked emesis was observed in 3/4 animals along with pronounced behavioral changes. These data are summarized in Table 3.

TABLE 3

Conscious ferret: Emetic episodes and behavioral changes following oral administration of Compound A

Treatment/dose (mg/kg)	Vomits	Retches	Salivation	Panting	Mouth clawing	Flattened posture	Ataxia	Lip licking	Backward walking
Vehicle (acetone/ cremophor/ dist. H_2O)	None	None	None	None	None	None	None	Mild (6/22)	Mild (7/22)

TABLE 3-continued

Conscious ferret: Emetic episodes and behavioral changes following oral administration of Compound A

Treatment/dose (mg/kg)	Vomits	Retches	Salivation	Panting	Mouth clawing	Flattened posture	Ataxia	Lip licking	Backward walking
Compound A (0.1 mg/kg)	None	None	None	None	None	Mild (2/5)	None	Mild (4/5)	Mild (3/5)
Compound A (0.3 mg/kg)	None	None	None	None	None	Mild (2/6)	None	Mild (3/6)	Mild (4/6)
Compound A (1.0 mg/kg)	None	None	None	None	None	Mild (2/6)	None	Mild (6/6)	Mild (4/6)
Compound A (3.0 mg/kg)	None	None	None	None	Mild (1/8)	Marked (7/8)	None	Mild (2/8)	Moderate (5/8)
Compound A (10 mg/kg)	None	Mild (2/6)	Mild (1/6)	None	Mild (1/6)	Marked (6/6)	None	Moderate (5/6)	Marked (6/6)
Compound A (30 mg/kg)	Moderate (3/4)	Marked (3/4)	Moderate (3/4)	Mild (1/4)	Marked (4/4)	Marked (4/4)	Mild (3/4)	Moderate (4/4)	Mild (2/4)

Animals were observed for up to three hours following dosing. Numbers in parentheses refer to the number of animals that responded. The numbers of animals in each group range from 4 to 22.

Therapeutic Index Calculation

From these experiments, a therapeutic index (TI) was determined for each compound by dividing the threshold dose for inducing emetic episodes by the ED_{50} value for inhibiting the pulmonary neutrophilia. The TI calculation is summarized in Table 4. Compound A had a TI of 12, causing no emetic episodes at an anti-inflammatory dose of 1 mg/kg.

TABLE 4

Summary of the effective doses (ED_{50}) for inhibition of LPS-induced pulmonary neutrophilia and induction of emesis and the therapeutic index derived from these values

Compound	Inhibition of LPS-induced neutrophilia (ED_{50} mg/kg)	Threshold emetic dose (mg/kg)	Therapeutic index
Compound A	0.8	10	12

5.10. Example 10

Biological Activity of Compound A in Patients with Severe Plaque-Type Psoriasis

Compound A is a novel oral agent that downregulates pro-inflammatory cytokine production in human cellular models. Compound A has been shown to decrease TNF-α, IL-12 and IFN-γ production as well as elevate production of IL-10. Psoriasis is strongly associated with dysregulation of cytokines and chemokines allowing for potential therapies with immunomodulatory compounds. This Phase 2, open-label, single arm, pilot study was designed to assess the biological activity of Compound A in patients with severe plaque-type psoriasis. Additional assessments for clinical outcomes were performed to evaluate the potential efficacy of Compound A in treating severe plaque-type psoriasis.

Compound A was administered 20 mg orally daily for 29 days with an additional 28-day observational follow-up period for patient safety. Skin punch biopsy specimens (6 mm) from target plaques were obtained at baseline, Day 15 and Day 29. A nonlesional skin biopsy was also taken at baseline. The primary pharmacodynamic endpoint was the percent change from baseline in epidermal thickness at Day 29. Epidermal skin thickness measurements and immunohistochemical analysis were carried out by a blinded reviewer to evaluate CD11c, CD83, K16, ICAM-1, HLA-DR, and fillagrin. Biopsy specimens were analyzed by RT-PCR for: TNF-α, p40-IL12/IL23, IL-10, IFN-γ, IP10, IL-2, IL-8, iNOS, p19-IL23, K16, CD 83, and hARP. PASI, PGA, and BSA measurements were performed to explore clinical efficacy during the 29-day treatment phase of the study. Adverse event reporting, clinical laboratory evaluations, physical examinations, ECG and vital sign measurements assessed safety. A total of 19 patients were enrolled: 15 patients had complete sets of evaluable biopsies and 17 patients had complete efficacy assessments.

Assessment of the change in epidermal thickness was the primary endpoint in this study. Nineteen patients were enrolled in the study, of which 15 had complete sets of evaluable biopsies at baseline and Day 29. Seventeen of the 19 subjects had clinical efficacy parameters measured at Baseline and Day 29. Eight (53.3%) of the patients with evaluable biopsies at baseline and Day 29 demonstrated a 20% reduction in epidermal skin thickness. The mean reduction of epidermal thickness among all 15 subjects with evaluable biopsies at baseline and Day 29 was 20.5% (p=0.015). FIG. **31** displays the change in epidermal thickness from baseline to Day 29 among subjects with evaluable biopsies.

Key inflammatory markers including epidermal and dermal T-cells, CD83+ and CD11c cells were evaluated in biopsy specimens. Results for 8 patients who responded showed a decrease of epidermal and dermal T-cells by 42.56% and 28.79% respectively in responders (≤20% epidermal thickness reduction). Mean reductions from baseline in epidermal and dermal CD83+ cells were 32.50% and 25.86% respectively in responders. CD11c cells were reduced by 40.16% in the epidermis and 18.50% in the dermis in responders. Table 5 lists reductions in key skin biopsy inflammatory markers in responders and nonresponders. In addition, one patient with abnormal K16 at baseline had normal K16 at Day 29. Three patients with abnormal ICAM-1 at baseline had normal ICAM-1 at Day 29. Two patients with abnormal HLA-DR had normal HLA-DR at Day 29 and three patients with abnormal fillagrin at baseline had normal fillagrin at Day 29.

TABLE 5

Percentage Reduction of Key Inflammatory Markers at Day 29

Cell		Epidermis	Dermis
T-cells	Responder	−42.56%	−28.79%
	Nonresponder	+8.74%	−17.34%
CD83+	Responder	−32.50%	−25.86%
	Nonresponder	−16.31%	+0.46%
CD11c	Responder	−40.16%	−18.50%
	Nonresponder	−2.54%	−21.19%

Biopsy specimens were evaluated for mRNA gene expression of key inflammatory markers by RT-PCR including: TNFα, p40-IL12/IL23, IL-10, IFNγ, IP10, IL-2, IL-8, iNOS, p19-IL23, K16 and CD83. The mRNA expression of iNOS was reduced 66.5% (p=0.025) in lesional skin after 29 days of treatment with Compound A. Reductions and increases in mRNA expression of other inflammatory markers showed overall trends of improvement. FIG. **32** graphically displays the change in iNOS expression during the study.

A total of 17 of the 19 subjects enrolled completed the 29-day treatment phase and had complete clinical efficacy assessments. Fourteen (73.7%) of the 19 subjects enrolled demonstrated improvement in their PASI with 3 (15.8%) of these patients showing a >50% reduction from baseline in their total Psoriasis Area and Severity Index (PASI) score at Day 29. FIG. **33** displays the percentage change in PASI scores among evaluable patients from baseline at Day 29. Additionally, 9 (52.9%) of the 17 evaluable patients demonstrated improvement in the static Physician's Global Assessment (sPGA) and 10 (58.8%) of the 17 evaluable patients showed a reduction from baseline in their psoriasis body surface area (BSA) after 29 days of treatment with Compound A. Safety was evaluated during treatment and follow-up phases through monitoring of adverse events, ECGs, laboratory tests, physical exams and vital signs. No deaths were reported nor did any patient prematurely discontinue due to an adverse event. Most common treatment-related adverse events included headache (26.3%), and nausea (15.8%).

In this clinical study, Compound A 20 mg p.o. QD for 29 days was safe in subjects with severe plaquetype psoriasis. The primary endpoint was reached with 8 (53.3%) of 15 subjects achieving a 20% reduction in epidermal thickness at Day 29. Reductions of key inflammatory markers in skin biopsies were noted including dermal and epidermal T-cells, CD83+ and CD11c cells. RT-PCR analysis revealed a statistically significant reduction of 66.5% in iNOS mRNA in skin biopsies at Day 29. A positive clinical efficacy signal was noted after 29 days of treatment with Compound A. 73.7% of enrolled patients demonstrated improvement in their psoriasis symptoms with 15.8% of these patients showing >50% reduction from baseline in their PASI score at Day 29. 47.4% of enrolled patients showed an improvement in their sPGA and 52.6% of enrolled patients showed a reduction from baseline in their psoriasis body surface area (BSA) at Day 29.

5.11. Example 11

A Phase 2 Study Demonstrating the Efficacy and Safety of Compound A in Subjects with Moderate-to-Severe Psoriasis

This phase 2, multicenter, randomized, double-blind, placebo-controlled, parallel-group, dose-comparison study evaluated the efficacy and safety of Compound A in subjects with moderate to severe plaque-type psoriasis who were candidates for systemic therapy.

This study included a 12-week treatment phase followed by a 4-week observational follow-up phase. A total of 260 subjects were randomized to receive Compound A 20 mg BID, Compound A 20 mg QD, or placebo for 12 weeks. The primary endpoint for this study was the proportion of subjects treated with Compound A who achieved a 75% reduction in Psoriasis Area and Severity Index score ("PASI-75") at week 12/last treatment in reference to the baseline visit. Last treatment is defined as the last PAST assessment completed during the 12-week treatment phase.

At week 12/last treatment, a significantly higher proportion of subjects treated with 20 mg BID (24%) achieved a PASI-75 compared with the placebo group (10%; P=0.023). Of the subjects receiving 20 mg BID or placebo, 57% versus 23% achieved PASI-50 at week 12/last treatment, respectively; whereas 14% versus 6% achieved PASI-90, respectively. At week 12/last treatment, subjects achieved a mean decrease of 52% versus 17% in PASI from baseline in the 20 mg BID versus placebo groups, respectively. Subjects receiving Compound A continued to improve over time, showing the greatest mean percent reduction in PASI score at week 12. Overall, the adverse event profiles were similar across all three treatment groups. The majority of adverse events reported were mild. No study drug-related serious adverse events were reported in this study. No subjects in the 20 mg BID group experienced psoriasis flare during the observational follow-up period.

In this clinical study, Compound A was shown to be well tolerated and safe in subjects with moderate to severe plaquetype psoriasis. The proportions of subjects that achieved 50%, 75%, and 90% improvement in PASI demonstrate the clinical activity of Compound A after 12 weeks of treatment.

5.12. Example 12

Solid Form Screening Studies

5.12.1. Experimental Methodology

Solubility Studies. A weighed sample of Compound A (about 100 mg) was treated with about 2 mL of the test solvent. The solvents used were either reagent or HPLC grade. The resulting mixture was agitated for at least 24 hours at about 25° C. When all of the solids were dissolved by visual inspection, the estimated solubilities were calculated. The solubilities were estimated from these experiments based on the total volume of solvent used to give a solution. The actual solubilities may be greater than those calculated due to the use of large amount of solvent or to a slow rate of dissolution. If dissolution did not occur during the experiment, the solubility was measured gravimetrically. A known volume of filtrate was evaporated to dryness and the weight of the residue was measured.

Solution Evaporation Studies. Solution evaporation was performed for solvents in which the solubility of Compound A was more than about 50 mg/mL, such as acetone, acetonitrile, methylene chloride and tetrahydrofuran. Solid samples were obtained by slowly evaporating the solvents at about 25° C. or about 50° C. in an open vial under nitrogen.

Equilibration Studies. Equilibration experiments were carried out by adding an excess of Compound A to about 2 mL of a test solvent. The resulting mixture was agitated for at least 24 hours at about 25° C. or about 50° C. Upon reaching equilibrium, the saturated solution was removed and allowed to evaporate slowly in an open vial under nitrogen at about 25° C. or about 50° C., respectively. The slurry resulting from the equilibration was filtered and dried in the air.

Cooling Crystallization Studies. Cooling crystallization studies were performed. The solid was dissolved in a solvent at an elevated temperature, about 65° C., and allowed to cool to about 25° C. Samples that did not crystallize at about 25° C. were placed in a refrigerator (about 0-5° C.). Solids were isolated by decantation and allowed to dry in the air.

Solvent/Anti-Solvent Precipitation Studies. Precipitations were carried out by solvent/anti-solvent combinations. The solid was dissolved in a solvent in which Compound A had a relatively high solubility, and then a selected solvent in which Compound A had a relatively low solubility (i.e., an anti-solvent) was added to the solution. A precipitate formed immediately in some solvent/anti-solvent systems. If the precipitation did not occur immediately, the resulting mixture was allowed to cool in a refrigerator (about 0-5° C.) until a precipitate formed. The precipitate was then isolated by decantation and allowed to dry in the air.

Interconversion Studies.

Interconversion experiments were performed by making slurries of a solid form in a saturated solvent. The slurries were agitated for at least 2 days at about 25° C. The saturated solution was removed by filtration and the solid was dried in the air.

Compression Studies. Compression tests were performed by pressing the sample under 2000 psi force for at least one minute with Carver Mini C presser. The sample was then analyzed by XRPD.

Hygroscopicity Studies. The hygroscopicity of various solid forms was studied using a Surface Measurement Systems DVS instrument. Typically a sample size of between about 10-50 mg was loaded into the DVS instrument sample pan and the sample was analyzed on a DVS automated sorption analyzer at about 25° C. The relative humidity was increased in increments of about 10% from about 0% to about 95% RH. The relative humidity was then decreased in a similar manner to accomplish a full adsorption/desorption cycle. The mass was recorded at periodic intervals throughout the experiment.

5.12.2. Characterization Methodology

Samples generated as described in the solid form screen were typically analyzed by X-Ray Powder Diffraction (XRPD). XRPD was conducted on a Thermo ARL X'TRA™ X-ray powder diffractometer using Cu Kα radiation at 1.54 Å. The instrument was equipped with a fine focus X-ray tube. The voltage and amperage of X-ray generator were set at 45 kV and 40 mA, respectively. The divergence slices were set at 4 mm and 2 mm and the measuring slices were set at 0.5 mm and 0.2 mm. The diffracted radiation was detected by a peltier-cooled Si(Li) solid-state detector. Typically, a theta-two theta continuous scan at 2.40°/min (0.5 sec/0.02° step) from 1.5 ° 2θ to 40° 2θ was used. A sintered alumina standard was used to check the peak position. In general, positions of XRPD peaks are expected to individually vary on a measurement-by-measurement basis by about ±0.2° 2θ. In general, as understood in the art, two XRPD patterns match one another if the characteristic peaks of the first pattern are located at approximately the same positions as the characteristic peaks of the second pattern. As understood in the art, determining whether two XRPD patterns match or whether individual peaks in two XRPD patterns match may require consideration of individual variables and parameters such as, but not limited to, preferred orientation, phase impurities, degree of crystallinity, particle size, variation in diffractometer instrument setup, variation in XRPD data collection parameters, and/or variation in XRPD data processing, among others. The determination of whether two patterns match may be performed by eye and/or by computer analysis. Examples of XRPD patterns collected and analyzed using these methods and parameters are provided herein, e.g., as FIG. **1**, FIG. **5**, FIG. **9**, FIG. **13**, FIG. **17**, FIG. **21** and FIG. **25**.

Differential Scanning calorimetry (DSC) analyses were performed on a TA Instruments Q1000™. About 5 mg of sample was placed into a tared DSC pan and the weight of the sample was accurately recorded. Typically, the sample was heated under nitrogen at a rate of about 10° C./min from about 25° C. up to a final temperature of about 200° C. Typically, thermal events were reported as extrapolated onset temperatures. Examples of DSC thermograms collected and analyzed using these methods and parameters are provided herein, e.g., as FIG. **2**, FIG. **6**, FIG. **10**, FIG. **14**, FIG. **18**, FIG. **22** and FIG. **26**.

Thermal Gravimetric Analyses (TGA) were performed on a TA Instruments Q500™. Calcium oxalate was used for calibration. About 10 mg of sample was placed on a pan, accurately weighed and loaded into the TGA furnace. The sample was heated under nitrogen at a rate of about 10° C./min from about 25° C. up to a final temperature of about 200° C. Examples of TGA thermograms collected and analyzed using these methods and parameters are provided herein, e.g., as FIG. **3**, FIG. **7**, FIG. **11**, FIG. **15**, FIG. **19**, FIG. **23** and FIG. **27**.

Solvation solvents were identified and quantified by TG-IR experiments using a TA Instruments Q500™ TGA interfaced with a Thermo Nicolet AEM Fourier transform IR spectrophotometer. Typically a sample size of about 20-50 mg was weighed into an aluminum pan and heated to about 200° C. During the TGA run, the vapor was transferred to the cell through a heated transfer line. The temperature of both transfer line and the cell were set at about 225° C. IR spectra were collected every 10-second repeat time. Volatiles were identified from a search of the Aldrich vapor phase spectral library and the library match results are presented to show the identified vapor.

Morphology and particle size analysis of the samples were carried out using an Olympus microscope. The instrument was calibrated with USP standards. D_{90} values were determined using the software Image Plus—Material Plus. The D_{90} value represents the 90th percentile of the particle size distribution as measured by length; i.e., 90% of the particles have a length of this value or less.

5.12.3. Solid Form Screening Study Results

Solid forms comprising Compound A which were prepared during the solid form screening studies included Forms A, B, C, D, E, F, G and an amorphous form. Representative XRPD patterns, DSC plots, TGA plots and DVS plots for each of Forms A, B, C, D, E, F and G are provided herein as FIG. **1**-FIG. **28**.

Solubility Studies. The approximate solubility of Form B of Compound A in various solvents at about 25° C. was determined. Results are shown in Table 6. Form B was found to be most soluble in acetone, acetonitrile, methylene chloride, methyl ethyl ketone and tetrahydrofuran (greater than about 50 mg/mL) followed by ethyl acetate (about 30.15 mg/mL). Form B was also found to have low solubility in several solvents including n-butanol, heptane, 2-propanol, toluene and water (less than about 1 mg/mL).

Solution Evaporation Studies. Results from solution evaporation studies performed at about 25° C. and about 50° C. are summarized in Table 7.

Equilibration Studies. Results from equilibration studies performed at about 25° C. and about 50° C. are summarized in Table 8.

Cooling Crystallization Studies. Results from cooling crystallization studies are summarized in Table 9. Cooling

crystallization studies yielded crystalline material from numerous solvents, including acetone, acetonitrile, n-butyl acetate, ethyl acetate, methanol, methylene chloride, methyl ethyl ketone (MEK) and tetrahydrofuran (THF). The crystalline materials obtained were typically characterized by XRPD, DSC and TGA.

Solvent/Anti-Solvent Precipitation Studies. Results from solvent/anti-solvent precipitation studies are summarized in Table 10. When heptane, water and toluene were added to Form B in THF solution at about 40° C., precipitates formed immediately. When heptane, methyl t-butyl ether (MTBE), toluene and water were added to Form B in acetonitrile solution separately at about 25° C., either a clear solution or a mixture formed. Crystalline material from MTBE/acetonitrile, water/acetonitrile and toluene/acetonitrile was obtained after stirring overnight. However, no crystallization occurred for heptane/acetonitrile mixture. When water was added to Form B in methanol solution at about 50° C., precipitates formed immediately and when heptane and toluene were added to Form B in methanol solution separately at about 50° C., either a clear solution or a mixture formed. Crystalline material from toluene/methanol and heptane/methanol was obtained after stirring overnight. When toluene was added to Form B in methylene chloride solution at about 25° C., precipitates formed immediately and when MTBE was added to Form B in methylene chloride solution at about 25° C., a clear solution was obtained. Crystalline material from MTBE/methylene chloride was obtained after stirred overnight. However, no crystallization occurred when heptane was added to Form B in methylene chloride solution. When heptane was added to Form B in MEK solution at about 50° C., precipitates formed immediately and when MTBE and toluene were added to Form B in MEK solution separately at about 50° C., clear solutions were obtained. Crystalline material from MTBE/MEK and toluene/MEK was obtained after stirring overnight. When heptane was added to Form B in n-butyl acetate solution at about 50° C., precipitates formed immediately and when MTBE and toluene were added to Form B in MEK solution separately at about 50° C., clear solutions were obtained. Crystalline material from MTBE/n-butyl acetate and toluene/n-butyl acetate was obtained after stirring overnight. When water and toluene were added to Form B in acetone solution separately at about 40° C., precipitates formed immediately and when ethanol and 2-propanol were added to Form B in acetone solution separately at about 40° C., clear solutions were obtained. Crystalline material from ethanol/acetone and 2-propanol/acetone were obtained after stirring overnight. Crystalline materials obtained were identified by XRPD, DSC, TGA.

Stability Studies. Stability study results are summarized in Table 11. The stabilities of Forms A, B, C and D were studied by exposing the solid samples to the stress condition of 40° C./75% RH for four weeks. Moreover, the stabilities of Forms A, B, C and D in different solvents were studied by equilibration in different solvents at 40° C. for four weeks. The slurries then were filtered and dried in the air. Solid samples obtained from the stability experiments were analyzed by XRPD and DSC.

Interconversion Studies. Results from interconversion studies are summarized in Table 12.

Compression Studies. Compression tests were performed on Forms A, B, C, D, E, F and G of Compound A. Each form studied was found to be substantially physically stable as observed by XRPD analysis.

Hygroscopicity Studies. Hygroscopicity (moisture sorption/desorption) studies were performed on Forms A, B, C, D, E, F and G. Each of the solid samples were analyzed by XRPD after undergoing a full adsorption/desorption cycle in the DVS system. XRPD results indicated that none of the forms analyzed underwent substantial solid-state transformation as a result of DVS analysis.

TABLE 6

Solubility Study on Form B

Solvent System	Approximate Solubility (mg/ml)
Acetone	>50
Acetonitrile	>50
n-Butanol	>0.72
n-Butyl acetate	9.75
Absolute ethanol	1.38
Ethyl acetate	30.15
Heptane	0.41
Methylene chloride	>50
Methyl ethyl ketone	>50
Methanol	4.05
Methyl t-butyl ether	1.17
2-Propanol	0.81
Tetrahydrofuran	>50
Toluene	0.90
Water	0.69
Ethanol:Water (1:1)	2.86

TABLE 7

Solution Evaporation Studies

Starting Form	Solvent System	Evaporation Temp. (° C.)	XRPD Analysis	DSC thermal events
B	Acetone	25	Form B	
B	Acetonitrile	25	Form B + Form E	77.28° C.; 151.84° C.
B	n-Butyl acetate	25	Form B	
B	Ethyl acetate	25	Form B	
B	Methylene chloride	25	Form D	93.11° C.
B	Methyl ethyl ketone	25	Form B	
B	Tetrahydrofuran	25	Form B	
B	Ethanol:Water (1:1)	25	Form B	
A	Acetonitrile	25	Form E	95.42° C. (TGA wt. loss = 3.56%)
A	Methylene chloride	25	Form D	97.23° C.
A	Acetone	50	Form B	
A	Acetonitrile	50	Form B	
A	n-Butyl acetate	50	Form B	
A	Ethyl acetate	50	Form B	
A	Methyl ethyl ketone	50	Form B	
A	Tetrahydrofuran	50	Form B	
A	Ethanol:Water (1:1)	50	Form B	

TABLE 8

Equilibration Studies

Starting Form	Solvent System	Equilib. Temp. ° C.	XRPD Analysis	DSC Thermal Events
B	n-Butanol	25	Form B	
B	n-Butyl acetate	25	Form B	
B	Ethanol	25	Form B	
B	Ethyl acetate	25	Form B	
B	Heptane	25	Form B	
B	Methanol	25	Form B	
B	Methyl t-butyl ether	25	Form B	
B	2-Propanol	25	Form B	
B	Toluene	25	Form C	159.31° C.
B	Toluene (evap. at 60° C.)	25	Form C	Broad multiplet

TABLE 8-continued

Equilibration Studies

Starting Form	Solvent System	Equilib. Temp. ° C.	XRPD Analysis	DSC Thermal Events
B	Toluene:Acetone (9:1) (evap. at 100° C.)	25	Form C	Broad multiplet (TGA wt. loss = 5.90%)
B	Water	25	Form B	
B	Water (50 days)	25	Form B	
A	Ethanol	25	Form F	145.06° C. (multiplet)
A	Heptane	25	Form A	
A	Ethyl acetate	25	Form G	108.96° C.
A	Water	25	Form A	
A	Toluene	25	Form C	170.18° C. (TGA wt. loss = 5.86%)
A	Toluene (evap. at 60° C.)	25	Form C	167.84° C.
A	Toluene:Acetone (9:1) (evap. at 100° C.)	25	Form C	Broad multiplet
A	Acetone:Ethanol (1:1)	25	Form B	154.00° C. (main)
A	Ethanol:Water (1:1)	25	Form F	145.22° C.
A	n-Butanol	50	Form B	
A	n-Butyl acetate	50	Form B	
A	Ethanol	50	Form B	
A	Heptane	50	Form B	
A	Methanol	50	Form B	

TABLE 8-continued

Equilibration Studies

Starting Form	Solvent System	Equilib. Temp. ° C.	XRPD Analysis	DSC Thermal Events
A	Methyl t-butyl ether	50	Form B	
A	2-Propanol	50	Form B	
A	Toluene	50	Form C	165.30° C. (multiplet)
A	Water	50	Form B	
A	Ethanol:Water (1:1)	50	Form B	

TABLE 9

Cooling Crystallization Studies

Starting Form	Solvent System	Analysis by XRPD	DSC Thermal Events
B	Acetone	Form E	
B	Acetonitrile	Form E	95.42° C.
B	n-Butyl acetate	Form B	
B	Ethyl acetate	Form B	
B	Methylene Chloride	Form D	100.90° C.
B	Methanol	Form B	
B	Methyl ethyl ketone	Form B	
B	THF	Form H	

TABLE 10

Solvent/Anti-Solvent Precipitation Studies

Starting Form	Solvent*	Anti-Solvent*	Ratio (Solvent: Antisolvent) & Temp.	Analysis by XRPD	DSC Thermal Events
B	Acetone	Ethanol	1:8 at 40° C.	Form B	
B	Acetone	2-Propanol	1:10 at 40° C.	Form B	
B	Acetone	Water	1:4 at 40° C.	Form B	
B	Acetone	Toluene	1:10 at 40° C.	Form C	167.57° C. (broad)
B	Acetonitrile	Heptane	1:8 at 25° C.	Form B	
B	Acetonitrile	MtBE	1:8 at 25° C.	Form B	
B	Acetonitrile	Water	1:6 at 25° C.	Form B	
B	Acetonitrile	Toluene	1:8 at 50° C.	Form C	167.97° C.
B	Methyl ethyl ketone	Heptane	1:3 at 50° C.	Form B	
B	MEK	MtBE	1:4 at 50° C.	Form B	
B	MEK	Toluene	1:3 at 50° C.	Form C	168.22° C.
B	n-Butyl acetate	Heptane	1:4 at 50° C.	Form B	
B	n-Butyl acetate	MtBE	1:4 at 50° C.	Form B	
B	n-Butyl acetate	Toluene	1:4 at 50° C.	Form B	
B	DCM	Heptane	1:8 at 25° C.	Form E + B	89.65° C.; 149.81° C.
B	DCM	MtBE	1:15 at 25° C.	Form B	
B	DCM	Toluene	1:15 at 25° C.	Form B	167.99° C. (multiplet)
B	Methanol	Heptane	1:3 at 50° C.	Form B	
B	Methanol	Water	1:3 at 50° C.	Form B	
B	Methanol	Toluene	1:3 at 50° C.	Form C	168.37° C. (multiplet)
B	Tetrahydrofuran	Heptane	1:6 at 40° C.	Form B	
B	Tetrahydrofuran	Water	1:6 at 40° C.	Form B	
B	Tetrahydrofuran	Toluene	1:6 at 40° C.	Form C	168.64° C. (multiplet)

*Abbreviations: MEK = methyl ethyl ketone; DCM = dichloromethane (i.e., methylene chloride); MtBE = methyl t-butyl ether

TABLE 11

Stability Studies

Starting Form	Test Conditions ("EQ" = equilibrate; "RH" = relative humidity)	Appearance	Analysis by XRPD
Form A	40° C./75% RH; 4 weeks	White solid	Form A
Form B	40° C./75% RH; 4 weeks	White solid	Form B
Form C	40° C./75% RH; 4 weeks	Yellow solid	Form C
Form D	40° C./75% RH; 4 weeks	White solid	Form D
Form A	EQ in ethanol at 40° C. for 4 weeks		Form F
Form A	EQ in heptane at 40° C. for 4 weeks		Form A
Form A	EQ in water at 40° C. for 4 weeks		Form A
Form A	EQ in toluene at 40° C. for 4 weeks		Form C
Form B	EQ in ethanol at 40° C. for 4 weeks		Form B
Form B	EQ in heptane at 40° C. for 4 weeks		Form B
Form B	EQ in water at 40° C. for 4 weeks		Form B
Form B	EQ in toluene at 40° C. for 4 weeks		Form B
Form C	EQ in ethanol at 40° C. for 4 weeks		Form C
Form C	EQ in heptane at 40° C. for 4 weeks		Form C
Form C	EQ in water at 40° C. for 4 weeks		Form C
Form C	EQ in toluene at 40° C. for 4 weeks		Form C
Form D	EQ in ethanol at 40° C. for 4 weeks		Form B
Form D	EQ in heptane at 40° C. for 4 weeks		Form B
Form D	EQ in water at 40° C. for 4 weeks		Form B
Form D	EQ in toluene at 40° C. for 4 weeks		Form C

TABLE 12

Interconversion Studies

Starting Form	Test Conditions ("EQ" = equilibrate)	Analysis by XRPD
Mixture of Forms A, B, C, D, E, F and G	EQ in acetone:ethanol (1:1) at 25° C.	Form B + C + F
Form A	EQ in acetone:ethanol (1:1) at 25° C.	Form B
Form C	EQ in acetone:ethanol (1:1) at 25° C.	Form C
Form D	EQ in acetone:ethanol (1:1) at 25° C.	Form B
Form E	EQ in acetone:ethanol (1:1) at 25° C.	Form B
Form F	EQ in acetone:ethanol (1:1) at 25° C.	Form F
Form G	EQ in acetone:ethanol (1:1) at 25° C.	Form B

5.13. Example 13

200 mg Dosage Capsule

Table 13 illustrates a batch formulation and single dosage formulation for a single dose unit containing 200 mg of a solid form comprising Compound A, i.e., about 40 percent by weight, in a size #0 capsule.

TABLE 13

Formulation for 200 mg capsule

Material	Percent By Weight	Quantity (mg/tablet)	Quantity (kg/batch)
Compound A	40.0%	200 mg	16.80 kg
Pregelatinized Corn Starch, NF5	9.5%	297.5 mg	24.99 kg
Magnesium Stearate	0.5%	2.5 mg	0.21 kg
Total	100.0%	500 mg	42.00 kg

The pregelatinized corn starch (SPRESS™ B-820) and Compound A components are passed through a 710 μm screen and then are loaded into a Diffusion Mixer with a baffle insert and blended for 15 minutes. The magnesium stearate is passed through a 210 μm screen and is added to the Diffusion Mixer. The blend is then encapsulated in a size #0 capsule, 500 mg per capsule (8400 capsule batch size) using a Dosator type capsule filling machine

5.14. Example 14

100 mg Oral Dosage Form

Table 14 illustrates a batch formulation and a single dose unit formulation containing 100 mg of a solid form comprising Compound A.

TABLE 14

Formulation for 100 mg tablet

Material	Percent by Weight	Quantity (mg/tablet)	Quantity (kg/batch)
Compound A	40%	100.00	20.00
Microcrystalline Cellulose, NF	53.5%	133.75	26.75
Pluronic F-68 Surfactant	4.0%	10.00	2.00
Croscarmellose Sodium Type A, NF	2.0%	5.00	1.00
Magnesium Stearate, NF	0.5%	1.25	0.25
Total	100.0%	250.00 mg	50.00 kg

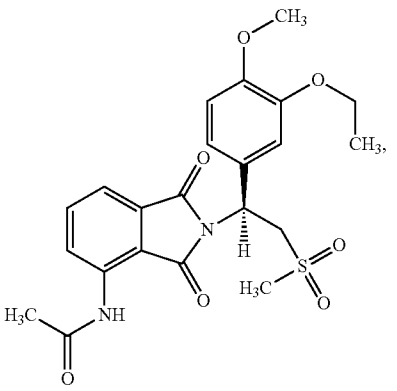
The microcrystalline cellulose, croscarmellose sodium, and Compound A components are passed through a #30 mesh screen (about 430μ to about 655μ). The Pluronic F-68® (manufactured by JRH Biosciences, Inc. of Lenexa, Kans.) surfactant is passed through a #20 mesh screen (about 457μ to about 1041μ). The Pluronic F-68® surfactant and 0.5 kgs of croscarmellose sodium are loaded into a 16 qt. twin shell tumble blender and are mixed for about 5 minutes. The mix is then transferred to a 3 cubic foot twin shell tumble blender where the microcrystalline cellulose is added and blended for about 5 minutes. The solid form comprising Compound A is added and blended for an additional 25 minutes. This pre-blend is passed through a roller compactor with a hammer mill attached at the discharge of the roller compactor and moved back to the tumble blender. The remaining croscarmellose sodium and magnesium stearate is added to the tumble blender and blended for about 3 minutes. The final mixture is compressed on a rotary tablet press with 250 mg per tablet (200,000 tablet batch size).

While the invention has been described with respect to the particular embodiments, it will be apparent to those skilled in the art that various changes and modifications may be made without departing from the spirit and scope of the invention as defined in the claims. Such modifications are also intended to fall within the scope of the appended claims.

What is claimed is:

1. A method of treating a disease or disorder selected from the group consisting of psoriasis, psoriatic arthritis, rheumatoid arthritis, Behcet's Disease, rheumatoid spondylitis, an arthritic condition, atopic dermatitis, and ulcerative colitis, wherein the method comprises administering a therapeutically or prophylactically effective amount of a Form B crystal form of the compound of Formula (I):

(I)



which is enantiomerically pure, and which has an X-ray powder diffraction pattern comprising peaks at about 10.1, 13.5, 20.7, and 26.9 degrees 2θ.

2. The method of claim **1**, wherein the crystal form has an X-ray powder diffraction pattern further comprising peaks at about 12.4, 15.7, 18.1, and 24.7 degrees 2θ.

3. The method of claim **2**, wherein the crystal form has an X-ray powder diffraction pattern further comprising peaks at about 16.3, 22.5, 26.2, and 29.1 degrees 2θ.

4. The method of claim **1**, wherein the crystal form has an X-ray powder diffraction pattern matching the pattern depicted in FIG **5**.

5. The method of claim **1**, wherein the crystal form has a differential scanning calorimetry plot comprising an endothermic event with an onset temperature of about 154° C.

6. The method of claim **1**, wherein the crystal form has a differential scanning calorimetry plot matching the plot depicted in FIG **6**.

7. The method of claim **1**, wherein the crystal form has a thermal gravimetric analysis plot comprising a mass loss of less than about 1% when heated from about 25° C. to about 140° C.

8. The method of claim **7**, wherein the mass loss is about 0.25%.

9. The method of claim **1**, wherein the crystal form has a thermal gravimetric analysis plot matching the plot depicted in FIG **7**.

10. The method of claim **1**, wherein the crystal form exhibits a mass increase of less than about 1% when subjected to an increase in relative humidity from about 0% to about 95% relative humidity.

11. The method of claim **10**, wherein the mass increase is about 0.6%.

12. The method of claim **1**, wherein the crystal form has a moisture sorption isotherm plot matching the plot depicted in FIG **8**.

13. The method of claim **1**, wherein the crystal form is stable upon exposure to about 40° C. and about 75% relative humidity for about 4 weeks.

14. The method of claim **1**, wherein the disease or disorder is psoriasis.

15. The method of claim **1**, wherein the disease or disorder is psoriatic arthritis.

16. The method of claim **1**, wherein the disease or disorder is rheumatoid arthritis.

17. The method of claim **1**, wherein the disease or disorder is Behcet's Disease.

18. The method of claim **1**, wherein the disease or disorder is rheumatoid spondylitis.

19. The method of claim **1**, wherein the disease or disorder is an arthritic condition.

20. The method of claim **1**, wherein the disease or disorder is atopic dermatitis.

21. The method of claim **1**, wherein the disease or disorder is ulcerative colitis.

* * * * *

EXHIBIT H

US009724330B2

(12) **United States Patent**
Muller et al.

(10) **Patent No.: US 9,724,330 B2**
(45) **Date of Patent: *Aug. 8, 2017**

(54) **METHODS OF USING (+)-2-[1-(3-ETHOXY-4-METHOXYPHENYL)-2-METHYL-SULFONYLETHYL]-4-ACETYLAMINO-ISOINDOLINE-1,3-DIONE**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

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Related U.S. Application Data

(60) Continuation of application No. 14/316,160, filed on Jun. 26, 2014, now Pat. No. 9,283,207, which is a continuation of application No. 13/682,652, filed on Nov. 20, 2012, now Pat. No. 8,802,717, which is a continuation of application No. 12/630,788, filed on Dec. 3, 2009, now Pat. No. 8,455,536, which is a continuation of application No. 12/098,379, filed on Apr. 4, 2008, now Pat. No. 7,659,303, which is a division of application No. 11/170,308, filed on Jun. 28, 2005, now Pat. No. 7,358,272, which is a division of application No. 10/392,195, filed on Mar. 19, 2003, now Pat. No. 6,962,940.

(60) Provisional application No. 60/438,450, filed on Jan. 7, 2003, provisional application No. 60/366,515, filed on Mar. 20, 2002.

(51) **Int. Cl.**

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C07D 209/44	(2006.01)
C07C 317/28	(2006.01)
C07D 209/48	(2006.01)
A61K 45/06	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 31/4035*** (2013.01); ***A61K 45/06*** (2013.01); ***C07C 317/28*** (2013.01); ***C07D 209/48*** (2013.01); *C07B 2200/07* (2013.01)

(58) **Field of Classification Search**

CPC A61K 31/4035; C07D 209/44

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,031,450	A	4/1962	Fischer et al.
3,322,755	A	5/1967	Roch et al.
3,920,636	A	11/1975	Takahashi et al.
4,001,237	A	1/1977	Partyka et al.
4,001,238	A	1/1977	Partyka et al.
4,047,404	A	9/1977	Hayashi
4,060,615	A	11/1977	Matier et al.
4,101,548	A	7/1978	Crenshaw et al.
4,162,316	A	7/1979	Nishimura et al.
4,209,623	A	6/1980	Juby
4,880,810	A	11/1989	Lowe, III
4,885,301	A	12/1989	Coates
5,147,875	A	9/1992	Coates et al.
5,354,571	A	10/1994	Morikawa et al.
5,401,774	A	3/1995	Pamukcu et al.
5,439,895	A	8/1995	Lee et al.
5,488,055	A	1/1996	Kumar et al.
5,608,914	A	3/1997	Keesler
5,614,530	A	3/1997	Kumar et al.
5,614,627	A	3/1997	Takase et al.
5,658,940	A	8/1997	Muller et al.
5,698,579	A	12/1997	Muller
5,703,098	A	12/1997	Muller et al.
5,710,170	A	1/1998	Guay et al.
5,728,844	A	3/1998	Muller et al.
5,728,845	A	3/1998	Muller et al.
5,736,570	A	4/1998	Muller et al.
5,798,373	A	8/1998	Warrellow
5,801,195	A	9/1998	Muller et al.
5,849,770	A	12/1998	Head et al.

(Continued)

FOREIGN PATENT DOCUMENTS

EP	0 347 146	6/1989
EP	0 349 239 A2	6/1989
EP	0 351 058	6/1989
EP	0 352 960 A2	7/1989
EP	0 395 328	4/1990

(Continued)

OTHER PUBLICATIONS

U.S. Appl. No. 60/366,515, filed Mar. 20, 2002, Muller, G. et al.
U.S. Appl. No. 60/436,975, filed Dec. 30, 2002, Muller, G. et al.
U.S. Appl. No. 60/438,448, filed Jan. 7, 2003, Muller, G. et al.
U.S. Appl. No. 60/454,149, filed Mar. 12, 2003, Muller, G. et al.
U.S. Appl. No. 60/454,155, filed Mar. 12, 2003, Muller, G. et al.

(Continued)

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(57) **ABSTRACT**

Stereomerically pure (+)-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, substantially free of its (−) isomer, and prodrugs, metabolites, polymorphs, salts, solvates, hydrates, and clathrates thereof are discussed. Also discussed are methods of using and pharmaceutical compositions comprising the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione are disclosed. The methods include methods of treating and/or preventing disorders ameliorated by the reduction of levels of TNF-α or the inhibition of PDE4.

33 Claims, 2 Drawing Sheets

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,877,200	A	3/1999	Muller	
5,891,896	A	4/1999	Warrellow et al.	
6,011,050	A	1/2000	Muller et al.	
6,020,339	A	2/2000	Perrier et al.	
6,020,358	A	2/2000	Muller et al.	
6,034,089	A	3/2000	Han et al.	
6,046,221	A	4/2000	Muller et al.	
6,069,156	A	5/2000	Oku et al.	
6,162,830	A	12/2000	Connor et al.	
6,166,041	A	12/2000	Cavalla et al.	
6,177,471	B1	1/2001	Menander et al.	
6,204,275	B1	3/2001	Friesen et al.	
6,218,400	B1	4/2001	Daugan et al.	
6,300,335	B1	10/2001	Campbell et al.	
6,316,472	B1	11/2001	Frenette et al.	
6,333,354	B1	12/2001	Schudt	
6,962,940	B2	11/2005	Muller et al.	
7,208,516	B2 *	4/2007	Muller	A61K 31/4035 514/417
7,276,529	B2	10/2007	Muller et al.	
7,358,272	B2	4/2008	Muller et al.	
7,427,638	B2	9/2008	Muller et al.	
7,507,759	B2 *	3/2009	Muller	A61K 31/4035 514/417
7,659,302	B2 *	2/2010	Muller	A61K 31/4035 514/417
7,659,303	B2 *	2/2010	Muller	A61K 31/4035 514/417
8,455,536	B2 *	6/2013	Muller	A61K 31/4035 514/417
8,802,717	B2 *	8/2014	Muller	A61K 31/4035 514/417
9,283,207	B2 *	3/2016	Muller	A61K 31/4035

FOREIGN PATENT DOCUMENTS

EP	0 428 268	A2	10/1990
EP	0 463 756	A1	6/1991
EP	0 526 004	A1	7/1992
EP	0 607 439	A1	9/1992
EP	0 722 937	A1	1/1996
EP	0 722 943	A1	1/1996
EP	0 722 944	A1	1/1996
EP	1752148	B1	6/2010
GB	2 063 249	A	6/1981
WO	WO 93/07149		4/1993
WO	WO 93/12095		6/1993
WO	WO 94/01728		1/1994
WO	WO 94/05661		3/1994
WO	WO 94/29277		12/1994
WO	WO 95/19978		7/1995
WO	WO 96/32379		10/1996
WO	WO 97/03070		1/1997
WO	WO 97/03675		2/1997
WO	WO 97/03985		2/1997
WO	WO 97/24334		7/1997
WO	WO 98/06722		2/1998
WO	WO 98/08848		3/1998
WO	WO 98/14448		4/1998
WO	WO 98/16521		4/1998
WO	WO 98/17668		4/1998
WO	WO 98/23597		6/1998
WO	WO 98/38168		9/1998
WO	WO 99/06041		2/1999
WO	WO 00/25777	A1	5/2000

OTHER PUBLICATIONS

Akazome, M. et al., 1997, "Asymmetric recognition of 1-arylethylamines by (R)-phenylglycyl-(R)-phenylglycine and its mechanism," Tetrahedron: Asymmetry, Elsevier Scince Publishers, Amsterdam, NL, 8(14):2331-2336.

Au et al., 1998, "Effect of PDE4 inhibitors on zymosan-induced IL-8 release from human neutrophils: synergism with prostanoids and salbutamol," *Brit. J. Pharm.* 123:1260-1266.

Baehr et al., 1979, "Isolation and characterization of cGMP phosphodiesterase from bovine rod outer segments," *J. Biol. Chem.* 254:11669.

Baughman et al., 1990, "Release of tumor necrosis factor by alveolar macrophages of patients iwth sarcoidosis," *J. Lab. Clin. Med.* 115:36-42.

Beavo and Reifsnyder, 1990 "Primary sequence of cyclic nucleotide phosphodiesterase isozymes and the design of selective inhibitors," *Trends in Pharm.*, 11:150-55.

Bissonnette et al., 1989, "Pulmonary inflammation and fibrosis in a murine model of asbestosis and silicosis. Possible role of tumor necrosis factor," *Inflammation* 13:329-339.

Bloom and Beavo 1996, "Identification and tissue-specific expression of PDE7 phosphodiesterase splice variants," *Proc. Natl. Acad. Sci. USA* 93:14188-14192.

Brackeen, M.F. et al., 1995, "Design and synthesis of conformationally constrained analogues of 4-(3-butoxy-4-methoxybenzyl) imidazolidin-2-one (Ro 20-1724) as potent inhibitors of cAMP-specific phosphodiesterase", *J. Med. Chem.* 38:4848-54.

Bryn et al., "Pharmaceutical solids: a strategic approach to regulatory considerations," *Pharm. Res.*, 12(7):945-954 (1995).

Caira, "Crystalline polymorphism of organic compounds," *Top. Curr. Chem.*, 198:163-208 (1998).

Carstensen, Jens T., 1995, *Drug Stability: Principles & Practice*, 2nd ed., Marcel Dekker, New York, NY pp. 379-380.

Casini et al., 1964, "Preparation of one of the optical antipodes of 2-phthalimidoglutarimide," Farmaco Ed. Sci. 19:563.

Celgene press release, "Apremilast ESTEEM program meets primary and major secondary endpoint in pivotal phase III psoriasis trials," dated Jan. 7, 2013.

Celgene press release, "Positive phase IIB topline clinical data for Celgene oral compound apremilast (CC-10004) reported for patients with moderate-to-severe psoriasis," Dec. 15, 2009.

Chemistry in Britain ,"Side-effects kill new prozac," p. 11 (2000).

Clouse et al., 1989, "Monokine regulation of human immunodeficiency virus-1 expression in a chronically infected human T cell clone," *J. Immunol.* 142:431-438.

Decision of the Appeal Board with Minutes of the Oral Proceedings, Mar. 17, 2015, in Opposition to EP Patent No. 1485087.

Decision with Minutes of the Oral Proceedings, dated May 19, 2011, in Opposition of EP Patent No. 1485087.

Derian et al., 1995, "Inhibition of chemotactic peptide-induced neutrophil adhesion to vascular endothelium by cAMP modulators," J. Immunol. 154:308-317.

Dredge et al., 2002, "Immunological effects of thalidomide and its chemical and functional analogs," *Crit. Rev. Immunol.* 22(5-6):425-437.

Dredge et al., 2002, "Recent developments in antiangiogenic therapy," *Expert Opin. Biol. Ther.* 2(8):953-966.

Dredge et al., 2003, "Angiogenesis inhibitors in cancer therapy," *Curr. Opin. Investig. Drugs* 4(6):667-674.

Duh et al., 1989, "Tumor necrosis factor alpha activates human immunodeficiency virus type 1 through induction of nuclear factor binding to the NF-kappa B sites in the long terminal repeat," *Proc. Nat. Acad. Sci.* 86:5974-5978.

European Commission's Decision to grant Marketing Authorization for Otezla, dated Jan. 15, 2015.

Featherstone, R.L., et al., 2000, "Comparison of phosphodiesterase inhibitors of differing isoenzyme selectivity added to St. Thomas' hospital cardioplegic solution used for hypothermic preservation of rat lungs", Am. J. Respir. Crit. Care Med. 162:850-6.

Gee et al., 2003, "Selective cytokine inhibitory drugs with enhanced antiangiogenic activity control tumor growth through vascular inhibition," *Cancer Res.* 63(23):8073-8078.

Gillespie et al., 1989, "Inhibition and stimulation of photoreceptor phosphodiesterases by dipyridamole and M&B 22,948," *Mol. Pharm.* 36:773-781.

(56) **References Cited**

OTHER PUBLICATIONS

Gottlieb et al., "An open-label, single-arm pilot study in patients with severe plaque-type psoriasis treated with an oral anti-inflammatory agent, apremilast," Current Medical Research and Opinion, 24(5):1529-1538 (2008).

Grant, "Theory and origin of polymorphosim," in *Polymoprhis in Pharmaceuticals Solids*, Ed. H. G. Brittain, Marcel Dekker, Inc., New York, NY, pp. 1-33 (1999).

Guillory, "Generation of polymophs, hydrates, solvates, and amorphous solids," in *Polymoprhis in Pharmaceuticals Solids*, Ed. H. G. Brittain, Marcel Dekker, Inc., New York, NY, pp. 183-226 (1999).

Hidaka and Asano 1976, "Human blood platelet 3': 5'-cyclic nucleotide phosphodiesterase. Isolation of low-Km and high-Km phosphodiesterase," *Biochem. Biophys. Acta* 429:485.

Hinshaw et al. 1990, "Survival of primates in LD100 septic shock following therapy with antibody to tumor necrosis factor (TNF alpha)," *Circ. Shock* 30:279-292.

Holler et al., 1990, "Increased serum levels of tumor necrosis factor alpha precede major complications of bone marrow transplantation," *Blood* 75:1011-1016.

Johnson et al., 1989, "Tumors producing human tumor necrosis factor induced hypercalcemia and osteoclastic bone resorption in nude mice," *Endocrinology* 124:1424-1427.

List et al., 1990, "The myelodysplastic syndromes: biology and implications for management.," *J. Clin. Oncol.* 8:1424-1441.

Lorenz et al., "Perspectives for TNF-α-targeting therapies," Arthritis Res., 4(suppl 3):S17-S24 (2002).

Luke, G.P. et al., 1999, "Synthesis of (S)-5-(1-aminoethyl)-2-(cyclohexylmethoxy) benzamide," Tetrahedron: Asymmetry, Elsevier Science Publishers, Amsterdam, NL, 10(22):4393-4403.

Marriott et al., 2001, "Immunotherapeutic and antitumour potential of thalidomide analogues," *Expert Opin. Biol. Ther.* 1(4):1-8.

Marriott et al., 2002, "Thalidomide and its analogues have distinct and opposing effects on TNF-alpha and TNFR2 during co-stimulation of both CD4(+) and CD8(+) T cells," *Clin. Exp. Immunol.* 130(1):75-84.

Merck Manual (1999) 17th ed., pp. 953.

Molostvov et al., 2004, "The effects of selective cytokine inhibitory drugs (CC-10004 and CC-1088) on VEGF and IL-6 expression and apoptosis in myeloma and endothelial cell co-cultures," *Br. J. Haematol.*124(3):366-375.

Monté et al., 1992, "Productive human immunodeficiency virus-1 infection of megakaryocytic cells is enhanced by tumor necrosis factor-alpha," *Blood* 79:2670-2679.

Muller et al., 1998, "Thalidomide analogs and PDE4 inhibition," *Bioorg. & Med Chem Lett.* 8:2669-2674.

Muller, et al., 1996, "Structural modifications of thalidomide produce analogs with enhanced tumor necrosis factor inhibitory activity," *J. Med. Chem.* 39:3238-3240.

Muller, George, et al., 1999, "Amino-substituted thalidomide analogs: potent inhibitors of TNF-alpha production.," *Bioorganic & Medicinal Chemistry Letters* 9:1625-1630.

Nicholson et al., 1991, "Differential modulation of tissue function and therapeutic potential of selective inhibitors of cyclic nucleotide phosphodiesterase isoenzymes," *Trends Pharmaco. Sci.* 12:19-27.

Notice of Opposition dated May 26, 2010, in Opposition of EP Patent No. 1485087.

Opponent's Response to Grounds of Appeal, dated Feb. 3, 2012, in Opposition of EP Patent No. 1485057.

Opponent's response to Summons to attend Oral Proceedings dated Feb. 3, 2015, in Opposition to EP Patent No. 1485087.

Papp et al., "Oral apremilast is active in the treatment of moderate to severe placque psoriasis," J. Am. Acad. Dermatol., p. 3308 (2011).

Patentee's Grounds of Appeal, dated Sep. 19, 2011, in Opposition of EP Patent No. 1485087.

Patentee's Petition for Review by Enlarged Appeal Board, dated Sep. 30, 2015, in Opposition to EP Patent No. 1485087.

Patentee's Response dated Jun. 25, 2012, in Opposition of EP Patent No. Patent No. 1485057; and revised Annex A, dated Jan. 16, 2013.

Patentee's Response to Notice of Opposition dated Oct. 29, 2010, in Opposition of EP Patent No. 1485087.

Patentee's Response to Summons to attend Oral Proceedings dated Feb. 16, 2015, in Opposition to EP Patent No. 1485087.

Poole et al., "Apremilast: first global approval," Drugs, 825-837 (2014).

Shealy et al., 1965, "D- and L-thalidomide." *Chem. Indus.* 12;24:1030-1031.

Strand et al., "Improvede quality of life with apremilast (APR) in the treatment of psoriasis: results from a phase IIb randomized controlled study," J. Am. Acad. Dermatol., p. 3337 (2011).

Summary of Product Characteristics (Otezla) dated Feb. 16, 2015.

Tencer et al., "Economic evaluation of sequencing strategies in the treatment of moderate to severe psoriasis in the United States," EADV (2014).

Tierney, et al., ed., 1998, Current Medical Diagnosis & Treatment, 37th ed., Appleton & Lange, pp. 499.

Van Dullemen et al., 1995, "Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2)," *Gastroenterology*, 109:129-135.

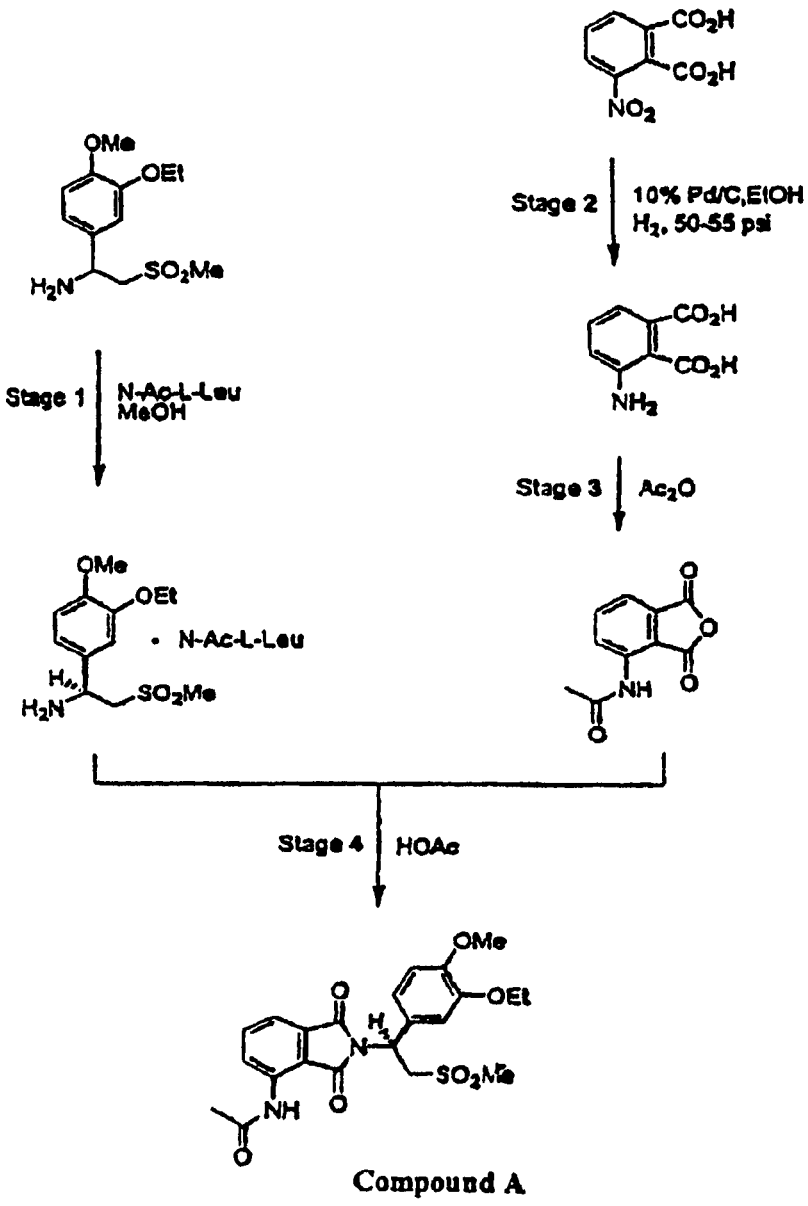
Verghese, et al.,1995, "Differential regulation of human monocyte-derived TNF alpha and IL-1 beta by type IV cAMP-phosphodiesterase (cAMP-PDE) inhibitors," *J. Pharmacol. Exp. Ther.*, 272(3), 1313-1320.

Wilen, S.H., 1972, *Tables of Resolving Agents and Optical Resoutions* (E.L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, IN) p. 268.

Wilen, S.H., et al., 1977, "Strategies in optical resolutions," *Tetrahedron* 33:2725-2736.

Wolff, Manfred E., *ed., Burger's Medicinal Chemistry and Drug Discovery*, 5th ed. 1995 172-178, 949-982.

* cited by examiner



Compound A

FIG. 1

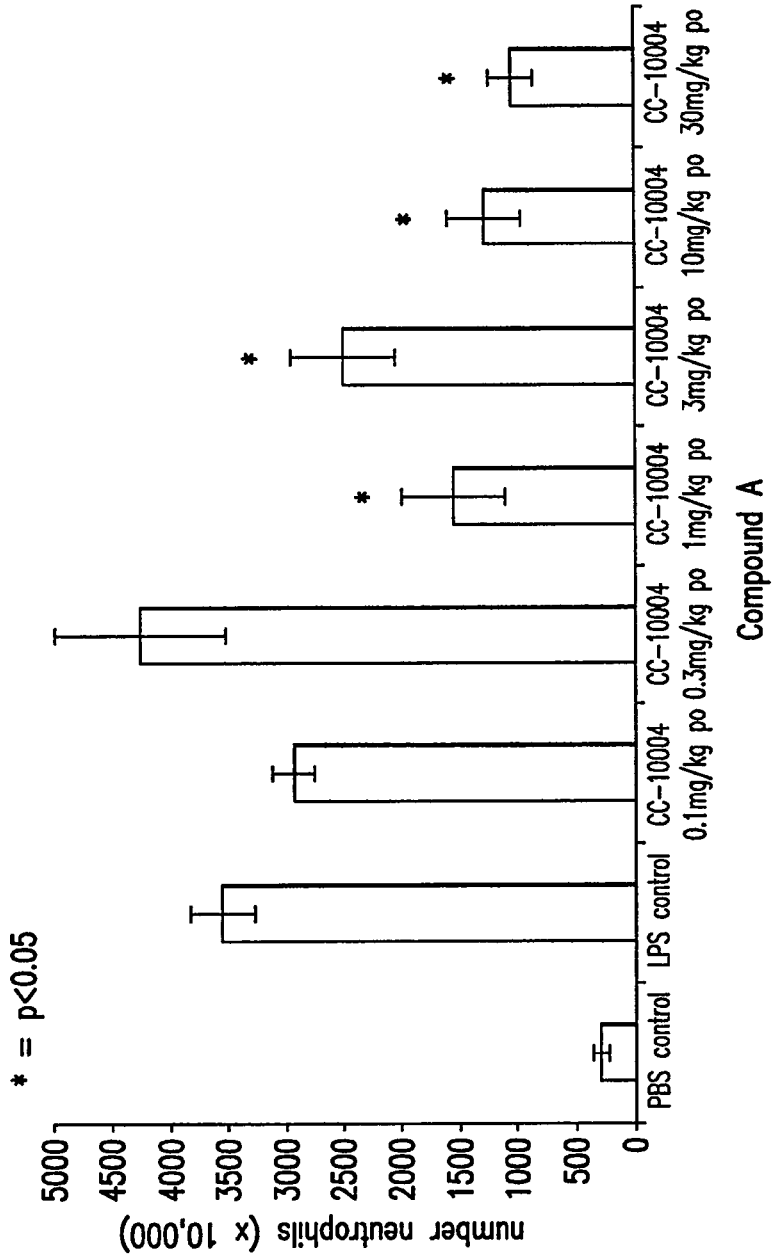

* = p<0.05
number neutrophils (x 10,000)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
PBS control
LPS control
CC-10004 0.1mg/kg po
CC-10004 0.3mg/kg po
CC-10004 1mg/kg po
CC-10004 3mg/kg po
CC-10004 10mg/kg po
CC-10004 30mg/kg po
Compound A

FIG.2

METHODS OF USING (+)-2-[1-(3-ETHOXY-4-METHOXYPHENYL)-2-METHYL-SULFONYLETHYL]-4-ACETYLAMINO-ISOINDOLINE-1,3-DIONE

This application is a continuation application of U.S. patent application Ser. No. 14/316,160, filed Jun. 26, 2014, which is a continuation of Ser. No. 13/682,652, filed Nov. 20, 2012, now U.S. Pat. No. 8,802,717, which is a continuation application of U.S. patent application Ser. No. 12/630,788, filed Dec. 3, 2009, now U.S. Pat. No. 8,455,536, which is a continuation application of U.S. patent application Ser. No. 12/098,379, filed Apr. 4, 2008, now U.S. Pat. No. 7,659,303, which is a divisional application of U.S. patent application Ser. No. 11/170,308, filed Jun. 28, 2005, now U.S. Pat. No. 7,358,272, which is a divisional application of U.S. patent application Ser. No. 10/392,195, filed Mar. 19, 2003, now U.S. Pat. No. 6,962,940, which claims the benefit of U.S. Provisional Application No. 60/366,515 filed Mar. 20, 2002 and U.S. Provisional Application No. 60/438,450 filed Jan. 7, 2003, all of which are incorporated herein by reference in their entireties.

1. FIELD OF INVENTION

The invention relates to methods of using and compositions comprising the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methysulfonylethyl]-4-acetylamin-oisoindoline-1,3-dione.

2. BACKGROUND OF THE INVENTION

Tumor necrosis factor alpha, (TNF-α) is a cytokine that is released primarily by mononuclear phagocytes in response to immunostimulators. TNF-α is capable of enhancing most cellular processes, such as differentiation, recruitment, proliferation, and proteolytic degradation. At low levels, TNF-α confers protection against infective agents, tumors, and tissue damage. But TNF-α also has a role in many diseases. When administered to mammals or humans, TNF-α causes or aggravates inflammation, fever, cardiovascular effects, hemorrhage, coagulation, and acute phase responses similar to those seen during acute infections and shock states. Enhanced or unregulated TNF-α production has been implicated in a number of diseases and medical conditions, for example, cancers, such as solid tumors and blood-born tumors; heart disease, such as congestive heat failure; and viral, genetic, inflammatory, allergic, and autoimmune diseases.

Adenosine 3',5'-cyclic monophosphate (cAMP) also plays a role in many diseases and conditions, such as but not limited to asthma and inflammation, and other conditions (Lowe and Cheng, *Drugs of the Future,* 17(9), 799-807, 1992). It has been shown that the elevation of cAMP in inflammatory leukocytes inhibits their activation and the subsequent release of inflammatory mediators, including TNF-α and NF-κB. Increased levels of cAMP also leads to the relaxation of airway smooth muscle.

It is believed that the primary cellular mechanism for the inactivation of cAMP is the breakdown of cAMP by a family of isoenzymes referred to as cyclic nucleotide phosphodiesterases (PDE) (Beavo and Reitsnyder, *Trends in Pharm.,* 11, 150-155, 1990). There are eleven known PDB families. It is recognized, for example, that the inhibition of PDE type IV is particularly effective in both the inhibition of inflammatory mediator release and the relaxation of airway smooth muscle (Verghese, et al., *Journal of Pharmacology and Experimental Therapeutics,* 272(3), 1313-1320, 1995). Thus, compounds that inhibit PDE4 (PDE IV) specifically, may inhibit inflammation and aid the relaxation of airway smooth muscle with a minimum of unwanted side effects, such as cardiovascular or anti-platelet effects. Currently used PDE4 inhibitors lack the selective action at acceptable therapeutic doses.

Cancer is a particularly devastating disease, and increases in blood TNF-α levels are implicated in the risk of and the spreading of cancer. Normally, in healthy subjects, cancer cells fail to survive in the circulatory system, one of the reasons being that the lining of blood vessels acts as a barrier to tumor-cell extravasation. But increased levels of cytokines have been shown to substantially increase the adhesion of cancer cells to endothelium in vitro. One explanation is that cytokines, such as TNF-α, stimulate the biosynthesis and expression of a cell surface receptors called ELAM-1 (endothelial leukocyte adhesion molecule). ELAM-1 is a member of a family of calcium-dependent cell adhesion receptors, known as LEC-CAMs, which includes LBCAM-1 and GMP-140. During an inflammatory response, ELAM-1 on endothelial cells functions as a "homing receptor" for leukocytes. Recently, ELAM-1 on endothelial cells was shown to mediate the increased adhesion of colon cancer cells to endothelium treated with cytokines (Rice et al., 1989, *Science* 246:1303-1306).

Inflammatory diseases such as arthritis, related arthritic conditions (e.g., osteoarthritis and rheumatoid arthritis), inflammatory bowel disease (e.g., Crohn's disease and ulcerative colitis), sepsis, psoriasis, atopic dermatitis, contact dermatitis, and chronic obstructive pulmonary disease, chronic inflammatory pulmonary diseases are also prevalent and problematic ailments. TNF-α plays a central role in the inflammatory response and the administration of their antagonists block chronic and acute responses in animal models of inflammatory disease.

Enhanced or unregulated TNF-α production has been implicated in viral, genetic, inflammatory, allergic, and autoimmune diseases. Examples of such diseases include but are not limited to: HIV; hepatitis; adult respiratory distress syndrome; bone-resorption diseases; chronic obstructive pulmonary diseases; chronic pulmonary inflammatory diseases; asthma, dermatitis; cystic fibrosis; septic shock; sepsis; endotoxic shock; hemodynamic shock; sepsis syndrome; post ischemic reperfusion injury, meningitis; psoriasis; fibrotic disease; cachexia; graft rejection; autoimmune disease; rheumatoid spondylitis; arthritic conditions, such as rheumatoid arthritis and osteoarthritis; osteoporosis; Crohn's disease; ulcerative colitis; inflammatory-bowel disease; multiple sclerosis; systemic lupus erythrematosus; ENL in leprosy; radiation damage; asthma; and hyperoxic alveolar injury. Tracey et al., 1987, *Nature* 330:662-664 and Hinshaw at al., 1990, *Circ. Shock* 30:279-292 (endotoxic shock); Dezube et al., 1990, *Lancet,* 335:662 (cachexia); Millar et al., 1989, *Lancet* 2:712-714 and Ferrai-Baliviera et al., 1989, *Arch. Surg.* 124:1400-1405 (adult respiratory distress syndrome); Bertolini et al., 1986, *Nature* 319:516-518, Johnson et al., 1989, *Endocrinology* 124:1424-1427, Holler et al., 1990, *Blood* 75:1011-1016, and Grau et al., 1989, *N. Engl. J. Med.* 320:1586-1591 (bone resorption diseases); Pignet at al, 1990, *Nature,* 344:245-247, Bissonnetto et al., 1989, *Inflammation* 13:329-339 and Baughman et at, 1990, *J. Lab. Clin. Med.* 115:36-42 (chronic pulmonary inflammatory diseases); Elliot et al., 1995, *Int. J. Pharmac.* 17:141-145 (rheumatoid arthritis); von Dullemen at al., 1995, *Gastroenterology,* 109:129-135 (Crohn's disease); Duh et al., 1989, *Proc. Nat. Acad. Sci.* 86:5974-5978,

Poll et al., 1990, *Proc. Nat. Acad. Sci.* 87:782-785, Monto et al., 1990, *Blood* 79:2670, Clouse st al., 1989, *J. Immunol.* 142, 431-438, Poll et al., 1992, *AIDS Res. Hum. Retrovirus,* 191-197, Poli et al. 1990, Proc. *Natl. Acad. Sci.* 87:782-784, Folks at al., 1989, PNAS 86:2365-2368 (HIV and opportunistic infections resulting from HIV).

Pharmaceutical compounds that can block the activity or inhibit the production of certain cytokines, including TNF-α, may be beneficial therapeutics. Many small-molecule inhibitors have demonstrated an ability to treat or prevent inflammatory diseases implicated by TNF-α (for a review, see Lowe, 1998 *Exp. Opin. Ther. Patents* 8:1309-1332). One such class of molecules are the substituted phenethylsulfones described in U.S. Pat. No. 6,020,358.

3. SUMMARY OF THE INVENTION

This invention relates to methods of treating diseases and disorders utilizing an enantiomer of a substituted phenethylsulfone compound and pharmaceutically acceptable salts, hydrates, solvates, clathrates, prodrugs and polymorphs thereof and methods for reducing the level of cytokines and their precursors in mammals. The invention also relates to pharmaceutical compositions comprising an enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione and a pharmaceutically acceptable carrier. The invention further relates to an enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione substantially free of its other enantiomer.

This invention particularly relates to the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione. This compound is believed to have increased potency and other benefits as compared to its racemate-2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

The invention encompasses the use of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione for treating or preventing diseases or disorders ameliorated by the inhibition of TNF-α production in mammals. In certain embodiments, this treatment includes the reduction or avoidance of adverse effects. Such disorders include, but are not limited to, cancers, including, but not limited to cancer of the head, thyroid, neck, eye, skin, mouth, throat, esophagus, cheat, bone, blood, bone marrow, lung, colon, sigmoid, rectum, stomach, prostate, breast, ovaries, kidney, liver, pancreas, brain, intestine, heart, adrenal, subcutaneous tissue, lymph nodes, heart, and combinations thereof. Specific cancers that can be treated by this method are multiple myeloma, malignant melanoma, malignant glioma, leukemia and solid tumors.

The invention also encompasses the use of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione in the treatment or prevention of heart disease, including, but not limited to congestive heart failure, cardiomyopathy, pulmonary edema, endotoxin-mediated septic shock, acute viral myocarditis, cardiac allograft rejection, and myocardial infarction.

The invention also encompasses the use of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione to treat diseases or disorders ameliorated by the inhibition of PDE4. For example, the compounds and compositions of the invention may be useful to treat or prevent viral, genetic, inflammatory, allergic, and autoimmune diseases. Examples of such diseases include, but are not limited to: HIV; hepatitis; adult respiratory distress syndrome; bone-resorption diseases; chronic obstructive pulmonary diseases; chronic pulmonary inflammatory diseases; dermatitis; inflammatory skin disease, atopic dermatitis, cystic fibrosis; septic shock; sepsis; endotoxic shock; hemodynamic shock; sepsis syndrome; post ischemic reperfusion injury; meningitis; psoriasis; fibrotic disease; cachexia; graft rejection including graft versus host disease; auto-immune disease; rheumatoid spondylitis; arthritic conditions, such as rheumatoid arthritis and osteoarthritis; osteoporosis; Crohn's disease; ulcerative colitis; inflammatory-bowel disease; multiple sclerosis; systemic lupus erythrematosus; erythema nodosum leprosum (ENL) in leprosy; radiation damage; asthma; and hyperoxic alveolar injury.

In yet another embodiment, the stereomerically pure (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is also useful in the treatment or prevention of microbial infections or the symptoms of microbial infections including, but not limited to, bacterial infections, fungal infections, malaria, mycobacterial infection, and opportunistic infections resulting from HIV.

The invention further encompasses pharmaceutical compositions and single unit dosage forms comprising an enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione and pharmaceutically acceptable polymorphs, prodrugs, salts, hydrates, clathrates, and solvates thereof.

In a separate embodiment, the invention encompasses the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

In a further embodiment, the invention encompasses a method of producing a stereomerically pure enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione which comprises contacting 1-(3-Ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethylamine with a chiral amino acid and contacting the product of the first step with N-(1,3-Dioxo-1,3-dihydro-isobenzofuran-4-yl)-acetamide. In a related embodiment the invention encompasses a chiral salt of 1-(3-Ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethylamine.

3.1. BRIEF DESCRIPTION OF THE FIGURES

FIG. **1**. illustrates the preparation of the (+) enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

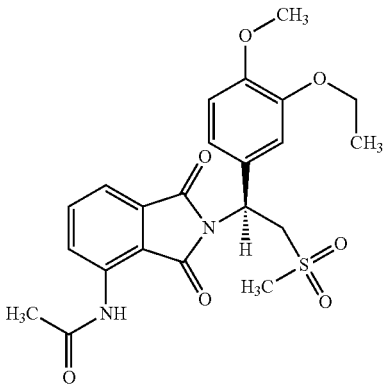
FIG. **2**. illustrates the effect of the enantiomer of the invention on LPS-induced neutrophilia in the lungs of conscious ferrets.

3.2. DEFINITIONS

As used herein, term "Compound A" refers to an enantiomerically pure form of 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione which comes off of an HPLC column at about 25.4 minutes when that column is a 150 mm×4.6 mm Ultron Chiral ES-OVS chiral HPLC column (Agilent Technology), the eluent is 15:85 ethanol: 20 mM KH_2PO_4 at pH 3.5, and the observation wavelength is 240 nm. The ^1H NMR spectrum of compound A is substantially as follows: δ($CDCl_3$): 1.47 (t, 3H), 2.26 (s, 3H), 2.87 (s, 3H), 3.68-3.75 (dd, 1H), 3.85 (s, 3H), 4.07-4.15 (q, 2H), 4.51-4.61 (dd, 1H), 5.84-5.90 (dd, 1H), 6.82-8.77 (m, 6H), 9.46 (s, 1H). The ^{13}C

NMR spectrum of Compound A is substantially as follows δ(DMSO-d_6): 14.66, 24.92, 41.61, 48.53, 54.46, 55.91, 64.51, 111.44, 112.40, 115.10, 118.20, 120.28, 124.94, 129.22, 131.02, 136.09, 137.60, 148.62, 149.74, 167.46, 169.14, 169.48. Compound A dissolved in methanol also rotates plane polarized light in the (+) direction.

Without being limited by theory, Compound A is believed to be S-{2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione}, which has the following structure:



As used herein, the term "patient" refers to a mammal, particularly a human.

As used herein, the term "pharmaceutically acceptable salts" refer to salts prepared from pharmaceutically acceptable non-toxic acids or bases including inorganic acids and bases and organic acids and bases. Suitable pharmaceutically acceptable base addition salts for the compound of the present invention include metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from lysine, N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. Suitable non-toxic acids include, but are not limited to, inorganic and organic acids such as acetic, alginic, anthranilic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethenesulfonic, formic, fumaric, furoic, galacturonic, gluconic, glucuronic, glutamic, glycolic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phenylacetic, phosphoric, propionic, salicylic, stearic, succinic, sulfanilic, sulfuric, tartaric acid, and p-toluenesulfonic acid. Specific non-toxic acids include hydrochloric, hydrobromic, phosphoric, sulfuric, and methanesulfonic acids. Examples of specific salts thus include hydrochloride and mesylate salts.

As used herein and unless otherwise indicated, the term "prodrug" means a derivative of a compound that can hydrolyze, oxidize, or otherwise react under biological conditions (in vitro or in vivo) to provide the compound. Examples of prodrugs include, but are not limited to, derivatives and metabolites of Compound A that include biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Prodrugs can typically be prepared using well-known methods, such as those described by 1 *Burger's Medicinal Chemistry and Drug Discovery,* 172-178, 949-982 (Manfred E. Wolff ed., 5th ed. 1995).

As used herein and unless otherwise indicated, the terms "biohydrolyzable amide," "biohydrolyzable ester," "biohydrolyzable carbamate," "biohydrolyzable carbonate," "biohydrolyzable ureide." "biohydrolyzable phosphate" mean an amide, ester, carbamate, carbonate, ureide, or phosphate, respectively, of a compound that either: 1) does not interfere with the biological activity of the compound but can confer upon that compound advantageous properties in vivo, such as uptake, duration of action, or onset of action; or 2) is biologically inactive but is converted in vivo to the biologically active compound. Examples of biohydrolyzable esters include, but are not limited to, lower alkyl esters, alkoxyacyloxy esters, alkyl acylamino alkyl esters, and choline esters. Examples of biohydrolyzable amides include, but are not limited to, lower alkyl amides, α-amino acid amides, alkoxyacyl amides, and alkylaminoalkylcarbonyl amides. Examples of biohydrolyzable carbamates include, but are not limited to, lower alkylamines, substituted ethylenediamines, aminoacids, hydroxyalkylamines, heterocyclic and heteroaromatic amines, and polyether amines.

As used herein and unless otherwise indicated, the term "stereomerically pure" means a composition that comprises one stereoisomer of a compound and is substantially free of other stereoisomers of that compound. For example, a stereomerically pure composition of a compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically pure composition of a compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, more preferably greater than about 90% by weight of one stereoisomer of the compound and less than about 10% by weight of the other stereoisomers of the compound, even more preferably greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, and most preferably greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound.

As used herein and unless otherwise indicated, the term "enantiomerically pure" means a stereomerically pure composition of a compound having one chiral center.

As used herein, term "adverse effects" includes, but is not limited to gastrointestinal, renal and hepatic toxicities, leukopenia, increases in bleeding times due to, e.g., thrombocytopenia, and prolongation of gestation, nausea, vomiting, somnolence, asthenia, dizziness, teratogenicity, extra-pyramidal symptoms, akathisia, cardiotoxicity including cardiovascular disturbances, inflammation, male sexual dysfunction, and elevated serum liver enzyme levels. The term "gastrointestinal toxicities" includes but is not limited to gastric and intestinal ulcerations and erosions. The term "renal toxicities" includes but is not limited to such conditions as papillary necrosis and chronic interstitial nephritis.

As used herein and unless otherwise indicated, the phrases "reduce or avoid adverse effects" and "reducing or avoiding adverse effects" mean the reduction of the severity of one or more adverse effects as defined herein.

It should be noted that if there is a discrepancy between a depicted structure and a name given that structure, the depicted structure is to be accorded more weight. In addition, if the stereochemistry of a structure or a portion of a structure is not indicated with, for example, bold or dashed

lines, the structure or portion of the structure is to be interpreted as encompassing all stereoisomers of it.

4. DETAILED DESCRIPTION OF THE INVENTION

This invention relates to stereomerically pure Compound A, which is an enantiomer of 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, substantially free of its other enantiomer, as well as novel methods using, and compositions comprising stereomerically pure Compound A. For example, the present invention encompasses the in vitro and in vivo use of Compound A, and the incorporation of Compound A into pharmaceutical compositions and single unit dosage forms useful in the treatment and prevention of a variety of diseases and disorders. Diseases and disorders which are ameliorated by the reduction of levels of TNF-α or inhibition of PDE4 are well known in the art and are described herein. Specific methods of the invention reduce or avoid the adverse effects associated with compounds used as TNF-α inhibitor. Other specific methods of the invention reduce or avoid the adverse effects associated with use of racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

Specific methods of the invention include methods of treating or preventing diseases and disorders including, but not limited to, solid tumor cancers, blood-born cancers and inflammatory diseases.

Pharmaceutical and dosage forms of the invention, which comprise Compound A or a pharmaceutically acceptable polymorph, prodrug, salt, clathrate, solvate or hydrate thereof, can be used in the methods of the invention.

Without being limited by theory, it is believed that Compound A can inhibit TNF-α production. Consequently, a first embodiment of the invention relates to a method of inhibiting TNF-α production which comprises contacting a cell exhibiting abnormal TNF-α production with an effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. In a particular embodiment, the invention relates to a method of inhibiting TNF-α production which comprises contacting a mammalian cell exhibiting abnormal TNF-α production with an effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof.

The invention also relates to a method of treating or preventing disorders ameliorated by the reduction of levels of TNF-α in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof.

A farther embodiment of the invention relates to a method of treating or preventing cancer, including but not limited to, solid tumor, blood-born tumor, leukemias, and in particular, multiple myeloma in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically effective amount of stereomerically pure compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof, in particular wherein the patient is a mammal.

In another embodiment, the invention relates to a method of inhibiting PDE4 which comprises contacting PDE4 with an effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof.

In another embodiment, the invention relates to a method of controlling cAMP levels in a cell which comprises contacting a cell with an effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. As used herein the term "controlling cAMP levels" includes preventing or reducing the rate of the breakdown of Adenosine 3',5'-cyclic monophosphate (cAMP) in a cell or increasing the amount of Adenosine 3',5'-cyclic monophosphate present in a cell, preferably a mammalian cell, more preferably a human cell. In a particular method, the rate of cAMP breakdown is reduced by about 10, 25, 50, 100, 200, or 500 percent as compared to the rate in comparable cells which have not been contacted with a compound of the invention.

A further embodiment of the invention relates to a method of treating or preventing diseases or disorders ameliorated by the inhibition of PDE4 in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. Disorders ameliorated by the inhibition of PDE4 include, but are not limited to, asthma, inflammation (e.g., inflammation due to reperfusion), chronic or acute obstructive pulmonary diseases, chronic or acute pulmonary inflammatory diseases, inflammatory bowel disease, Crohn's Disease, Bechet's Disease, or colitis.

A further embodiment of the invention relates to a method of treating or preventing depression, asthma, inflammation (e.g., contact dermatitis, atopic dermatitis, psoriasis, rheumatoid arthritis, osteoarthritis, inflammatory skin disease, inflammation due to reperfusion), chronic or acute obstructive pulmonary diseases, chronic or pulmonary inflammatory diseases, inflammatory bowel disease, Crohn's Disease, Bechet's Disease or colitis in a patient which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof, in particular wherein the patient is a mammal.

A separate embodiment of the invention encompasses methods of treating or preventing Myelodysplastic syndrome (MDS) which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. MDS refers to a diverse group of hematopoietic stem cell disorders. MDS is characterized by a cellular marrow with impaired morphology and maturation (dysmyelopoiesis), peripheral blood cytopenias, and a variable risk of progression to acute leukemia, resulting from ineffective blood cell production. See The Merck Manual 953 (17th ed. 1999) and List et al., 1990, *J. Clin. Oncol.* 8:1424.MDS

A separate embodiment of the invention encompasses methods of treating or preventing Myeloproliferative disease (MPD) which comprises administering to a patient in need of such treatment or prevention a therapeutically or prophylactically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. Myeloproliferative disease (MPD) refers to a group of disorders

characterized by clonal abnormalities of the hematopoietic stem cell. See e.g., Current Medical Diagnosis & Treatment, pp. 499 (37th ed., Tierney et al. ed, Appleton & Lange, 1998).

The invention also encompasses a method of treating, preventing or managing complex regional pain syndrome, which comprises administering to a patient in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of a stereomerically pure Compound A, or a pharmaceutically acceptable salt, solvate, hydrate, stereoisomer, clathrate, or prodrug thereof. In a specific embodiment, the administration is before, during or after surgery or physical therapy directed at reducing or avoiding a symptom of complex regional pain syndrome in the patient.

In particular methods of the invention, stereomerically pure Compound A, or a pharmaceutically acceptable polymorph, prodrug, salt, solvate, hydrate, or clathrate thereof is adjunctively administered with at least one additional therapeutic agent. Examples of additional therapeutic agents include, but are not limited to, anti-cancer drugs, anti-inflammatories, antihistamines and decongestants.

4.1. Synthesis and Preparation

Racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is readily prepared using the methods in U.S. Pat. No. 6,020,358, which is incorporated herein by reference.

Compound A can be isolated from the racemic compound by techniques known in the art. Examples include, but are not limited to, the formation of chiral salts and the use of chiral or high performance liquid chromatography "HPLC" and the formation and crystallization of chiral salts. See, e.g., Jacques, J., et al., *Enantiomers, Racemates and Resolutions* (Wiley-Interscience, New York, 1981); Wilen, S. H., et al., *Tetrahedron* 33:2725 (1977); Eliel, E. L., *Stereochemistry of Carbon Compounds* (McGraw-Hill, N.Y., 1962); and Wilen, S. H., *Tables of Resolving Agents and Optical Resolutions* p. 268 (E. L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, Ind., 1972).

In a specific method, Compound A is synthesized from 3-acetamidophthalic anhydride and a chiral amino acid salt of (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine. Chiral amino acid salts of (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine include, but not limited to salts formed with the L isomers of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, omithine, 4-aminobutyric acid, 2 amino isobutyric acid, 3 amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, and N-acetyl-leucine. A specific chiral amino acid salt is (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine N-acetyl-L-leucine salt, which is resolved from 2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine and N-acetyl-L-leucine in methanol.

4.2. Methods of Treatment

The invention encompasses methods of treating and preventing diseases or disorders ameliorated by the reduction of levels of TNF-α in a patient which comprise administering to a patient in need of such treatment or prevention a therapeutically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof.

Disorders ameliorated by the inhibition of TNF-α include, but are not limited to: heart disease, such as congestive heart failure, cardiomyopathy, pulmonary edema, endotoxin-mediated septic shock, acute viral myocarditis, cardiac allograft rejection, and myocardial infarction; solid tumors, including but not limited to, sarcoma, carcinomas, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, Kaposi's sarcoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma; and blood-born tumors including but not limited to, acute lymphoblastic leukemia "ALL", acute lymphoblastic B-cell leukemia, acute lymphoblastic T-cell leukemia, acute myeloblastic leukemia "AML", acute promyelocytic leukemia "APL", acute monoblastic leukemia, acute erythroleukemic leukemia, acute megakaryoblastic leukemia, acute myelomonocytic leukemia, acute nonlymphocyctic leukemia, acute undifferentiated leukemia, chronic myelocytic leukemia "CML", chronic lymphocytic leukemia "CLL", hairy cell leukemia, multiple myeloma and acute and chronic leukemias, for example, lymphoblastic, myelogenous, lymphocytic, and myelocytic leukemias.

Specific methods of the invention further comprise the administration of an additional therapeutic agent (i.e., a therapeutic agent other than Compound A). Examples of additional therapeutic agents include, but are not limited to, anti-cancer drugs such as, but are not limited to: alkylating agents, nitrogen mustards, ethylenimines, methylmelamines, alkyl sulfonates, nitrosoureas, triazenes, folic acid analofs, pyrimidine analogs, purine analogs, vinca alkaloids, epipodophyllotoxins, antibiotics, topoisomerase inhibitors and anti-cancer vaccines.

Specific additional therapeutic agents include, but are not limited to: acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer, carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cisplatin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; dactinomycin; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; docetaxel; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflomithine hydrochlo-

ride; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; flurocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; ilmofosine; interleukin II (including recombinant interleukin II or rIL2), interferon alfa-2a; interferon alfa-2b; interferon alfa-n1; interferon alfa-n3; interferon beta-I a; interferon gamma-1 b; iproplatin; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedepa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper, mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; paclitaxel; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; rogletimide; safingol; safingol hydrochloride; semustine, simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur, talisomycin; tecogalan sodium; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; triciribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin hydrochloride. Other anti-cancer drugs include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor, bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor, carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor, cytostatin; daclximab; decitabine; dehydrodidemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl spiromustine; docetaxel; docosanol; dolasetron doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflomithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor, interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor, multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor, platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; pro-

tein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor, retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rehitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor, stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

The invention further encompasses a method of treating or preventing diseases or disorders ameliorated by the inhibition of PDE4 in a patient which comprise administering to a patient in need of such treatment or prevention a therapeutically effective amount of stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. Disorders ameliorated by the inhibition of PDE4 include, but are not limited to, asthma, inflammation, chronic or acute obstructive pulmonary disease, chronic or acute pulmonary inflammatory disease, inflammatory bowel disease, Crohn's Disease, Bechet's Disease, colitis, ulcerative colitis and arthritis or inflammation due to reperfusion. In a preferred embodiment, the disease or disorder to be treated or prevented is chronic obstructive pulmonary disease.

Specific methods of the invention can comprise the administration of an additional therapeutic agent such as, but not limited to, anti-inflammatory drugs, antihistamines and decongestants. Examples of such additional therapeutic agents include, but are not limited to: antihistamines including, but not limited to, ethanolamines, ethylenediamines, piperazines, and phenothiazines; antinflammatory drugs; NSAIDS, including, but not limited to, aspirin, salicylates, acetominophen, indomethacin; sulindac, etodolac, fenamates, tolmetin, ketorolac, diclofenac, ibuprofen, naproxen, fenoprofen, ketoprofen, flurbiprofen, oxaprozin, piroxicam, meloxicam, pyrazolon derivatives; and steroids including, but not limited to, cortical steroids and adrenocortical steroids.

Specific methods of the invention avoid or reduce drug-drug interactions and other adverse effects associated with agents used in the treatment of such disorders, including racemic substituted phenylethylsulfones. Without being limited by any theory, stereomerically pure Compound A may further provide an overall improved therapeutic effectiveness, or therapeutic index, over racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione. For example, a smaller amount of the drug may in some circumstances be administered to attain the same level of effectiveness.

As stated above, the active compound of the invention (i.e., Compound A) may be used in the treatment or prevention of a wide range of diseases and conditions. The magnitude of a prophylactic or therapeutic dose of a particular active ingredient of the invention in the acute or chronic management of a disease or condition will vary, however, with the nature and severity of the disease or condition, and the route by which the active ingredient is administered. The dose, and perhaps the dose frequency, will also vary according to the age, body weight, and response of the individual patient. Suitable dosing regimens can be readily selected by those skilled in the art with due consideration of such factors. In general, the recommended daily dose range for the conditions described herein lie within the range of from about 1 mg to about 1000 mg per day, given as a single once-a-day dose preferably as divided doses throughout a day. More specifically, the daily dose is administered twice daily in equally divided doses. Specifically, a daily dose range should be from about 5 mg to about 500 mg per day, more specifically, between about 10 mg and about 200 mg per day. Specifically, the daily dose may be administered in 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 50 mg, or 100 mg dosage forms. In managing the patient, the therapy should be initiated at a lower dose, perhaps about 1 mg to about 25 mg, and increased if necessary up to about 200 mg to about 1000 mg per day as either a single dose or divided doses, depending on the patient's global response. Alternatively, the daily dose is from 0.01 mg/kg to 100 mg/kg.

It may be necessary to use dosages of the active ingredient outside the ranges disclosed herein in some cases, as will be apparent to those of ordinary skill in the art. Furthermore, it is noted that the clinician or treating physician will know how and when to interrupt, adjust, or terminate therapy in conjunction with individual patient response.

The phrases "therapeutically effective amount", "prophylactically effective amount" and "therapeutically or prophylactically effective amount," as used herein encompasses the above described dosage amounts and dose frequency schedules. Different therapeutically effective amounts may be applicable for different diseases and conditions, as will be readily known by those of ordinary skill in the art. Similarly, amounts sufficient to treat or prevent such disorders, but insufficient to cause, or sufficient to reduce, adverse effects associated with racemic 2-[1-(3-Ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione are also encompassed by the above described dosage amounts and dose frequency schedules.

4.3. Pharmaceutical Compositions

Pharmaceutical compositions and single unit dosage forms comprising Compound A, or a pharmaceutically acceptable polymorph, prodrug, salt, solvate, hydrate, or clathrate thereof, are encompassed by the invention. Individual dosage forms of the invention may be suitable for oral, mucosal (including rectal, nasal, or vaginal), parenteral

(including subcutaneous, intramuscular, bolus injection, intraarterial, or intravenous), sublingual, transdermal, buccal, or topical administration.

Pharmaceutical compositions and dosage forms of the invention comprise stereomerically pure Compound A, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate, hydrate, or clathrate thereof. Pharmaceutical compositions and dosage forms of the invention typically also comprise one or more pharmaceutically acceptable excipients.

A particular pharmaceutical composition encompassed by this embodiment comprises stereomerically pure Compound A, or a pharmaceutically acceptable polymorph, prodrug, salt, solvate, hydrate, or clathrate thereof, and at least one additional therapeutic agent. Examples of additional therapeutic agents include, but are not limited to: anti-cancer drugs and anti-inflammation therapies including, but not limited to, those listed above in section 4.2.

Single unit dosage forms of the invention are suitable for oral, mucosal (e.g., nasal, sublingual, vaginal, buccal, or rectal), parenteral (e.g., subcutaneous, intravenous, bolus injection, intramuscular, or intraarterial), or transdermal administration to a patient. Examples of dosage forms include, but are not limited to: tablets; caplets; capsules, such as soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suppositories; ointments; cataplasms (poultices); pastes; powders; dressings; creams; plasters; solutions; patches; aerosols (e.g., nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a patient, including suspensions (e.g., aqueous or non-aqueous liquid suspensions, oil-in-water emulsions, or a water-in-oil liquid emulsions), solutions, and elixirs; liquid dosage forms suitable for parenteral administration to a patient; and sterile solids (e.g., crystalline or amorphous solids) that can be reconstituted to provide liquid dosage forms suitable for parenteral administration to a patient.

The composition, shape, and type of dosage forms of the invention will typically vary depending on their use. For example, a dosage form used in the acute treatment of inflammation or a related disorder may contain larger amounts of one or more of the active ingredients it comprises than a dosage form used in the chronic treatment of the same disease. Similarly, a parenteral dosage form may contain smaller amounts of one or more of the active ingredients it comprises than an oral dosage form used to treat the same disease or disorder. These and other ways in which specific dosage forms encompassed by this invention will vary from one another will be readily apparent to those skilled in the art. See, e.g., *Remington's Pharmaceutical Sciences,* 18th ed., Mack Publishing, Easton Pa. (1990).

Typical pharmaceutical compositions and dosage forms comprise one or more excipients. Suitable excipients are well known to those skilled in the art of pharmacy, and non-limiting examples of suitable excipients are provided herein. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a patient. For example, oral dosage forms such as tablets may contain excipients not suited for use in parenteral dosage forms. The suitability of a particular excipient may also depend on the specific active ingredients in the dosage form.

Lactose-free compositions of the invention can comprise excipients that are well known in the art and are listed, for example, in the U.S. Pharmocopia (USP) SP (XXI)/NF (XVI). In general, lactose-free compositions comprise an active ingredient, a binder/filler, and a lubricant in pharmaceutically compatible and pharmaceutically acceptable amounts. Preferred lactose-free dosage forms comprise an active ingredient, microcrystalline cellulose, pre-gelatinized starch, and magnesium stearate.

This invention further encompasses anhydrous pharmaceutical compositions and dosage forms comprising active ingredients, since water can facilitate the degradation of some compounds. For example, the addition of water (e.g., 5%) is widely accepted in the pharmaceutical arts as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. See, e.g., Jens T. Carstensen, *Drug Stability: Principles & Practice,* 2d. Ed., Marcel Dekker, NY, N.Y., 1995, pp. 379-80. In effect, water and heat accelerate the decomposition of some compounds. Thus, the effect of water on a formulation can be of great significance since moisture and/or humidity are commonly encountered during manufacture, handling, packaging, storage, shipment, and use of formulations.

Anhydrous pharmaceutical compositions and dosage forms of the invention can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms that comprise lactose and at least one active ingredient that comprises a primary or secondary amine are preferably anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected.

An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions are preferably packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Examples of suitable packaging include, but are not limited to, hermetically sealed foils, plastics, unit dose containers (e.g., vials), blister packs, and strip packs.

The invention further encompasses pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will decompose. Such compounds, which are referred to herein as "stabilizers," include, but are not limited to, antioxidants such as ascorbic acid, pH buffers, or salt buffers.

Like the amounts and types of excipients, the amounts and specific types of active ingredients in a dosage form may differ depending on factors such as, but not limited to, the route by which it is to be administered to patients. However, typical dosage forms of the invention comprise compound A, or a pharmaceutically acceptable salt, solvate, clathrate, hydrate, polymorph or prodrug thereof lie within the range of from about 1 mg to about 1000 mg per day, given as a single once-a-day dose in the morning but preferably as divided doses throughout the day taken with food. More specifically, the daily dose is administered twice daily in equally divided doses. Specifically, a daily dose range should be from about 5 mg to about 500 mg per day, more specifically, between about 10 mg and about 200 mg per day. In managing the patient, the therapy should be initiated at a lower dose, perhaps about 1 mg to about 25 mg, and increased if necessary up to about 200 mg to about 1000 mg per day as either a single dose or divided doses, depending on the patient's global response.

4.3.1. Oral Dosage Forms

Pharmaceutical compositions of the invention that are suitable for oral administration can be presented as discrete

dosage forms, such as, but are not limited to, tablets (e.g., chewable tablets), caplets, capsules, and liquids (e.g., flavored syrups). Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of pharmacy well known to those skilled in the art. See generally, *Remington's Pharmaceutical Sciences,* 18th ed., Mack Publishing, Easton Pa. (1990).

Typical oral dosage forms of the invention are prepared by combining the active ingredient(s) in an intimate admixture with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of preparation desired for administration. For example, excipients suitable for use in oral liquid or aerosol dosage forms include, but are not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, and coloring agents. Examples of excipients suitable for use in solid oral dosage forms (e.g., powders, tablets, capsules, and caplets) include, but are not limited to, starches, sugars, micro-crystalline cellulose, diluents, granulating agents, lubricants, binders, and disintegrating agents.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid excipients are employed. If desired, tablets can be coated by standard aqueous or non-aqueous techniques. Such dosage forms can be prepared by any of the methods of pharmacy. In general, pharmaceutical compositions and dosage forms are prepared by uniformly and intimately admixing the active ingredients with liquid carriers, finely divided solid carriers, or both, and then shaping the product into the desired presentation if necessary.

For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as powder or granules, optionally mixed with an excipient. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

Examples of excipients that can be used in oral dosage forms of the invention include, but are not limited to, binders, fillers, disintegrants, and lubricants. Binders suitable for use in pharmaceutical compositions and dosage forms include, but are not limited to, corn starch, potato starch, or other starches, gelatin, natural and synthetic gums such as acacia, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre-gelatinized starch, hydroxypropyl methyl cellulose, (e.g., Nos. 2208, 2906, 2910), microcrystalline cellulose, and mixtures thereof.

Examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein include, but are not limited to, talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, and mixtures thereof. The binder or filler in pharmaceutical compositions of the invention is typically present in from about 50 to about 99 weight percent of the pharmaceutical composition or dosage form.

Suitable forms of microcrystalline cellulose include, but are not limited to, the materials sold as AVICEL-PH-101, AVICEL-PH-103 AVICEL RC-581, AVICEL-PH-105 (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, Pa.), and mixtures thereof. An specific binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC-581. Suitable anhydrous or low moisture excipients or additives include AVICEL-PH-103™ and Starch 1500 LM.

Disintegrants are used in the compositions of the invention to provide tablets that disintegrate when exposed to an aqueous environment. Tablets that contain too much disintegrant may disintegrate in storage, while those that contain too little may not disintegrate at a desired rate or under the desired conditions. Thus, a sufficient amount of disintegrant that is neither too much nor too little to detrimentally alter the release of the active ingredients should be used to form solid oral dosage forms of the invention. The amount of disintegrant used varies based upon the type of formulation, and is readily discernible to those of ordinary skill in the art. Typical pharmaceutical compositions comprise from about 0.5 to about 15 weight percent of disintegrant, specifically from about 1 to about 5 weight percent of disintegrant.

Disintegrants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, agar-agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrilin potassium, sodium starch glycolate, potato or tapioca starch, pro-gelatinized starch, other starches, clays, other algins, other celluloses, gums, and mixtures thereof.

Lubricants that can be used in pharmaceutical compositions and dosage forms of the invention include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, and mixtures thereof. Additional lubricants include, for example, a syloid silica gel (AEROSIL 200, manufactured by W.R. Grace Co. of Baltimore, Md.), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Plano, Tex.), CAB-O-SIL (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, Mass.), and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about 1 weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated.

4.3.2. Delayed Release Dosage Forms

Active ingredients of the invention can be administered by controlled release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Pat. Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; and 4,008,719, 5,674,533, 5,059,595, 5,591,767, 5,120,548, 5,073,543, 5,639,476, 5,354,556, and 5,733,566, each of which is incorporated herein by reference. Such dosage forms can be used to provide slow or controlled-release of one or more active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the active ingredients of the invention. The invention thus encompasses single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled-release.

All controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug sub-

stance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition, controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the drug, and can thus affect the occurrence of side (e.g., adverse) effects.

Most controlled-release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled-release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, temperature, enzymes, water, or other physiological conditions or compounds.

4.3.3. Parenteral Dosage Forms

Parenteral dosage forms can be administered to patients by various routes including, but not limited to, subcutaneous, intravenous (including bolus injection), intramuscular, and intraarterial. Because their administration typically bypasses patients' natural defenses against contaminants, parenteral dosage forms are preferably sterile or capable of being sterilized prior to administration to a patient. Examples of parenteral dosage forms include, but are not limited to, solutions ready for injection, dry products ready to be dissolved or suspended in a pharmaceutically acceptable vehicle for injection, suspensions ready for injection, and emulsions.

Suitable vehicles that can be used to provide parenteral dosage forms of the invention are well known to those skilled in the art. Examples include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

Compounds that increase the solubility of one or more of the active ingredients disclosed herein can also be incorporated into the parenteral dosage forms of the invention.

4.3.4. Transdermal, Topical, and Mucosal Dosage Forms

Transdermal, topical, and mucosal dosage forms of the invention include, but are not limited to, ophthalmic solutions, sprays, aerosols, creams, lotions, ointments, gels, solutions, emulsions, suspensions, or other forms known to one of skill in the art. See, e.g., *Remington's Pharmaceutical Sciences,* 16th and 18th eds., Mack Publishing, Easton Pa. (1980 & 1990); and *Introduction to Pharmaceutical Dosage Forms.* 4th ed., Lee & Febiger, Philadelphia (1985). Dosage forms suitable for treating mucosal tissues within the oral cavity can be formulated as mouthwashes or as oral gels. Further, transdermal dosage forms include "reservoir type" or "matrix type" patches, which can be applied to the skin and worn for a specific period of time to permit the penetration of a desired amount of active ingredients.

Suitable excipients (e.g., carriers and diluents) and other materials that can be used to provide transdermal, topical, and mucosal dosage forms encompassed by this invention are well known to those skilled in the pharmaceutical arts, and depend on the particular tissue to which a given pharmaceutical composition or dosage form will be applied. With that fact in mind, typical excipients include, but are not limited to, water, acetone, ethanol, ethylene glycol, propylene glycol, butane-1,3-diol, isopropyl myristate, isopropyl palmitate, mineral oil, and mixtures thereof to form lotions, tinctures, creams, emulsions, gels or ointments, which are non-toxic and pharmaceutically acceptable. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well known in the art. See, e.g., *Remington's Pharmaceutical Sciences,* 16th and 18th eds., Mack Publishing, Easton Pa. (1980 & 1990).

Depending on the specific tissue to be treated, additional components may be used prior to, in conjunction with, or subsequent to treatment with active ingredients of the invention. For example, penetration enhancers can be used to assist in delivering the active ingredients to the tissue. Suitable penetration enhancers include, but are not limited to: acetone; various alcohols such as ethanol, oleyl, and tetrahydrofuryl; alkyl sulfoxides such as dimethyl sulfoxide; dimethyl acetamide; dimethyl formamide; polyethylene glycol; pyrrolidones such as polyvinylpyrrolidone; Kollidon grades (Povidone, Polyvidone); urea; and various water-soluble or insoluble sugar esters such as Tween 80 (polysorbate 80) and Span 60 (sorbitan monostearate).

The pH of a pharmaceutical composition or dosage form, or of the tissue to which the pharmaceutical composition or dosage form is applied, may also be adjusted to improve delivery of one or more active ingredients. Similarly, the polarity of a solvent carrier, its ionic strength, or tonicity can be adjusted to improve delivery. Compounds such as stearates can also be added to pharmaceutical compositions or dosage forms to advantageously alter the hydrophilicity or lipophilicity of one or more active ingredients so as to improve delivery. In this regard, stearates can serve as a lipid vehicle for the formulation, as an emulsifying agent or surfactant, and as a delivery-enhancing or penetration-enhancing agent. Different salts, hydrates or solvates of the active ingredients can be used to further adjust the properties of the resulting composition.

4.3.5. Kits

Typically, active ingredients of the invention are preferably not administered to a patient at the same time or by the same mute of administration. This invention therefore encompasses kits which, when used by the medical practitioner, can simplify the administration of appropriate amounts of active ingredients to a patient.

A typical kit of the invention comprises a unit dosage form of compound A, or a pharmaceutically acceptable salt, solvate, hydrate, clathrate, polymorph or prodrug thereof, and a unit dosage form of a second active ingredient. Examples of second active ingredients include, but are not limited to, those listed in section 4.2 above.

Kits of the invention can further comprise devices that are used to administer the active ingredient(s). Examples of such devices include, but are not limited to, syringes, drip bags, patches, and inhalers.

Kits of the invention can further comprise pharmaceutically acceptable vehicles that can be used to administer one or more active ingredients. For example, if an active ingredient is provided in a solid form that must be reconstituted for parenteral administration, the kit can comprise a sealed container of a suitable vehicle in which the active ingredient can be dissolved to form a particulate-free sterile solution that is suitable for parenteral administration. Examples of

pharmaceutically acceptable vehicles include, but are not limited to: Water for Injection USP; aqueous vehicles such as, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

5. EXAMPLES

5.1. Example 1

Synthesis of 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione

A stirred solution of 1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethylamine (1.0 g, 3.7 mmol) and 3-acetamidophthalic anhydride (751 mg, 3.66 mmol) in acetic acid (20 mL) was heated at reflux for 15 h. The solvent was removed in vacuo to yield an oil. Chromatography of the resulting oil yielded the product as a yellow solid (1.0 g. 59% yield): mp, 144° C.; ^{1}H NMR ($CDCl_3$) δ1.47 (t, J=7.0 Hz, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.88 (s, 3H, CH_3), 3.75 (dd, J=4.4, 14.3 Hz, 1H, CHH), 3.85 (s, 3H, CH3), 4.11 (q, J=7 Hz, 2H, CH2), 5.87 (dd, J=4.3, 10.5 Hz, 1H, NCH), 6.82-6.86 (m, 1H, Ar), 7.09-7.11 (m, 2H, Ar), 7.47 (d, J=7 Hz, 1H, Ar), 7.64 (t, J=8 Hz, 1H, Ar), 8.74 (d, J=8 Hz, 1H, Ar), 9.49 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ14.61, 24.85, 41.54, 48.44, 54.34, 55.85, 64.43, 111.37, 112.34, 115.04, 118.11, 120.21, 124.85, 129.17, 130.96, 136.01, 137.52, 148.54, 149.65, 167.38, 169.09, 169.40; Anal Calc'd. for $C_{22}H_{24}NO_7S$: C, 57.38; H, 5.25; N, 6.08. Found: C, 57.31; H, 5.34; N, 5.83.

5.2. Example 2

Synthesis of (+)2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione

Preparation of 3-Aminopthalic Acid

10% Pd/C (2.5 g), 3-nitrophthalic acid (75.0 g, 355 mmol) and ethanol (1.5 L) were charged to a 2.5 L Parr hydrogenator, under a nitrogen atmosphere. Hydrogen was charged to the reaction vessel for up to 55 psi. The mixture was shaken for 13 hours, maintaining hydrogen pressure between 50 and 55 psi. Hydrogen was released and the mixture was purged with nitrogen 3 times. The suspension was filtered through a celite bed and rinsed with methanol. The filtrate was concentrated in vacuo. The resulting solid was reslurried in ether and isolated by vacuum filtration. The solid was dried in vacuo to a constant weight, affording 54 g (84% yield) of 3-aminopthalic acid as a yellow product. ^{1}H-NMR (DMSO-d6) δ: 3.17 (s, 2H), 6.67 (d, 1H), 6.82 (d, 1H), 7.17 (t, 1H), 8-10 (brs, 2H). ^{13}C-NMR (DMSO-d6) δ: 112.00, 115.32, 118.20, 131.28, 135.86, 148.82, 169.15, 170.09.

Preparation of 3-Acetamidophthalic Anhydride

A 1 L 3-necked round bottom flask was equipped with a mechanical stirrer, thermometer, and condenser and charged with 3-aminophthalic acid (108 g, 596 mmol) and acetic anhydride (550 mL). The reaction mixture was heated to reflux for 3 hours and cooled to ambient temperature and further to 0-5° C. for another 1 hour. The crystalline solid was collected by vacuum filtration and washed with ether. The solid product was dried in vacuo at ambient temperature to a constant weight, giving 75 g (61% yield) of 3-acetamidophthalic anhydride as a white product. ^{1}H-NMR ($CDCl_3$) δ: 2.21 (s, 3H), 7.76 (d, 1H), 7.94 (t, 1H), 8.42 (d, 1H), 9.84 (s, 1H).

Resolution of 2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine

A 3 L 3-necked round bottom flask was equipped with a mechanical stirrer thermometer, and condenser and charged with 2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine (137.0 g, 500 mmol), N-acetyl-L-leucine (52 g, 300 mmol), and methanol (1.0 L). The stirred slurry was heated to reflux for 1 hour. The stirred mixture was allowed to cool to ambient temperature and stirring was continued for another 3 hours at ambient temperature. The slurry was filtered and washed with methanol (250 mL). The solid was air-dried and then dried in vacuo at ambient temperature to a constant weight, giving 109.5 g (98% yield) of the crude product (85.8% ee). The crude solid (55.0 g) and methanol (440 mL) were brought to reflux for 1 hour, cooled to room temperature and stirred for an additional 3 hours at ambient temperature. The slurry was filtered and the filter cake was washed with methanol (200 mL). The solid was air-dried and then dried in vacuo at 30° C. to a constant weight, yielding 49.6 g (90% recovery) of (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine-N-acetyl-L-leucine salt (98.4% ee). Chiral HPLC (1/99 EtOH/20 mM KH_2PO_4@pH 7.0, Ultron Chiral ES-OVS from Agilent Technologies, 150 mm×4.6 mm, 0.5 mL/min., @240 nm): 18.4 min (S-isomer, 99.2%), 25.5 min (R-isomer, 0.8%).

Preparation of Compound A

A 500 mL 3-necked round bottom flask was equipped with a mechanical stirrer, thermometer, and condenser. The reaction vessel was charged with (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-yl amine N-acetyl-L-leucine salt (25 g, 56 mmol, 98% ee), 3-acetamidophthalic anhydride (12.1 g 58.8 mmol), and glacial acetic acid (250 mL). The mixture was refluxed over night and then cooled to <50° C. The solvent was removed in vacuo, and the residue was dissolved in ethyl acetate. The resulting solution was washed with water (250 mL×2), saturated aqueous $NaHCO_3$(250 mL×2), brine (250 mL×2), and dried over sodium sulphate. The solvent was evaporated in vacuo, and the residue recrystallized from a binary solvent containing ethanol (150 mL) and acetone (75 mL). The solid was isolated by vacuum filtration and washed with ethanol (100 mL×2). The product was dried in vacuo at 60° C. to a constant weight, affording 19.4 g (75% yield) of S-{2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-aminoisoindoline-1,3-dione with 98% ee. Chiral HPLC (15/85 EtOH/20 mM KH_2PO_4 @pH 3.5, Ultron Chiral ES-OVS from Agilent Technology, 150 mm×4.6 mm, 0.4 mL/min., @240 nm): 25.4 min (S-isomer, 98.7%), 29.5 min (R-isomer, 1.2%). ^{1}H-NMR ($CDCl_3$) δ: 1.47 (t, 3H), 2.26 (s, 3H), 2.87 (s, 3H), 3.68-3.75 (dd, 1H), 3.85 (s, 3H), 4.07-4.15 (q, 2H), 4.51-4.61 (dd, 1H), 5.84-5.90 (dd, 1H), 6.82-8.77 (m, 6H), 9.46 (s, 1H). ^{13}C-NMR (DMSO-d6) δ: 14.66, 24.92, 41.61, 48.53, 54.46, 55.91, 64.51, 111.44, 112.40, 115.10, 118.20, 120.28, 124.94, 129.22, 131.02, 136.09, 137.60, 148.62, 149.74, 167.46, 169.14, 169.48.

5.3. Example 3

TNF-α Inhibition

Human Whole Blood LPS-Induced TNF-α Assay

The ability of compounds to inhibit LPS-induced TNF-α production by human whole blood was measured essentially as described below for the LPS-induced TNF-α assay in human PBMC, except that freshly drawn whole blood was

used instead of PBMC. (George Muller, et al. 1999, *Bioorganic & Medicinal Chemistry Letters* 9; 1625-1630.) Human whole blood LPS-induced TNF-α IC_{50}-294 nM

Mouse LPS-Induced Serum TNF-α Inhibition

Compounds were tested in this animal model according to previously described methods (Corral et al. 1996, *Mol. Med* 2:506-515). Mouse LPS-induced serum TNF-α inhibition (ED_{50}, mg/kg, p.o.)=0.05.

LPS-Induced TNF-α Production

Lipopolysaccharide (LPS) is an endotoxin produced by gram-negative bacteria such as *E. coli* which induces production of many pro-inflammatory cytokines, including TNF-α. In peripheral blood mononuclear cells (PBMC), the TNF-α produced in response to LPS is derived from monocytes, which comprise approximately 5-20% of the total PBMC. Compounds were tested for the ability to inhibit LPS-induced TNF-α production from human PBMC as previously described (Muller et al. 1996, *J. Med Chem.* 39:3238). PBMC from normal donors were obtained by Ficoll Hypaque (Pharmacia, Piscataway, N.J., USA) density centrifugation. Cells were cultured in RPMI (Life Technologies, Grand Island, N.Y., USA) supplemented with 10% AB±human serum (Gemini Bio-products, Woodland, Calif., USA), 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Life Technologies).

PBMC (2×10^5 cells) were plated in 96-well flat-bottom Costar tissue culture plates (Corning, N.Y., USA) in triplicate. Cells were stimulated with LPS (Sigma, St. Louis, Mo., USA) at 100 ng/ml in the absence or presence of compounds. Compounds (Celgene Corp., Warren, N.J., USA) were dissolved in DMSO (Sigma) and further dilutions were done in culture medium immediately before use. The final DMSO concentration in all samples was 0.25%. Compounds were added to cells 1 hour before LPS stimulation. Cells were incubated for 18-20 hours at 37° C. in 5% CO_2 and supernatants were then collected, diluted with culture medium and assayed for TNF-α levels by ELISA (Endogen, Boston, Mass., USA). LPS-induced TNF-α IC_{50}-77 nM.

IL-1β-Induced TNF-α Production

During the course of inflammatory diseases, TNF-α production is often stimulated by the cytokine IL-1β, rather than by bacterially derived LPS. Compounds were tested for the ability to inhibit IL-1β-induced TNF-α production from human PBMC as described above for LPS-induced TNF-α production, except that the PBMC were isolated from source leukocyte units (Sera-Tec Biologicals, North Brunswick, N.J., USA) by centrifugation on Ficoll-Paque Plus (Amersham Pharmacia, Piscataway, N.J., USA), plated in 96-well tissue culture plates at 3×10^5 cells/well in RPMI-1640 medium (BioWhittaker, Walkersville, Md., USA) containing 10% heat-inactivated fetal bovine serum (Hyclone), 2 mM L-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin (complete medium), pretreated with compounds at 10, 2, 0.4, 0.08, 0.016, 0.0032, 0.00064, and 0 μM in duplicate at a final DMSO concentration of 0.1% at 37° C. in a humidified incubator at 5% CO_3 for 1 hour, then stimulated with 50 ng/ml recombinant human IL-1β (Endogen) for 18 hours. IL-β-induced TNF-α IC_{50}=83 nM.

5.4. Example 4

PDE Selectivity

PDE1, 2, 3, 5, and 6 Enzyme Assays

The specificity of compounds for PDE4 was assessed by testing at a single concentration (10 μM) against bovine PDE1, human PDE2, PDE3, and PDE5 from human platelets (Hidaka and Asano 1976, *Biochem. Biophys. Acta* 429:485, and Nicholsen et al. 1991, *Trends Pharmaco. Sci.* 12:19), and PDE6 from bovine retinal rod outer segments (Baehr at al. 1979, J. Biol. Chem. 254:11669, and Gillespie at al. 1989, *Mol. Pharm.* 36:773). Results are listed in Table 1.

PDE7 Enzyme Assay

PDE7 is a cAMP-selective PDB expressed mainly in T cells and in skeletal muscle. T cell-derived cytokines such as IL-2 and IFN-γ are potentially regulatable via PDE7 inhibition. PDE7 was purified from Hut78 human T cells by anion exchange chromatography as previously described (Bloom and Beavo 1996, *Poc. Natl. Acad Sci. USA* 93:14188-14192). Compounds were tested against the PDE7 preparation in the presence of 10 nM cAMP as described for PDE4 in Table 1 below.

TABLE 1

	Racemic Compound	Compound A	Compound B*
PDE Inhibition			
PDE4 IC_{50} (from U937 cells) (nM)	81.8	73.5	611
PDE1 (% inhib at 10 μM)	9%	23%	27%
PDE2 (% inhib at 10 μM)	19%	6%	10%
PDE3 (% inhib at 10 μM)	21%	20%	31%
PDE5 (% inhib at 10 μM)	3%	3%	−9%
PDE6 (% inhib at 10 μM)	ND	−6%	10%
PDE7 IC_{50} (nM)	22110	20500	ND
PDE Specificity Ratios from above data (*fold)			
PDE4/PDE1	>2700	>500	>50
PDF4/PDE2	>800	>10000	>260
PDP4/PDE3	>670	>1200	>45
PDE4/PDE5	>12000	>30000	>39000
PDE4/PDE6	ND	>40000	>250
PDE7 IC_{50}/PDE4 IC_{50}	270	279	ND

*Compound B is the opposite enantiomer of Compound A.

5.5. Example 5

PDE4 Inhibition

PDE4 (U937 Cell-Derived) Enzyme Assay

PDE4 enzyme was purified from U937 human monocytic cells by gel filtration chromatography as previously described (Muller at al. 1998, *Bioorg. & Med Chem Lett* 8:2669-2674). Phosphodiesterase reactions were carried out in 50 mM Tris HCl pH 7.5, 5 mM $MgCl_2$, 1 μM cAMP, 10 nM [^{3}H]-cAMP for 30 min at 30° C., terminated by boiling, treated with 1 mg/ml snake venom, and separated using AG-IXS ion exchange resin (BioRad) as described (Muller at al. 1998, *Bioorg. & Med Chem Lett* 8:2669-2674). Reactions consumed less than 15% of available substrate. Results are listed in Table 1.

5.6. Example 6

Human T Cell Assays

SEB-Induced IL-2 and IFN-γ Production

Staphylococcal Enterotoxin 8 (SEB) is a superantigen derived from gram-positive bacteria *Staphylococcus aureus*. SEB provides a convenient physiological stimulus specific for T cells expressing particular T cell receptor Vβ chains. Human PBMC (consisting of approximately 50% T cells)

were isolated from source leukocyte units as described above and plated in 96-well tissue culture plates at 3×10^5 cells/well in complete medium, pretreated with compounds at 10, 2, 0.4, 0.08, 0.016, 0.0032, 0.00064, and 0 μM in duplicate at a final DMSO concentration of 0.1% at 37° C. in a humidified incubator at 5% CO_2 for 1 hour, then stimulated with 100 ng/ml SEB (Sigma Chemical Co., St. Louis, Mo., USA) for 18 hours. IL-2 and IFN-γ levels were measured by ELISA (R&D Systems, Minneapolis, Minn., USA). IL-2 IC_{50}=291 nM. IFN-γ IC_{50}=46 nM.

5.7. Example 6

cAMP Elevation Assays

PGE_2-Induced cAMP Elevation

Prostaglandin E_2(PGE_2) binds to prostanoid receptors on monocytes, T cells and other leukocytes and consequently elevates intracellular cAMP levels, resulting in inhibition of cellular responses. The combination of PGE_2 and a PDE4 inhibitor synergistically elevates cAMP levels in these cell types, and the elevation of cAMP in PBMC caused by PDE4 inhibitors in the presence of PGE_2 is proportional to the inhibitory activity of that PDE4 inhibitor. Intracellular cAMP was measured in human PBMC as follows. PBMC were isolated as described above and plated in 96-well plates at 1×10^6 cells per well in RPMI-1640. The cells were pre-treated with compounds at 100, 10, 1, 0.1, 0.01, and 0 μM in a final concentration of 2% DMSO in duplicate at 37° C. in a humidified incubator at 5% CO_2 for one hour. The cells were then stimulated with PGE_2 (10 μM) (Sigma) for 1 h. The cells were lysed with HCl, 0.1 N final concentration to inhibit phosphodiesterase activity and the plates were frozen at −20° C. The cAMP produced was measured using cAMP (low pH) Immunoassay kit (R&D Systems). PBMC cAMP EC_{50} for racemate is 3.09 μM. PBMC cAMP EC_{50} for Compound A is 1.58 μM.

Elevation of cAMP in human neutrophils was measured as follows. PBMC were removed from source leukocytes (Sera-Tee Biologicals) by centrifugation on Ficoll-Paque Plus (Amersham Pharmacia). The resulting erythrocyte/polymorphonuclear cell (PMN) pellet was resuspended in Hank's Balanced Salt Solution (BioWhittaker) and mixed with an equal volume of 3% Dextran T-500 (Amersham Pharmacia) in 0.9% saline. Erythrocytes were allowed to sediment for 20 minutes, and the PMN were removed and centrifuged at 120 rpm for 8 minutes at 4° C. The remaining erythrocytes were lysed in cold 0.2% saline for 30 seconds, and the cells restored to isotonicity by the addition of an equal volume of 1.6% saline. The PMN were centrifuged at 1200 rpm for 8 minutes at 4° C., then resuspended in RPMI-1640 and assayed for cAMP elevation as described for PBMC above. PMN were found to be approximately 74% CD18/CD11b$^+$, 71% CD16$^+$CD9$^+$ neutrophils by flow cytometzy on a FACSCalibur (Becton Dickinson, San Jose, Calif., USA). Results are shown in Table 2.

fMLF-Induced LTB4 Production

N-formyl-methionine-leucine-phenylalanine (fMLF) is a bacterially derived peptide that activates neutrophils to rapidly degranulate, migrate, adhere to endothelial cells, and release leukotriene LTB4, a product of arachidonic acid metabolism and itself a neutrophil chemoattractant. Compounds were tested for the ability to block fMLF-induced neutrophil LTB4 production as previously described (Hatzelmann and Schudt 2001, *J. Pharm. Exp. Ther.* 297:267-279), with the following modifications. Neutrophils were isolated as described above and resuspended in phosphate-buffered saline without calcium or magnesium (BioWhittaker) containing 10 mM HEPES pH7.2 and plated in 96-well tissue culture plates at a concentration of 1.7×10^6 cells/well. Cells were treated with 50 μM thimerosal (Sigma)/1 mM $CaCl_2$/1 mM $MgCl_2$ for 15 minutes at 37° C. 5% CO_2, then treated with compounds at 1000, 200, 40, 8, 1.6, 0.32, 0.064, and 0 nM in a final DMSO concentration of 0.01% in duplicate for 10 minutes. Neutrophils were stimulated with 1 μM fMLF for 30 minutes, then lysed by the addition of methanol (20% final concentration) and frozen in a dry ice/isopropanol bath for 10 minutes. Lysates were stored at −70° C. until the LTB4 content was measured by competitive LTB4 ELISA (R&D Systems). Results are shown in Table 2.

Zymosan-Induced IL-8 Production

Zymosan A, or the heat-killed yeast *Saccharomyces cerevisiae*, binds to the adhesion molecule Mac-1 on the neutrophil surface and triggers phagocytosis, cell activation and IL-8 production. Zymosan-induced IL-8 production was measured as previously described (Au et al. 1998, *Brit. J. Pharm.* 123:1260-1266) with the following modifications. Human neutrophils were purified as described above, plated in 96-well tissue culture plates at 3×10^5 cells/well in complete medium, treated with compounds at 10, 2, 0.4, 0.08, 0.016, 0.0032, 0.00064, and 0 μM in duplicate in a final DMSO concentration of 0.1% for 1 hour at 37° C. 5% CO_2. Neutrophils were then stimulated with unopsonized, boiled Zymosan A (Sigma) at 2.5×10^5 particles/well for 18 hours. Supernatants were harvested and tested for IL-8 by ELISA (R&D Systems). Results are shown in Table 2.

fMLF-Induced CD18/CD11b Expression

CD18/CD11b (Mac-1) expression on neutrophils was measured as previously described (Derian at al. 1995, *J. Immunol.:* 154:308-317) with the following modifications. Neutrophils were isolated as described above, then resuspended in complete medium at 1×10^6 cells/ml, pretreated with compounds at 10, 1, 0.1, 0.01, and 0 μM in duplicate at a final DMSO concentration of 0.1% for 10 minutes at 37° C. 5% CO_2. Cells were then stimulated with 30 nM fMLF for 30 minutes and then chilled to 4° C. Cells were treated with rabbit IgG (Jackson ImmunoResearch Labs, West Grove, Pa., USA) (10 μg/1×10^6 cells) to block Fc receptors, stained with CD18-FITC and CD11b-PB (Becton Dickinson), and analyzed by flow cytometry on a FACSCalibur. CD18/CD11b expression (mean fluorescence) in the absence of stimulation was subtracted from all samples to obtain inhibition curves and calculate IC_{50}s. Results are shown in Table 2.

fMLF-Induced Adhesion to HUVEC

Human umbilical vein endothelial cells (HUVEC) were used as a substrate for neutrophil adhesion as previously described (Derian et al. 1995, *J. Immunol.:* 154:308-317) with the following modifications. HUVEC cells were obtained from Anthrogenesis (Cedar Knolls, N.J., USA), and neutrophils were not treated with cytochalasin B. Cells were treated with compounds at 10, 1, 0.1, 0.01, 0.001, and 0 μM in a final DMSO concentration of 0.1% in duplicate for 10 minutes, stimulated with 500 nM fMLF for 30 minutes, and washed twice with PBS before measuring fluorescence on an FLX800 plate reader (Bio-Tek Instruments, Winooski, Vt. USA). Results are shown in Table 2.

TABLE 2

Human Neutrophil Assays (all values in nM)	Racemic Compound	Compound A
PGE_2-induced cAMP EC_{50}	12589	4570
fMLF-induced LTB4 IC_{50}	20.1	2.48
Zymosan-induced IL-8 IC_{50}	ND	94
fMLF-induced CD18 expression IC_{50}	ND	390
fMLF-induced CD11b expression IC_{50}	ND	74
fMLF-induced adhesion to HUVEC IC_{50}	ND	150

5.8. Example 8

Aqueous Solubility

Equilibrium solubilities were measured in pH 7.4 aqueous buffer. The pH 7.4 buffer was prepared by adjusting the pH of a 0.07 M NaH_2PO_4 solution to 7.4 with 10 N NaOH. The ionic strength of the solution was 0.15. At least 1 mg of powder was combined with 1 ml of buffer to make >1 mg/ml mixture. These samples were shaken for >2 hours and left to stand overnight at room temperature. The samples were then filtered through a 0.45-μm Nylon syringe filter that was first saturated with the sample. The filtrate was sampled twice, consecutively. The filtrate was assayed by HPLC against standards prepared in 50% methanol. Compound A has 3.5-fold greater aqueous solubility than the racemic mixture. Measured solubility Compound A=0.012 mg/mL; racemic mixture=0.0034 mg/mL.

5.9. Example 8

LPS-Induced Lung Neutrophilia Ferret Model

The conscious ferret model has been used to investigate anti-inflammatory, emetic and behavioral effects of PDE4 inhibitors when administered by the oral (p.o.) route. From these experiments, a therapeutic index (TI) for each PDE4 inhibitor may be determined. The TI has been calculated by dividing the threshold dose for causing emetic episodes and behavioral changes by the anti-inflammatory dose (dose that causes 50% inhibition of the LPS-induced neutrophilia).

Animal Husbandry

Male ferrets (Mustela Pulorius Euro, weighing 1-2 kg). Ferrets were supplied either by Bury Green Farm or Misay Consultancy. Following transport, the animals were allowed to acclimatize in the holding rooms for a period of not less than 7 days. The Diet comprised SDS diet C pelleted food given ad lib with Whiskers cat food given 3 times per week. Water was pasteurized animal grade drinking water and was changed daily.

Dosing with PDE4 Inhibitor

PDE4 inhibitors were administered orally (p.o.), at doses initially of 1-10 mg/kg, but subsequently up to 30 m&/kg in order to establish whether the TI was 10 or higher, and/or at lower doses to establish the minimum dose to cause 50% inhibition of neutrophilia. Ferrets were fasted overnight but allowed free access to water. The animals were orally dosed with vehicle or PDE4 inhibitor using a 15 cm dosing needle that was passed down the back of the throat into the oesophagus. After dosing, the animals were returned to holding cages fitted with Perspex doors to allow observation, and given free access to water. After dosing, the animals were constantly observed and any emesis or behavioural changes were recorded. The animals were allowed access to food 60-90 minutes after p.o. dosing

Exposure to LPS

Thirty minutes after p.o. dosing with compound or vehicle control, the ferrets were placed into sealed Perspex containers and exposed to an aerosol of LPS (100 μg/ml) for 10 minutes. Aerosols of LPS were generated by a nebulizer (DeVilbiss, USA) and this was directed into the Perspex exposure chamber. Following a 10 minute exposure period, the animals were returned to the holding cages and allowed free access to water, and at a later stage, food. Observation continued for a period of at least 2.5 hours post p.o. dosing and emetic episodes and behavioral changes were recorded.

Bronchoalveolar Lavage

Six hours after LPS exposure the animals were killed by overdose of sodium pentobarbitone administered intraperitoneally. The trachea was then cannulated with polypropylene tubing and the lungs lavaged twice with 20 ml heparinized (10 units/ml) phosphate buffered saline (PBS).

Blood Sampling/Tissue Removal

A terminal blood sample (10 ml) was removed by transthoracic cardiac puncture. The blood was spun at 2500 rpm for 15 minutes and the plasma removed and stored at −20° C. The brain also removed and frozen at −20° C. for analysis of compound content.

Cell Counts

The bronchoalveolar lavage (BAL) samples were centrifuged at 1500 rpm for 5 minutes. The supernatant was removed and the resulting cell pellet re-suspended in 1 ml PBS. A cell smear of the re-suspended fluid was prepared and stained with Leishmans stain to allow differential cell counting. A total cell count was made using the remaining re-suspended sample. From this, the total number of neutrophils in the BAL was determined.

Parameters Measured:

1. % Inhibition of LPS-induced pulmonary neutrophilia.
2. Emetic episodes—the number of vomits and retches were counted.
3. Behavioral changes—the following behavioral effects were noted: salivation, panting, mouth clawing, flattened posture, ataxia, arched back and backward walking. Any behavioral changes were semi-quantified by applying a severity rating (mild, moderate or severe).
4. The TI was calculated as the highest dose found to not cause emetic episodes divided by the lowest dose found to inhibit pulmonary neutrophilia by 50% or more.

The effect of Compound A on LPS-induced neutrophilia in the lungs of conscious ferrets is demonstrated in FIG. **1**.

Emesis and Behavioral Changes

Following p.o. dosing of the PDE4, the ferrets were observed for at least 2 hours and emetic episodes (vomits and retches) and behavioral changes were recorded.

No emetic episodes (retching or vomiting) were observed in the ferrets pre-treated p.o. with the relevant vehicle (acetone/cremophor/distilled water). In a small proportion of the control-treated animals (7/22), mild behavioral changes (lip licking and backward walking) were seen.

Compound A (0.1-3 mg/kg, p.o.), caused no emetic episodes (retching and vomiting). Some behavioral changes (flattened posture, lip licking and backward walking) were observed and classified as mild. At 10 mg/kg in 2/6 ferrets, some retching but no frank emesis was observed along with salivation and behavioral changes (scored as mild or moderate). At the highest dose tested (30 mg/kg) moderate to marked emesis was observed in 3/4 animals along with pronounced behavioral changes. These data are summarized in Table III.

TABLE III

Conscious ferret: Emetic episodes and behavioural changes following oral administration of Compound A.

Treatment/dose (mg/kg)	Vomits	Retching	Salivation	Fasting	Megth clawing	Flattened posture	Ataxle	Lip licking	Backward walking
Vehicle (acetone/cremophor/ dist.H2O)	None	None	None	None	None	None	None	Mild (6/22)	Mild (7/22)

TABLE III-continued

Conscious ferret: Emetic episodes and behavioural changes following oral administration of Compound A.

Treatment/dose (mg/kg)	Vomits	Retching	Salivation	Fasting	Megth clawing	Flattened posture	Ataxle	Lip licking	Backward walking
Compound A (0.1 mg/kg)	None	None	None	None	None	Mild (2/5)	None	Mild (4/5)	Mild (3/5)
Compound A (0.3 mg/kg)	None	None	None	None	None	Mild (2/6)	None	Mild (3/6)	Mild (4/6)
Compound A (1.0 mg/kg)	None	None	None	None	None	Mild (2/6)	None	Mild (6/6)	Mild (4/6)
Compound A (3.0 mg/kg)	None	None	None	None	Mild (1/8)	Marked (7/8)	None	Mild (2/8)	Moderate (5/8)
Compound A (10 mg/kg)	None	Mild (2/6)	Mild (1/6)	None	Mild (1/6)	Marked (6/6)	None	Moderate (5/6)	Marked (6/6)
Compound A (30 mg/kg)	Moderate (3/4)	Marked (3/4)	Moderate (3/4)	Mild (1/4)	Marked (4/4)	Marked (4/4)	Mild (3/4)	Moderate (4/4)	Mild (2/4)

Animals were observed for up to 3 hours following dosing. Numbers in parentheses refer to the number of animals that responded The numbers of animals in each group range from 4-22.

Therapeutic Index Calculation

From these experiments, a therapeutic index (TI) was determined for each compound by dividing the threshold dose for inducing emetic episodes by the ED_{50} value for inhibiting the pulmonary neutrophilia. The TI calculation is summarized in Table IV. Compound A had a TI of 12, causing no emetic episodes at an anti-inflammatory dose of 1 mg/kg.

TABLE IV

Summary of the effective doses (ED_{50}) for inhibition of LPS-induced pulmonary neutrophilia and induction of emesis and the therapeutic index derived from these values.

Compound	Inhibition of LPS-induced neutrophilia (ED_{50} mg/kg)	Threshold emetic dose (mg/kg)	Therapeutic Index
Compound A	0.8	10	12

5.10. Example 9

200 Mg Dosage Capsule

Table V illustrates a batch formulation and single dosage formulation for a 200 mg Compound A single dose unit, i.e., about 40 percent by weight, in a size #0 capsule.

TABLE V

Formulation for 200 mg capsule

Material	Percent By Weight	Quantity (mg/tablet)	Quantity (kg/batch)
Compound A	40.0%	200 mg	16.80 kg
Pregelatinized Corn Starch, NF5	9.5%	297.5 mg	24.99 kg
Magnesium Stearate	0.5%	2.5 mg	0.21 kg
Total	100.0%	500 mg	42.00 kg

The pregelatinized corn starch (SPRESS B-820) and Compound A components are passed through a 710 μm screen and then are loaded into a Diffusion Mixer with a baffle insert and blended for 15 minutes. The magnesium stearate is passed through a 210 μm screen and is added to the Diffusion Mixer. The blend is then encapsulated in a size #0 capsule, 500 mg per capsule (8400 capsule batch size) using a Dosator type capsule filling machine.

5.11. Example 10

100 Mg Oral Dosage Form

Table VI illustrates a batch formulation and a single dose unit formulation containing 100 mg of Compound A.

TABLE VI

Formulation for 100 mg tablet

Material	Percent by Weight	Quantity (mg/tablet)	Quantity (kg/batch)
Compound A	40%	100.00	20.00
Microcrystalline Cellulose, NF	53.5%	133.75	26.75
Pluronic F-68 Surfactant	4.0%	10.00	2.00
Croscarmellose Sodium Type A, NF	2.0%	5.00	1.00
Magnesium Stearate, NF	0.5%	1.25	0.25
Total	100.0%	250.00 mg	50.00 kg

The microcrystalline cellulose, croscarmellose sodium, and Compound A components are passed through a #30 mesh screen (about 430μ to about 655μ). The Pluronic F-68® (manufactured by JRH Biosciences, Inc. of Lenexa, Kans.) surfactant is passed through a #20 mesh screen (about 457μ to about 1041μ). The Pluronic F-68® surfactant and 0.5 kgs of croscarmellose sodium are loaded into a 16 qt. twin shell tumble blender and are mixed for about 5 minutes. The mix is then transferred to a 3 cubic foot twin shell tumble blender where the microcrystalline cellulose is added and blended for about 5 minutes. The thalidomide is added and blended for an additional 25 minutes. This pre-blend is passed through a roller compactor with a hammer mill attached at the discharge of the roller compactor and moved back to the tumble blender. The remaining croscarmellose sodium and magnesium stearate is added to the tumble blender and blended for about 3 minutes. The final mixture is compressed on a rotary tablet press with 250 mg per tablet (200,000 tablet batch size).

5.12. Example 11

Aerosol Dosage Form

A concentrate is prepared by combining Compound A, and a 12.6 kg portion of the trichloromonofluoromethane in a sealed stainless steel vessel equipped with a high shear mixer. Mixing is carried out for about 20 minutes. The bulk suspension is then prepared in the sealed vessel by combining the concentrate with the balance of the propellants in a bulk product tank that is temperature controlled to 21° to 27° C. and pressure controlled to 2.8 to 4.0 BAR. 17 ml aerosol containers which have a metered valve which is designed to provide 100 inhalations of the composition of the invention. Each container is provided with the following:

Compound A	0.0120 g
trichloromonofluoromethane	1.6939 g
dichlorodifluoromethane	3.7175 g
dichlorotetrafluoroethane	1.5766 g
total	7.0000 g

While the invention has been described with respect to the particular embodiments, it will be apparent to those skilled in the art that various changes and modifications may be made without departing from the spirit and scope of the invention as defined in the claims. Such modifications are also intended to fall within the scope of the appended claims.

What is claimed is:

1. A method of treating an autoimmune disease, the method comprising administering to a patient having the autoimmune disease a therapeutically effective amount of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

2. The method of claim **1** further comprising administering to the patient a therapeutically effective amount of an antihistamine, anti-inflammatory drug, non-steroid anti-inflammatory drug, or steroid.

3. The method of claim **1** wherein the patient is a mammal.

4. The method of claim **1** wherein the compound is administered parenterally, transdermally, mucosally, nasally, buccally, sublingually, or orally.

5. The method of claim **4** wherein the compound is administered orally.

6. The method of claim **5** wherein the compound is administered orally in a tablet or capsule form.

7. The method of claim **1** wherein the therapeutically effective amount is from about 1 mg to about 1000 mg per day.

8. The method of claim **7** wherein the therapeutically effective amount is from about 5 mg to about 500 mg per day.

9. The method of claim **8** wherein the therapeutically effective amount is from about 10 mg to about 200 mg per day.

10. The method of claim **2** wherein the non-steroid anti-inflammatory drug is selected from aspirin, salicylates, acetominophen, indomethacin, sulindac, etodolac, fenamates, tolmetin, ketorolac, diclofenac, ibuprofen, naproxen, fenoprofen, ketoprofen, flurbiprofen, oxaprozin, piroxicam, meloxicam and a pyrazolon derivative.

11. The method of claim **5**, wherein the compound is administered twice daily in equally divided doses.

12. The method of claim **5**, wherein the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione comprises greater than about 90% by weight of (+) isomer based on the total weight percent of the compound.

13. The method of claim **5**, wherein the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione comprises greater than about 95% by weight of (+) isomer based on the total weight percent of the compound.

14. The method of claim **5**, wherein the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione comprises greater than about 97% by weight of (+) isomer based on the total weight percent of the compound.

15. The method of claim **6**, wherein the capsule contains about 10 mg of the compound.

16. The method of claim **6**, wherein the capsule contains about 20 mg of the compound.

17. The method of claim **6**, wherein the capsule contains about 25 mg of the compound.

18. The method of claim **6**, wherein the capsule contains about 50 mg of the compound.

19. The method of claim **6**, wherein the tablet contains about 10 mg of the compound.

20. The method of claim **6**, wherein the tablet contains about 20 mg of the compound.

21. The method of claim **6**, wherein the tablet contains about 25 mg of the compound.

22. The method of claim **6**, wherein the tablet contains about 50 mg of the compound.

23. The method of claim **1**, wherein the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione comprises about 98.7% by weight of (+) isomer based on the total weight percent of the compound.

24. The method of claim **23**, wherein the compound is administered orally in a tablet form.

25. The method of claim **24**, wherein the tablet is administered twice daily in equally divided doses.

26. The method of claim **25**, wherein the tablet contains about 10 mg of the compound.

27. The method of claim **25**, wherein the tablet contains about 20 mg of the compound.

28. The method of claim **24**, wherein the tablet is administered in a single, once-a-day dose.

29. The method of claim **28**, wherein the tablet contains about 100 mg of the compound.

30. The method of claim **24**, wherein the therapeutically effective amount is from about 1 mg to about 1000 mg per day.

31. The method of claim **24**, wherein the therapeutically effective amount is from about 5 mg to about 500 mg per day.

32. The method of claim **24**, wherein the therapeutically effective amount is from about 10 mg to about 200 mg per day.

33. The method of claim **24**, wherein the therapeutically effective amount is about 100 mg per day.

* * * * *

EXHIBIT I

US010092541B2

(12) **United States Patent**
Day

(10) **Patent No.: US 10,092,541 B2**
(45) **Date of Patent: *Oct. 9, 2018**

(54) **METHODS FOR THE TREATMENT OF DISEASES AMELIORATED BY PDE4 INHIBITION USING DOSAGE TITRATION OF APREMILAST**

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This patent is subject to a terminal disclaimer.

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(58) **Field of Classification Search**

None

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,536,809	A	10/1970	Applezweig	
3,598,123	A	8/1971	Zaffaroni	
3,845,770	A	11/1974	Theeuwes et al.	
3,916,899	A	11/1975	Theeuwes et al.	
4,008,719	A	2/1977	Theeuwes et al.	
5,059,595	A	10/1991	Le Grazie	
5,073,543	A	12/1991	Marshall et al.	
5,120,548	A	6/1992	McClelland et al.	
5,354,556	A	10/1994	Sparks et al.	
5,591,767	A	1/1997	Mohr et al.	
5,639,476	A	6/1997	Oshlack et al.	
5,674,533	A	10/1997	Santus et al.	
5,733,566	A	3/1998	Lewis	
6,020,358	A	2/2000	Muller et al.	
6,962,940	B2	11/2005	Muller et al.	
7,208,516	B2	4/2007	Muller et al.	
7,276,529	B2	10/2007	Muller et al.	
7,358,272	B2	4/2008	Muller et al.	
7,427,638	B2	9/2008	Muller et al.	
7,507,759	B2	3/2009	Muller et al.	
7,659,302	B2	2/2010	Muller et al.	
7,659,303	B2	2/2010	Muller et al.	
7,893,101	B2	2/2011	Muller et al.	
8,093,283	B2	1/2012	Muller et al.	
8,455,536	B2 *	6/2013	Muller	A61K 31/4035 514/417
8,629,173	B2	1/2014	Muller et al.	
2006/0148882	A1	7/2006	Zeldis et al.	
2006/0183787	A1	8/2006	Muller et al.	
2008/0234359	A1	9/2008	Muller et al.	
2009/0186923	A1	7/2009	Armer et al.	
2009/0239926	A1	11/2009	Schafer et al.	
2010/0168475	A1	7/2010	Saindane et al.	
2014/0024695	A1	1/2014	Muller et al.	
2014/0100259	A1	4/2014	Muller et al.	

FOREIGN PATENT DOCUMENTS

WO	WO 03/080049	A1	10/2003
WO	WO 2006/065814	A1	6/2006
WO	WO 2012/149251	A1	11/2012
WO	WO 2014/151180	A1	9/2014

OTHER PUBLICATIONS

Papp et al. (The Lancet, 380(9843), 738-746, 2012).*

Schett et al. (Arthritis & Rheumatism, 64(10): 3156-03167, 2012).*

FDA label for Otezla® (apremalist) tablets (Mar. 2014).*

"Clinical Pharmacology Review NDA 205437 Apremilast Application No. 205437Orig1s000," Clinical Pharmacology and Biopharmaceutics Review(s), Nov. 21, 2013, Retrieved from the Internet: URL:http://www.accessdata.fda.gov/drugsatfda_docs/nda/2014/205437Orig1s000ClinPharmR.pdf, retrieved on Sep. 22, 2015, 114 pages.

Anonymous, "Highlights of prescribing information—Otezla," Mar. 1, 2014, Retrieved from the Internet: URL:http//web.archive.org/web/20140327021029/http://www.otezla.com/otezla-prescribing-information.pdf, retrieved on Sep. 22, 2015, 9 pages.

(Continued)

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(57) **ABSTRACT**

Methods of treating, managing or preventing diseases ameliorated by inhibiting PDE4 such as psoriasis, ankylosing spondylitis, Behcet's disease, rheumatoid arthritis, atopic dermatitis, Crohn's disease, and ulcerative colitis are disclosed. Specific methods encompass the administration of apremilast in specific dosage titration schedule, alone or in combination with a second active agent.

24 Claims, No Drawings

(56) **References Cited**

OTHER PUBLICATIONS

Anonymous, "Oral Otezla® (apremilast) demostrated clinically meaningful and sustained improvements in skin, nail and scalp of adult patients with moderate to severe plaque psoriasis," Mar. 23, 2014, Retrieved from the Internet: URL:http://ir.celgene.com/releasedetail.cfm?releaseid=834900, retrieved on Sep. 22, 2015, pp. 1-6.

Baughman et al., "Release of tumor necrosis factor by alveolar macrophages of patients with sarcoidosis," *J. Lab. Clin. Med.* 115:36-42 (1990).

Beavo and Reifsnyder, "Primary sequence of cyclic nucleotide phosphodiesterase isozymes and the design of selective inhibitors," *Trends in Pharm.,* 11(4):150-155 (1990).

Bertolini et al., "Stimulation of bone resorption and inhibition of bone formation in vitro by human tumour necrosis factors," *Nature*, 319(6053):516-518 (1986).

Bissonnette et al., "Pulmonary inflammation and fibrosis in a murine model of asbestosis and silicosis. Possible role of tumor necrosis factor," *Inflammation* 13:329-339 (1989).

Carstensen, *Drug Stability: Principles & Practices*, Second Edition, Marcel Dekker, New York, NY, pp. 379-380 (1995).

Clouse et al., "Monokine regulation of human immunodeficiency virus-1 expression in a chronically infected human T cell clone," *J. Immunol.* 142:431-438 (1989).

Dezube et al., "Pentoxifylline and wellbeing in patients with cancer," Lancet, 335(8690):662 (1990).

Duh et al., "Tumor necrosis factor alpha activates human immunodeficiency virus type 1 through induction of nuclear factor binding to the NF-kappa B sites in the long terminal repeat," *Proc. Nat. Acad. Sci. USA*, 86:5974-5978 (1989).

Elliot et al., "TNF alpha blockade in rheumatoid arthritis: rationale, clinical outcomes and mechanisms of action," *Int. J. Immunopharmacol.*, 17:141-145 (1995).

Ferrari-Baliviera et al., "Tumor necrosis factor induces adult respiratory distress syndrome in rats," *Arch. Surg.*, 124(12):1400-1405 (1989).

Folks et al., "Tumor necrosis factor alpha induces expression of human immunodeficiency virus in a chronically infected T-cell clone," *Proc. Natl. Acad. Sci. USA*, 86(7):2365-2368 (1989).

Gottlieb et al., "Efficacy, tolerability, and pharmacodynamics of apremilast in recalcitrant plaque psoriasis: a phase II open-label study," *J. Drugs Dermatology*,12(8):888-897 (2013).

Grau et al., "Tumor necrosis factor and disease severity in children with falciparum malaria," *N. Engl. J. Med.*, 320(24):1586-1591 (1989).

Hinshaw et al., "Survival of primates in LD100 septic shock following therapy with antibody to tumor necrosis factor (TNF alpha)," *Circ. Shock* 30:279-292 (1990).

Holler et al., "Increased serum levels of tumor necrosis factor alpha precede major complications of bone marrow transplantation," *Blood*, 75(4):1011-1016 (1990).

Johnson et al., "Tumors producing human tumor necrosis factor induced hypercalcemia and osteoclastic bone resorption in nude mice," *Endocrinology* 124:1424-1427 (1989).

Kavanaugh et al., "Treatment of psoriatic arthritis in a phase 3 randomised, placebo-controlled trial with apremilast, an oral phosphodiesterase 4 inhibitor," *Ann. Rhuem. Dis.*, 73:1020-1026 (2014).

Lowe et al., Drugs of the Future, 17(9):799-807 (1992).

Lowe, "Tumour necrosis factor-α antagonists and their therapeutic applications," *Exp. Opin. Ther. Patents*, 8:1309-1322 (1998).

Millar et al., "Tumour necrosis factor in bronchopulmonary secretions of patients with adult respiratory distress syndrome," *Lancet*, 2(8665):712-714 (1989).

Monte et al., "Productive human immunodeficiency virus-1 infection of megakaryocytic cells is enhanced by tumor necrosis factor-alpha," *Blood* 79:2670-2679 (1992).

Nikolov, "Application No. 205437Orig1s000 Cross discipline team leader review," Center for Drug Evaluation and Research, Feb. 6, 2014, Retrieved from the Internet: URL:http://www.accessdata.fda.gov/drugsatfda_docs/nda/2014/205437Orig1s000CrossR.pdf, retrieved on Sep. 24, 2015, 42 pages.

Papp et al., "Efficacy of apremilast in the treatment of moderate to severe psoriasis: a randomised controlled trial," *Lancet*, 380:738-746 (2012).

Piguet et al., "Requirement of tumour necrosis factor for development of silica-induced pulmonary fibrosis," *Nature*, 344:245-247 (1990).

Poli et al., "The effect of cytokines and pharmacologic agents on chronic HIV infection," *AIDS Res. Hum. Retrovirus.*, 8(2):191-197 (1992).

Poli et al., "Tumor necrosis factor alpha functions in an autocrine manner in the induction of human immunodeficiency virus expression," *Proc. Nat. Acad. Sci. USA*, 87(2):782-785 (1990).

The Merck Manual, 17th Edition, Merck & Company, West Point, PA, pp. 448, 944-952 (1999).

Tracey et al., "Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia," *Nature*, 330(6149):662-664 (1987).

Van Dullemen et al., "Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2)," *Gastroenterology*, 109:129-135 (1995).

Verghese et al., "Differential regulation of human monocyte-derived TNF alpha and IL-1 beta by type IV cAMP-phosphodiesterase (cAMP-PDE) inhibitors," *J. Pharmacol. Exp. Ther.*, 272(3), 1313-1320 (1995).

Wolff ed., Burger's Medicinal Chemistry and Drug Discovery, 5th Edition, John Wiley & Sons, Inc., pp. 172-178, 949-982 (1995).

U.S. Appl. No. 60/366,515, filed Mar. 20, 2002, Schafer et al.

U.S. Appl. No. 60/438,450, filed Jan. 7, 2003, Schafer et al.

U.S. Appl. No. 60/634,982, filed Dec. 13, 2004, Zeldis et al.

Anonymous, "A Phase 3, Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group, Efficacy and Safety Study of Two Doses of Apremilast (CC-10004) in Subjects With Active Psoriatic Arthritis Who Have Not Been Previously Treated With Disease Modifying Anti-rheumatic Drugs," retrieved from http://clinicaltrials.gov/archive/NCT01307423/2012_09_06 on May 22, 2014.

STN File CA, Registry No. 608141-41-9, entered STN on Oct. 23, 2003, Chemical Abstracts Index Name "Acetamide, N-[2-[(1S)-1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl)ethyl]-2,3-dihydro-1,3-dioxo-1H-isoindol-4-yl]".

Dong et al., "Inhibition of PDE3, PDE4 and PDE7 potentiates glucocorticoid-induced apoptosis and overcomes glucocorticoid resistance in CEM T leukemic cells," Biochem. Pharmacol., 79(3):321-329 (2010).

Fox et al., "Combined oral cyclosporin and methotrexate therapy in patients with rheumatoid arthritis elevates methotrexate levels and reduces 7-hydroxymethotrexate levels when compared with methotrexate alone," Rheumatology (Oxford), 42(8):989-994 (2003).

Gladman et al., Kelley's Textbook of Rheumatology, 2 vols. 6th Edition, W. B. Saunders Company, Chapter 71, pp. 1071-1077 (2001).

Gladman, "Current concepts in psoriatic arthritis," Curr. Opin. Rheumatol., 14(4):361-366 (2002).

Kowalczyk et al., "Effect of phosphodiesterase antagonists on glucocorticoid mediated growth inhibition in murine skin cell lines," Eur. J. Pharmacol., 610(1-3):29-36 (2009).

Long et al., "Human articular chondrocytes produce IL-7 and respond to IL-7 with increased production of matrix metalloproteinase-13," Arthritis Res. Ther., 10(1):R23 (2008).

McCann et al., "Apremilast, a novel PDE4 inhibitor, inhibits spontaneous production of tumour necrosis factor-alpha from human rheumatoid synovial cells and ameliorates experimental arthritis," Arthritis Res. Ther., 12(3):R107 (2010).

Meyers et al., "Phosphodiesterase 4 inhibitors augment levels of glucocorticoid receptor in B cell chronic lymphocytic leukemia but not in normal circulating hematopoietic cells," Clin. Cancer Res., 13(16):4920-4927 (2007).

Patel et al., "Psoriatic arthritis—emerging concepts," Rheumatology (Oxford), 40(3):243-246 (2001).

(56) **References Cited**

OTHER PUBLICATIONS

Rihl et al., "Identification of interleukin-7 as a candidate disease mediator in spondylarthritis," Arthritis Rheum., 58(11):3430-3435 (2008).
Tierney et al. (eds), Current Medical Diagnosis & Treatment 1998, 37th Edition, Appleton & Lange, Stamford, CT, p. 793 (1998).
Wilen et al., "Strategies in optical resolutions," Tetrahedron, 33:2725-2736 (1977).
Wilen, Tables of Resolving Agents and Optical Resolutions, (E.L. Eliel, Ed.), University of Notre Dame Press, Notre Dame, IN, p. 268 (1972).

* cited by examiner

METHODS FOR THE TREATMENT OF DISEASES AMELIORATED BY PDE4 INHIBITION USING DOSAGE TITRATION OF APREMILAST

This application claims priority to U.S. Provisional Patent Application No. 62/038,176, filed Aug. 15, 2014, the entirety of which is incorporated herein by reference.

1. FIELD

Provided herein are methods for treating, preventing and/or managing diseases ameliorated by PDE4 inhibition such as psoriasis, ankylosing spondylitis, Behcet's disease, rheumatoid arthritis, atopic dermatitis, Crohn's disease, and ulcerative colitis by administering apremilast in a specific dosage titration schedule. Also provided herein are pharmaceutical compositions and dosage forms comprising specific amounts of apremilast suitable for use in methods of treating, preventing and/or managing psoriasis, ankylosing spondylitis, Behcet's disease, rheumatoid arthritis, atopic dermatitis, Crohn's disease, and ulcerative colitis. The compounds disclosed herein are for use in the methods of the invention.

2. BACKGROUND

Adenosine 3',5'-cyclic monophosphate (cAMP) plays a role in many diseases and conditions, such as but not limited to inflammation, and other conditions (Lowe and Cheng, *Drugs of the Future,* 17(9), 799-807, 1992). It has been shown that the elevation of cAMP in inflammatory leukocytes inhibits their activation and the subsequent release of inflammatory mediators, including TNF-α and NF-κB.

It is believed that the primary cellular mechanism for the inactivation of cAMP is the breakdown of cAMP by a family of isoenzymes referred to as cyclic nucleotide phosphodiesterases (PDE) (Beavo and Reitsnyder, *Trends in Pharm.,* 11, 150-155, 1990). It is recognized, for example, that the inhibition of PDE type IV is particularly effective in the inhibition of inflammatory mediator release (Verghese, et al., *Journal of Pharmacology and Experimental Therapeutics,* 272(3), 1313-1320, 1995). Thus, compounds that inhibit PDE4 (PDE IV) specifically may inhibit inflammation with a minimum of unwanted side effects such as cardiovascular or anti-platelet effects.

Inflammatory diseases such as arthritis, related arthritic conditions (e.g., ankylosing spondylitis, osteoarthritis and rheumatoid arthritis), Behcet's disease, inflammatory bowel disease (e.g., Crohn's disease and ulcerative colitis), psoriasis, atopic dermatitis and contact dermatitis are prevalent and problematic ailments. TNF-α plays a central role in the inflammatory response and the administration of their antagonists block chronic and acute responses in animal models of inflammatory disease. Enhanced or unregulated TNF-α production has been implicated in a number of diseases, for example psoriasis, ankylosing spondylitis, Behcet's disease, rheumatoid arthritis, atopic dermatitis, Crohn's disease, and ulcerative colitis. Tracey et al., 1987, *Nature* 330:662-664 and Hinshaw et al., 1990, *Circ. Shock* 30:279-292 (endotoxic shock); Dezube et al., 1990, *Lancet,* 335:662 (cachexia); Millar et al., 1989, *Lancet* 2:712-714 and Ferrai-Baliviera et al., 1989, *Arch. Surg.* 124:1400-1405 (adult respiratory distress syndrome); Bertolini et al., 1986, *Nature* 319:516-518, Johnson et al., 1989, *Endocrinology* 124:1424-1427, Holler et al., 1990, *Blood* 75:1011-1016, and Grau et al., 1989, *N. Engl. J. Med.* 320:1586-1591 (bone resorption diseases); Pignet et al., 1990, *Nature,* 344:245-247, Bissonnette et al., 1989, *Inflammation* 13:329-339 and Baughman et al., 1990, *J. Lab. Clin. Med.* 115:36-42 (chronic pulmonary inflammatory diseases); Elliot et al., 1995, *Int. J. Pharmac.* 17:141-145 (rheumatoid arthritis); von Dullemen et al., 1995, *Gastroenterology,* 109:129-135 (Crohn's disease); Duh et al., 1989, *Proc. Nat. Acad. Sci.* 86:5974-5978, Poll et al., 1990, *Proc. Nat. Acad. Sci.* 87:782-785, Monto et al., 1990, *Blood* 79:2670, Clouse et al., 1989, *J. Immunol.* 142, 431-438, Poll et al., 1992, *AIDS Res. Hum. Retrovirus,* 191-197, Poli et al. 1990, *Proc. Natl. Acad. Sci.* 87:782-784, Folks et al., 1989, *PNAS* 86:2365-2368 (HIV and opportunistic infections resulting from HIV).

Therefore, pharmaceutical compounds that can inhibit PDE4 or TNF-α, may be beneficial therapeutics. Small-molecule inhibitors have demonstrated an ability to treat or prevent inflammatory diseases implicated by PDE4 or TNF-α (for a review, see Lowe, 1998 *Exp. Opin. Ther. Patents* 8:1309-1332). One such class of molecules is the substituted phenethylsulfones described in U.S. Pat. No. 6,020,358.

There is a significant need for safe and effective methods of treating, preventing and managing psoriasis, ankylosing spondylitis, Behcet's disease, rheumatoid arthritis, atopic dermatitis, Crohn's disease, and ulcerative colitis, particularly for patients that are refractory to conventional treatments. In addition, there is a need to treat such disease while reducing or avoiding the toxicity and/or side effects associated with conventional therapies.

3. SUMMARY

Provided herein are methods of treating, preventing and/or managing psoriasis, ankylosing spondylitis, Behcet's disease, rheumatoid arthritis, atopic dermatitis, Crohn's disease, and ulcerative colitis in humans in need thereof using specific dosage titration schedule of apremilast. The methods comprise administering to a patient in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of apremilast, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate (e.g., hydrate) or clathrate thereof in specific dosage titration schedule.

In some embodiments, provided herein are methods of treating psoriasis, ankylosing spondylitis, Behcet's disease, rheumatoid arthritis, atopic dermatitis, Crohn's disease, and ulcerative colitis, which comprises orally administering to a patient having psoriasis, ankylosing spondylitis, Behcet's disease, rheumatoid arthritis, atopic dermatitis, Crohn's disease, or ulcerative colitis escalating doses of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, or a pharmaceutically acceptable prodrug, polymorph, salt, or solvate thereof, wherein a starting dose is between about 10 mg/day and about 20 mg/day, and a maximum dose is between about 40 mg/day and about 100 mg/day.

In some embodiments, the method comprises the following initial titration schedule:

(i) 10 mg in the morning on the first day of administration;

(ii) 10 mg in the morning and 10 mg after noon on the second day of administration;

(iii) 10 mg in the morning and 20 mg after noon on the third day of administration;

(iv) 20 mg in the morning and 20 mg after noon on the fourth day of administration;

(v) 20 mg in the morning and 30 mg after noon on the fifth day of administration; and

(vi) 30 mg in the morning and 30 mg after noon on the sixth and every subsequent day of administration.

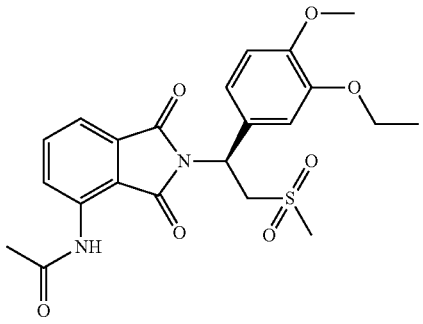
In some embodiments, the methods further comprise the administration of a therapeutically or prophylactically effective amount of at least a second active agent, including but not limited to, an anti-inflammatory agent, an immunosuppressant, mycophenolate mofetil, a biologic agent, or a Cox-2 inhibitor.

In another embodiment, apremilast, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate (e.g., hydrate) or clathrate thereof is administered orally in a dosage form such as a tablet and a capsule.

4. DETAILED DESCRIPTION

4.1 Definitions

As used herein, the term "apremilast" refers to (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, also known as N-[2-[(1S)-1-(3-ethoxy-4-methoxylphenyl)-2-(methylsulfonyl) ethyl]-2,3-dihydro-1,3-dioxo-1H-isoindol-4-yl]acetamide. Apremilast has the following structure:



As used herein and unless otherwise indicated, the term "pharmaceutically acceptable salt" includes, but is not limited to, salts prepared from pharmaceutically acceptable non-toxic acids or bases including inorganic acids and bases and organic acids and bases. Suitable pharmaceutically acceptable base addition salts provided herein include metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from lysine, N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. Suitable non-toxic acids include, but are not limited to, inorganic and organic acids such as acetic, alginic, anthranilic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethenesulfonic, formic, fumaric, furoic, galacturonic, gluconic, glucuronic, glutamic, glycolic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phenylacetic, phosphoric, propionic, salicylic, stearic, succinic, sulfanilic, sulfuric, tartaric acid, and p-toluenesulfonic acid. Specific non-toxic acids include hydrochloric, hydrobromic, phosphoric, sulfuric, and methanesulfonic acids. Examples of specific salts thus include hydrochloride and mesylate salts.

As used herein and unless otherwise indicated, the term "hydrate" means a compound provided herein or a salt thereof that further includes a stoichiometric or non-stoichiometric amount of water bound by non-covalent intermolecular forces.

As used herein and unless otherwise indicated, the term "solvate" means a solvate formed from the association of one or more solvent molecules to a compound provided herein. The term "solvate" includes hydrates (e.g., monohydrate, dihydrate, trihydrate, tetrahydrate and the like).

As used herein and unless otherwise indicated, the term "polymorph" means solid crystalline forms of a compound provided herein or complex thereof. Different polymorphs of the same compound can exhibit different physical, chemical and/or spectroscopic properties.

As used herein and unless otherwise specified, the term "prodrug" means a derivative of a compound that can hydrolyze, oxidize, or otherwise react under biological conditions (in vitro or in vivo) to provide the compound. Examples of prodrugs include, but are not limited to, derivatives and metabolites of apremilast that include biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. Prodrugs can typically be prepared using well-known methods, such as those described by 1 *Burger's Medicinal Chemistry and Drug Discovery,* 172-178, 949-982 (Manfred E. Wolff ed., 5th ed. 1995).

As used herein, and unless otherwise specified, the term "enantiomer," "isomer" or "stereoisomer" encompasses all enantiomerically/stereomerically pure and enantiomerically/stereomerically enriched compounds provided herein.

As used herein, and unless otherwise indicated, the term "stereomerically pure" or "enantiomerically pure" means that a compound comprises one stereoisomer and is substantially free of its counter stereoisomer or enantiomer. For example, a compound is stereomerically or enantiomerically pure, when the compound contains greater than or equal to 80%, 90%, 95%, 96%, 97%, 98% or 99% of one stereoisomer, and 20%, 10%, 5%, 4%, 3%, 2%, 1% or less of the counter stereoisomer. "Substantially free of its (R) enantiomer" is encompassed by the term stereomerically pure or enantiomerically pure.

As used herein, term "adverse effect" includes, but is not limited to gastrointestinal, renal and hepatic toxicities, leukopenia, increases in bleeding times due to, e.g., thrombocytopenia, and prolongation of gestation, nausea, vomiting, somnolence, asthenia, dizziness, teratogenicity, extra-pyramidal symptoms, akathisia, cardiotoxicity including cardiovascular disturbances, inflammation, male sexual dysfunction, and elevated serum liver enzyme levels. The term "gastrointestinal toxicities" includes but is not limited to gastric and intestinal ulcerations and erosions. The term "renal toxicities" includes but is not limited to such conditions as papillary necrosis and chronic interstitial nephritis.

As used herein, the term "patient" refers to a mammal, particularly a human. In some embodiments, the patient is a female. In further embodiments, the patient is a male. In further embodiments, the patient is a pediatric (a newborn, an infant, a child, or an adolescent).

As used herein, and unless otherwise specified, the term "pediatrics" or "pediatric medicine" refers to the branch of medicine that deals with the medical care of newborns, infants, children, and adolescents. The approximate age range of a newborn is from birth to 1 month of age. The approximate age range of an infant is greater than 1 month to 2 years of age. The approximate age range of a child is greater than 2 years to 12 years of age. The approximate age range of an adolescent is greater than 12 years to 21 years of age.

As used herein, and unless otherwise specified, the terms "treat," "treating" and "treatment" contemplate an action

that occurs while a patient is suffering from the specified disease or disorder, which reduces the severity or symptoms of the disease or disorder, or retards or slows the progression or symptoms of the disease or disorder.

As used herein, unless otherwise specified, the terms "prevent," "preventing" and "prevention" contemplate an action that occurs before a patient begins to suffer from the specified disease or disorder, which inhibits or reduces the severity or symptoms of the disease or disorder.

As used herein, and unless otherwise indicated, the terms "manage," "managing," and "management" encompass preventing the recurrence of the specified disease or disorder in a patient who has already suffered from the disease or disorder, and/or lengthening the time that a patient who has suffered from the disease or disorder remains in remission. The terms encompass modulating the threshold, development and/or duration of the disease or disorder, or changing the way that a patient responds to the disease or disorder.

4.2 Methods of Treatment and Prevention

Provided herein are methods of treating, managing and/or preventing psoriasis, ankylosing spondylitis, Behcet's disease, rheumatoid arthritis, atopic dermatitis, Crohn's disease, and ulcerative colitis, which comprise administering to a patient in need of such treatment, management or prevention a therapeutically or prophylactically effective amount of apremilast, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate or clathrate thereof in a specific dosage titration schedule. Apremilast, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate or clathrate thereof, is provided for use in the methods of treatment, management and/or prevention disclosed herein.

Provided herein are methods of treating psoriasis, which comprise administering to a patient in need of such treatment a therapeutically effective amount of apremilast, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate or clathrate thereof in a specific dosage titration schedule.

Provided herein are methods of treating ankylosing spondylitis, which comprise administering to a patient in need of such treatment a therapeutically effective amount of apremilast, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate or clathrate thereof in a specific dosage titration schedule.

Provided herein are methods of treating Behcet's disease, which comprise administering to a patient in need of such treatment a therapeutically effective amount of apremilast, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate or clathrate thereof in a specific dosage titration schedule.

Provided herein are methods of treating rheumatoid arthritis, which comprise administering to a patient in need of such treatment a therapeutically effective amount of apremilast, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate or clathrate thereof in a specific dosage titration schedule.

Provided herein are methods of treating atopic dermatitis, which comprise administering to a patient in need of such treatment a therapeutically effective amount of apremilast, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate or clathrate thereof in a specific dosage titration schedule.

Provided herein are methods of treating Crohn's disease, which comprise administering to a patient in need of such treatment a therapeutically effective amount of apremilast, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate or clathrate thereof in a specific dosage titration schedule.

Provided herein are methods of treating ulcerative colitis, which comprise administering to a patient in need of such treatment a therapeutically effective amount of apremilast, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate or clathrate thereof in a specific dosage titration schedule.

Apremislast is provided for use in the methods of treatment of the invention.

In some embodiments apremilast is provided for use in the methods of treatment, management and/or prevention of a disease disclosed herein.

In some embodiments the disease is psoriasis. In some embodiments the disease is ankylosing spondylitis. In some embodiments the disease is Behcet's disease. In some embodiments the disease is rheumatoid arthritis. In some embodiments the disease is atopic dermatitis. In some embodiments the disease is Crohn's disease. In some embodiments the disease is ulcerative colitis.

In some embodiments apremilast is present as a pharmaceutically acceptable prodrug thereof. In some embodiments apremilast is present as a pharmaceutically acceptable metabolite thereof. In some embodiments apremilast is present as a pharmaceutically acceptable polymorph thereof. In some embodiments apremilast is present as a pharmaceutically acceptable salt thereof. In some embodiments apremilast is present as a pharmaceutically acceptable solvate thereof. In some embodiments apremilast is present as a pharmaceutically acceptable clathrate thereof.

In some embodiments, the methods also encompass inhibiting or averting signs and symptoms of psoriasis, ankylosing spondylitis, Behcet's disease, rheumatoid arthritis, atopic dermatitis, Crohn's disease, or ulcerative colitis as well as addressing the disease itself, prior to the onset of symptoms by administering apremilast, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate or clathrate thereof in a specific dosage titration schedule. Patients having history of psoriasis, ankylosing spondylitis, Behcet's disease, rheumatoid arthritis, atopic dermatitis, Crohn's disease, or ulcerative colitis are preferred candidates for preventive regimens. In some embodiments, apremilast, or a pharmaceutically acceptable prodrug, metabolite, polymorph, salt, solvate or clathrate thereof is provided for use in the methods of inhibiting or averting signs and symptoms of psoriasis, ankylosing spondylitis, Behcet's disease, rheumatoid arthritis, atopic dermatitis, Crohn's disease, or ulcerative colitis as well as addressing the disease itself, prior to the onset of symptoms.

In certain embodiments, apremilast is orally administered to a patient having psoriasis, ankylosing spondylitis, Behcet's disease, rheumatoid arthritis, atopic dermatitis, Crohn's disease, or ulcerative colitis in a twice daily dose of 30 mg per day (i.e., 60 mg per day). In certain embodiments, apremilast is for use in the methods of oral administration.

In some embodiments, the patient is an adult.

In some embodiments, the patient is a newborn.

In some embodiments, the patient is an infant.

In some embodiments, the patient is a child.

In some embodiments, the patient is an adolescent.

In some embodiments, provided herein are methods of treating psoriasis, ankylosing spondylitis, Behcet's disease, rheumatoid arthritis, atopic dermatitis, Crohn's disease, and ulcerative colitis, which comprises orally administering to a patient having psoriasis, ankylosing spondylitis, Behcet's disease, rheumatoid arthritis, atopic dermatitis, Crohn's dis-

ease, or ulcerative colitis escalating doses of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, or a pharmaceutically acceptable prodrug, polymorph, salt, or solvate thereof, wherein a starting dose is between about 10 mg/day and about 20 mg/day, and a maximum dose is between about 40 mg/day and about 100 mg/day. Stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, or a pharmaceutically acceptable prodrug, polymorph, salt, or solvate thereof, is provided for use in the methods of treatment disclosed herein above.

In some embodiments, the method comprises the following initial titration schedule:

(i) 10 mg in the morning on the first day of administration;

(ii) 10 mg in the morning and 10 mg after noon on the second day of administration;

(iii) 10 mg in the morning and 20 mg after noon on the third day of administration;

(iv) 20 mg in the morning and 20 mg after noon on the fourth day of administration;

(v) 20 mg in the morning and 30 mg after noon on the fifth day of administration; and

(vi) 30 mg in the morning and 30 mg after noon on the sixth and every subsequent day of administration.

In some embodiments, the dosing schedule may be represented as follows:

Day 1	Day 2		Day 3		Day 4		Day 5		Day 6 & thereafter	
AM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
10 mg	10 mg	10 mg	10 mg	20 mg	20 mg	20 mg	20 mg	30 mg	30 mg	30 mg

In some embodiments, the method comprises the following initial titration schedule:

(i) 10 mg in the morning on the first day of administration;

(ii) 10 mg in the morning and 10 mg after noon on the second day of administration;

(iii) 10 mg in the morning and 20 mg after noon on the third day of administration;

(iv) 20 mg in the morning and 20 mg after noon on the fourth day of administration;

(v) 20 mg in the morning and 30 mg after noon on the fifth day of administration;

(vi) 30 mg in the morning and 30 mg after noon on the sixth day of administration;

(vii) 30 mg in the morning and 40 mg after noon on the seventh day of administration;

(viii) 40 mg in the morning and 40 mg after noon on the eighth and every subsequent day of administration.

In some embodiments, the method comprises the following initial titration schedule:

(i) 10 mg in the morning on the first day of administration;

(ii) 10 mg in the morning and 10 mg after noon on the second day of administration;

(iii) 10 mg in the morning and 20 mg after noon on the third day of administration;

(iv) 20 mg in the morning and 20 mg after noon on the fourth and every subsequent day of administration.

In some embodiments, the method comprises the following initial titration schedule:

(i) 10 mg in the morning and 10 mg after noon on the first and second day of administration;

(ii) 20 mg in the morning and 20 mg after noon on the third and fourth day of administration;

(iii) 30 mg in the morning and 30 mg after noon on the fifth and every subsequent day of administration.

In one embodiment, stereomerically pure apremilast, (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, is administered according the above schedule. In one embodiment, stereomerically pure apremilast, (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is provided for use in methods of administration according to the above schedules.

In one embodiment, the dosage of apremilast, (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, may be reduced to 30 mg once daily in patients with severe renal impairment.

In one embodiment, apremilast, (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, can be administered without regard to meals according the above schedule.

In some embodiments, the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione comprises greater than about 90% by weight of (+) isomer based on the total weight percent of the compound.

In some embodiments, the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione comprises greater than about 95% by weight of (+) isomer based on the total weight percent of the compound.

In some embodiments, the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione comprises greater than about 96% by weight of (+) isomer based on the total weight percent of the compound.

In some embodiments, the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione comprises greater than about 97% by weight of (+) isomer based on the total weight percent of the compound.

In some embodiments, the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione comprises greater than about 98% by weight of (+) isomer based on the total weight percent of the compound.

In some embodiments, the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione comprises greater than about 99% by weight of (+) isomer based on the total weight percent of the compound.

In some embodiments, the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is administered in an amount of about 20 mg twice a day following the initial titration schedule.

In some embodiments, the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-

acetylaminoisoindoline-1,3-dione is administered in an amount of about 30 mg twice a day following the initial titration schedule.

In some embodiments, the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is administered once or twice daily.

In some embodiments, the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is administered in tablet form. In some embodiments, the tablet comprises a 10 mg, 20 mg or 30 mg dose of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

In some embodiments, the methods provided herein, further comprise administering to the patient a therapeutically effective amount of a second active agent. In some embodiments, the second active agent is an anti-inflammatory agent, an immunosuppressant, mycophenolate mofetil, a biologic agent, or a Cox-2 inhibitor. In some embodiments, the second active agent is a nonsteroidal anti-inflammatory agent. In some embodiments, the second active agent is a disease-modifying anti-rheumatic agent. In some embodiments, the second active agent is methotrexate. In some embodiments, the second active agent is sulfasalazine. In some embodiments, the second active agent is leflunomide. In some embodiments, the second active agent is etanercept. In some embodiments, the second active agent is an oral corticosteroid. In some embodiments, the second active agent is prednisone. In some embodiments, a second active agent is provided for administration in the methods disclosed herein.

In some embodiments, the patient has received prior treatment for psoriasis, ankylosing spondylitis, Behcet's disease, rheumatoid arthritis, atopic dermatitis, Crohn's disease, or ulcerative colitis. In some embodiments, the prior treatment is with a disease-modifying antirheumatic drug. In some embodiments, the psoriasis, ankylosing spondylitis, Behcet's disease, rheumatoid arthritis, atopic dermatitis, Crohn's disease, or ulcerative colitis is refractory to the prior treatment.

In some embodiments, the method comprises administering stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, substantially free of any salt, solvate, or prodrug forms of (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

In some embodiments, the method comprises administering a pharmaceutically acceptable salt of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

In some embodiments, the method comprises administering a pharmaceutically acceptable solvate of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

4.2.1 Combination Therapy

In particular methods encompassed by this embodiment, apremilast is administered in combination with another drug ("second active agent") for treating, managing and/or preventing psoriasis, ankylosing spondylitis, Behcet's disease, rheumatoid arthritis, atopic dermatitis, Crohn's disease, or ulcerative colitis. In some embodiments, apremilast is provided for use in methods of treatment, management, and/or prevention any of the above diseases, wherein the method comprises administering apremilast in combination with another drug ("second active agent").

In certain embodiments, the methods encompass synergistic combinations for the treatment, prevention and/or management of psoriasis, ankylosing spondylitis, Behcet's disease, rheumatoid arthritis, atopic dermatitis, Crohn's disease, or ulcerative colitis. Apremilast may also be used to alleviate adverse effects associated with some second active agents.

One or more second active agents can be used in the methods together with apremilast. Second active agents can be administered before, after or simultaneously with apremilast. In some embodiments, the one or more second active agents are selected from the group consisting of anti-inflammatories such as nonsteroidal anti-inflammatory drugs (NSAIDs), immunosuppressants, topical corticosteroids, calcineurin inhibitors, Cox-2 inhibitors, TNF-alpha inhibitors, antirheumatics, antipsoriatics, interleukin inhibitors, narcotic analgesic combinations, salicylates, glucocorticoids and topical rubefacients.

In one embodiment, the second active agent is selected from the group consisting of an anti-inflammatory agent, an immunosuppressant, mycophenolate mofetil, a biologic agent, or a Cox-2 inhibitor.

In one embodiment, the second active agent is sulfasalazine.

In one embodiment, the second active agent is leflunomide.

In one embodiment, the second active agent is an oral corticosteroid.

In one embodiment, the second active agent is etanercept.

In some embodiments, the second active agents may include, but are not limited to, anti-inflammatories such as NSAIDs including, but not limited to, diclofenac (e.g., ARTHROTEC®), diflunisal (e.g., DOLOBID®), etodolac (e.g., LODINE®), fenoprofen (e.g., NALFON®), ibuprofen (e.g., ADVIL, CHILDREN'S ADVIL/MOTRIN, MEDIPREN, MOTRIN, NUPRIN or PEDIACARE FEVER®), indomethacin (e.g., ARTHREXIN®), ketoprofen (e.g., ORUVAIL®), ketorolac (e.g., TORADOL®), fosfomycin tromethamine (e.g., MONURAL®), meclofenamate (e.g., Meclomen®), nabumetone (e.g., RELAFEN®), naproxen (e.g., ANAPROX®, ANAPROX® DS, EC-NAPROSYN®, NAPRELAN® or NAPROSYN®), oxaprozin (e.g., DAYPRO®), piroxicam (e.g., FELDENE®), sulindac (e.g., CLINORIL®), and tolmetin (e.g., TOLECTIN® DS or TOLECTIN®).

In other embodiments, the second active agents may include, but are not limited to, disease-modifying antirheumatic drugs (DMARDs) or immunosuppressants such as, but not limited to, methotrexate (Rheumatrex®), sulfasalazine (Azulfidine®), leflunomide (Arava®), and cyclosporine (Sandimmune® or Neoral®).

In other embodiments, the second active agent is an oral corticosteroid, such as, but not limited to, budesonide (Entocort®), dexamethasone, fludrocortisone (Florinef®, Florinef® acetate), hydrocortisone, methylprednisone, prednisolone, and prednisone.

In other embodiments, the second active agents may include, but are not limited to, mycophenolate mofetil (CellCept®), an immunosuppressive agent widely used in organ transplantation and gaining favor in treating autoimmune and inflammatory skin disorders.

In further embodiments, the second active agents may include, but are not limited to, biologic agents such as etanercept (Enbrel®), infliximab (Remicade®) and adalimumab (Humira®).

In further embodiments, the second active agents may include, but are not limited to, Cox-2 inhibitors such as celecoxib (Celebrex®), valdecoxib (Bextra®) and meloxicam (Mobic®).

In some embodiments, the one or more selective active agents is selected from the group consisting of acitretin, adalimumab, alclometasone, alefacept, aloe vera, amcinonide, ammonium lactate/urea, ammonium lactate/halobetasol, anthralin, benzocaine/pyrilamine/zinc oxide, betamethasone, betamethasone/calcipotriene, calcipotriene, clobetasol, clocortolone, coal tar, coal tar/salicylic acid, corticotropin, cyclosporine, desonide, desoximetasone, diflorasone, fluocinonide, flurandrenolide, halcinonide, halobetasol, hydrocortisone, hydrocortisone/pramoxine, hydroxyurea, infliximab, methotrexate, methoxsalen, mometasone, pramoxine, prednisone, prednisolone, prednicarbate, resorcinol, tazarotene, triamcinolone and ustekinumab.

In some embodiments, the one or more selective active agents is selected from the group consisting of abatacept, acetaminophen, acetaminophen/hydrocodone, acetaminophen/tramadol, adalimumab, alemtuzumab, aluminum hydroxide/aspirin/calcium carbonate/magnesium hydroxide, anakinra, aspirin, auranofin, aurothioglucose, atorvastatin, azathioprine, celecoxib, certolizumab, chondroitin, cortisone, corticotropin, cyclophosphamide, cyclosporine, daclizumab, dexamethasone, diclofenac, diclofenac/misoprostol, diflunisal, doxycycline, esomeprazole, esomeprazole/naproxen, etanercept, etodolac, famotidine, famotidine/ibuprofen, fenoprofen, flurbiprofen, glucosamine, gold sodium thiomalate, golimumab, hydroxychloroquine, ibuprofen, indomethacin, infliximab, interferon, interferon gamma-1b, ketoprofen, lansoprazole, lansoprazole/naproxen, leflunomide, levamisole, meclofenamate, meloxicam, methotrexate, methylprednisone, methylprednisolone, methyl salicylate, minocycline, mycophenolate mofetil, nabumetone, naproxen, oxaprozin, penicillamine, phenytoin, piroxicam, prednisone, primrose oil, rituximab, rofecoxib, salsalate, sulindac, sulfasalazine, tetracycline, tocilizumab, tofacitinib, tolmetin, tramadol, triamcinolone, trolamine salicylate, valdecoxib and pharmaceutically acceptable prodrugs and salts thereof.

In some embodiments, the one or more selective active agents is selected from the group consisting of abatacept, acetaminophen, acetaminophen/hydrocodone, acetaminophen/tramadol, acitretin, adalimumab, alclometasone, alefacept, alemtuzumab, aloe vera, aluminum hydroxide/aspirin/calcium carbonate/magnesium hydroxide, amcinonide, ammonium lactate/urea, ammonium lactate/halobetasol, anakinra, anthralin, aspirin, auranofin, aurothioglucose, atorvastatin, azathioprine, benzocaine/pyrilamine/zinc oxide, betamethasone, betamethasone/calcipotriene, calcipotriene, celecoxib, certolizumab, chondroitin, clobetasol, clocortolone, coal tar, coal tar/salicylic acid, corticotropin, cortisone, cyclophosphamide, cyclosporine, daclizumab, desonide, desoximetasone, dexamethasone, diclofenac, diclofenac/misoprostol, diflorasone, diflunisal, doxycycline, esomeprazole, esomeprazole/naproxen, etanercept, etodolac, famotidine, famotidine/ibuprofen, fenoprofen, fluocinonide, flurandrenolide, flurbiprofen, fostamatinib, glucosamine, gold sodium thiomalate, golimumab, halcinonide, halobetasol, hydrocortisone, hydrocortisone/pramoxine, hydroxyurea, hydroxychloroquine, ibuprofen, indomethacin, infliximab, interferon, interferon gamma-1b, ibrutinib, ketoprofen, lansoprazole, lansoprazole/naproxen, leflunomide, lenalidomide, levamisole, meclofenamate, meloxicam, methotrexate, methoxsalen, methylprednisone, methylprednisolone, methyl salicylate, minocycline, mometasone, mycophenolate mofetil, nabumetone, naproxen, oxaprozin, penicillamine, phenytoin, piroxicam, pomalidomide, pramoxine, prednisone, prednisolone, prednicarbate, primrose oil, resorcinol, rituximab, rofecoxib, salsalate, sulindac, sulfasalazine, tazarotene, tetracycline, tocilizumab, tofacitinib, tolmetin, tramadol, triamcinolone, trolamine salicylate, ustekinumab, valdecoxib, 3-(5-amino-2-methyl-4-oxo-4H-quinazolin-3-yl)-piperidine-2,6-dione, (S)-3-(4-((4-(morpholinomethyl)benzyl)oxy)-1-oxoisoindolin-2-yl)piperidine-2,6-dione and pharmaceutically acceptable prodrugs and salts thereof.

In some embodiments, the one or more selective active agents is selected from the group consisting of a PDE7 inhibitor, a Btk inhibitor, a cereblon targeting agent, a Tyk2 inhibitor, a Syk inhibitor, a JAK inhibitor, a JNK inhibitor, a MK2 inhibitor, an ERP5 inhibitor, a PD-1 inhibitor, a TIMP-3 inhibitor, an IL23p19 inhibitor, an IL-17 blocker, an IKK-2 inhibitor, a LH2B inhibitor, a PKC-theta inhibitor, an IRAK4 inhibitor, a ROCK inhibitor, and a ROR-gamma-T inhibitor.

Administration of apremilast and a second active agent to a patient can occur simultaneously or sequentially by the same or different routes of administration. The suitability of a particular route of administration employed for a particular second active agent will depend on the second active agent itself (e.g., whether it can be administered orally or topically without decomposing) and the subject being treated. Particular routes of administration for the second active agents or ingredients are known to those of ordinary skill in the art. See, e.g., *The Merck Manual,* 448 (17th ed., 1999).

The amount of second active agent administered can be determined based on the specific agent used, the subject being treated, the severity and stage of disease and the amount(s) of apremilast and any optional additional second active agents concurrently administered to the patient. Those of ordinary skill in the art can determine the specific amounts according to conventional procedures known in the art. In the beginning, one can start from the amount of the second active agent that is conventionally used in the therapies and adjust the amount according to the factors described above. See, e.g., *Physician's Desk Reference* (59th Ed., 2005).

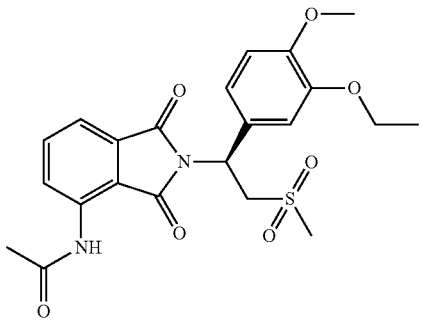
In certain embodiments, the second active agent is administered orally, topically, transdermally, intravenously or subcutaneously. In certain embodiments, the second active agent is administered once to four times daily. In certain embodiments, the second active agent is administered once to four times monthly. In certain embodiments, the second active agent is administered once every week. In certain embodiments, the second active agent is administered once every other week. In certain embodiments, the second active agent is administered once every month. In certain embodiments, the second active agent is administered once every two months. In certain embodiments, the second active agent is administered once every three months. In certain embodiments, the second active agent is administered in an amount of from about 1 to about 1,000 mg, from about 5 to about 500 mg, from about 10 to about 350 mg or from about 50 to about 200 mg. The specific amount of the second active agent will depend on the specific agent used, the age of the subject being treated, the severity and stage of disease and the amount(s) of apremilast and any optional additional second active agents concurrently administered to the patient.

4.3 Apremilast

Apremilast is an inhibitor of phosphodiesterase 4 (PDE4) specific for cyclic adenosine monophosphate (cAMP).

PDE4 inhibition results in increased intracellular cAMP levels and is effective in the inhibition of inflammatory mediator release.

Without being limited by theory, apremilast is believed to be (+) enantiomer of 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methanesulfonylethyl]-4-acetylaminoisoindolin-1,3-dione having the following structure:



Apremilast may be prepared according to methods disclosed in U.S. Pat. Nos. 6,962,940; 7,208,516; 7,427,638; or 7,893,101, the entirety of each which is incorporated herein by reference. In a specific method, apremilast may be prepared, for example, by the following process.

A stirred solution of 1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethylamine (1.0 g, 3.7 mmol) and 3-acetamidophthalic anhydride (751 mg, 3.66 mmol) in acetic acid (20 mL) was heated at reflux for 15 h. The solvent was removed in vacuo to yield an oil. Chromatography of the resulting oil yielded the product as a yellow solid (1.0 g, 59% yield): mp, 144° C.; ^{1}H NMR ($CDCl_3$) δ: 1.47 (t, J=7.0 Hz, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.88 (s, 3H, CH_3), 3.75 (dd, J=4.4, 14.3 Hz, 1H, CH), 3.85 (s, 3H, CH3), 4.11 (q, J=7 Hz, 2H, CH_2), 5.87 (dd, J=4.3, 10.5 Hz, 1H, NCH), 6.82-6.86 (m, 1H, Ar), 7.09-7.11 (m, 2H, Ar), 7.47 (d, J=7 Hz, 1H, Ar), 7.64 (t, J=8 Hz, 1H, Ar), 8.74 (d, J=8 Hz, 1H, Ar), 9.49 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ: 14.61, 24.85, 41.54, 48.44, 54.34, 55.85, 64.43, 111.37, 112.34, 115.04, 118.11, 120.21, 124.85, 129.17, 130.96, 136.01, 137.52, 148.54, 149.65, 167.38, 169.09, 169.40; Anal Calc'd. for $C_{22}H_{24}NO_7S$: C, 57.38; H, 5.25; N, 6.08. Found: C, 57.31; H, 5.34; N, 5.83.

Preparation of 3-aminophthalic acid: 10% Pd/C (2.5 g), 3-nitrophthalic acid (75.0 g, 355 mmol) and ethanol (1.5 L) were charged to a 2.5 L Parr hydrogenator under a nitrogen atmosphere. Hydrogen was charged to the reaction vessel for up to 55 psi. The mixture was shaken for 13 hours, maintaining hydrogen pressure between 50 and 55 psi. Hydrogen was released and the mixture was purged with nitrogen 3 times. The suspension was filtered through a celite bed and rinsed with methanol. The filtrate was concentrated in vacuo. The resulting solid was reslurried in ether and isolated by vacuum filtration. The solid was dried in vacuo to a constant weight, affording 54 g (84% yield) of 3-aminopthalic acid as a yellow product. ^{1}H-NMR (DMSO-d6) δ: 3.17 (s, 2H), 6.67 (d, 1H), 6.82 (d, 1H), 7.17 (t, 1H), 8-10 (br, s, 2H); ^{13}C-NMR (DMSO-d6) δ: 112.00, 115.32, 118.20, 131.28, 135.86, 148.82, 169.15, 170.09.

Preparation of 3-aminophthalic anhydride: A 1 L 3-necked round bottom flask was equipped with a mechanical stirrer, thermometer, and condenser and charged with 3-aminophthalic acid (108 g, 596 mmol) and acetic anhydride (550 mL). The reaction mixture was heated to reflux for 3 hours and cooled to about 25° C. and further to 0-5° C. for another 1 hour. The crystalline solid was collected by vacuum filtration and washed with ether. The solid product was dried in vacuo at ambient temperature to a constant weight, giving 75 g (61% yield) of 3-acetamidopthalic anhydride as a white product. ^{1}H-NMR ($CDCl_3$) δ: 2.21 (s, 3H), 7.76 (d, 1H), 7.94 (t, 1H), 8.42 (d, 1H), 9.84 (s, 1H).

Resolution of 2-(3-ethoxy-4-methoxyphenyl-1-(methylsulphonyl)-eth-2-ylamine: A 3 L 3-necked round bottom flask was equipped with a mechanical stirrer, thermometer, and condenser and charged with 2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine (137.0 g, 500 mmol), N-acetyl-L-leucine (52 g, 300 mmol), and methanol (1.0 L). The stirred slurry was heated to reflux for 1 hour. The stirred mixture was allowed to cool to ambient temperature and stirring was continued for another 3 hours at ambient temperature. The slurry was filtered and washed with methanol (250 L). The solid was air-dried and then dried in vacuo at ambient temperature to a constant weight, giving 109.5 g (98% yield) of the crude product (85.8% ee). The crude solid (55.0 g) and methanol (440 mL) were brought to reflux for 1 hour, cooled to room temperature and stirred for an additional 3 hours at ambient temperature. The slurry was filtered and the filter cake was washed with methanol (200 mL). The solid was air-dried and then dried in vacuo at 30° C. to a constant weight, yielding 49.6 g (90% recovery) of (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-ylamine-N-acetyl-L-leucine salt (98.4% ee). Chiral HPLC (1/99 EtOH/20 mM KH_2PO_4 @ pH 7.0, Ultron Chiral ES-OVS from Agilent Technologies, 150 mm×4.6 mm, 0.5 mL/min., @ 240 nm): 18.4 min (S-isomer, 99.2%), 25.5 min (R-isomer, 0.8%).

Final preparation of apremilast: A 500 mL 3-necked round bottom flask was equipped with a mechanical stirrer, thermometer, and condenser. The reaction vessel was charged with (S)-2-(3-ethoxy-4-methoxyphenyl)-1-(methylsulphonyl)-eth-2-yl amine N-acetyl-L-leucine salt (25 g, 56 mmol, 98% ee), 3-acetamidophthalic anhydride (12.1 g, 58.8 mmol), and glacial acetic acid (250 mL). The mixture was refluxed over night and then cooled to <50° C. The solvent was removed in vacuo, and the residue was dissolved in ethyl acetate. The resulting solution was washed with water (250 mL×2), saturated aqueous $NaHCO_3$ (250 mL×2), brine (250 mL×2), and dried over sodium sulphate. The solvent was evaporated in vacuo, and the residue recrystallized from a binary solvent containing ethanol (150 mL) and acetone (75 mL). The solid was isolated by vacuum filtration and washed with ethanol (100 mL×2). The product was dried in vacuo at 60° C. to a constant weight, affording 19.4 g (75% yield) of apremilast with 98% ee. Chiral HPLC (15/85 EtOH/20 mM KH_2PO_4 @ pH 5, Ultron Chiral ES-OVS from Agilent Technology, 150 mm×4.6 mm, 0.4 mL/min, @ 240 nm): 25.4 min (S-isomer, 98.7%), 29.5 min (R-isomer, 1.2%). ^{1}H-NMR ($CDCl_3$) δ: 1.47 (t, 3H), 2.26 (s, 3H), 2.87 (s, 3H), 3.68-3.75 (dd, 1H), 3.85 (s, 3H), 4.07-4.15 (q, 2H), 4.51-4.61 (dd, 1H), 5.84-5.90 (dd, 1H), 6.82-8.77 (m, 6H), 9.46 (s, 1H); ^{13}C-NMR (DMSO-d6) δ: 14.66, 24.92, 41.61, 48.53, 54.46, 55.91, 64.51, 111.44, 112.40, 115.10, 118.20, 120.28, 124.94, 129.22, 131.02, 136.09, 137.60, 148.62, 149.74, 167.46, 169.14, 169.48.

Pharmaceutical Compositions and Dosage Forms

Pharmaceutical compositions can be used in the preparation of individual, single unit dosage forms. Pharmaceutical compositions and dosage forms can comprise apremilast or a pharmaceutically acceptable salt or solvate thereof and a second active agent. Examples of the optional second active agents are disclosed herein (see, e.g., section 4.2.1). Phar-

maceutical compositions and dosage forms can further comprise one or more carriers, excipients or diluents.

The pharmaceutical compositions provided herein are suitable for oral administration can be presented as discrete dosage forms, such as, but not limited to, tablets (e.g., chewable tablets), caplets, capsules and liquids (e.g., flavored syrups). Such dosage forms contain predetermined amounts of active ingredients and can be prepared by methods of pharmacy well known to those skilled in the art. See generally, *Remington's Pharmaceutical Sciences,* 20th ed., Mack Publishing, Easton Pa. (2,000).

Typical oral dosage forms are prepared by combining the active ingredients in an intimate admixture with at least one excipient according to conventional pharmaceutical compounding techniques. Excipients can take a wide variety of forms depending on the form of preparation desired for administration. Non-limiting examples of excipients suitable for use in oral liquid or aerosol dosage forms include water, glycols, oils, alcohols, flavoring agents, preservatives and coloring agents. Non-limiting examples of excipients suitable for use in solid oral dosage forms (e.g., powders, tablets, capsules and caplets) include starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders and disintegrating agents.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid excipients are employed. If desired, tablets can be coated by standard aqueous or non-aqueous techniques. Such dosage forms can be prepared by any of the methods of pharmacy. In general, pharmaceutical compositions and dosage forms are prepared by uniformly and intimately admixing the active ingredients with liquid carriers, finely divided solid carriers or both and then shaping the product into the desired presentation if necessary.

For example, a tablet can be prepared by compression or molding. Compressed tablets can be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as powder or granules, optionally mixed with an excipient. Molded tablets can be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

Non-limiting examples of excipients that can be used in oral dosage forms include binders, fillers, disintegrants and lubricants. Non-limiting examples of binders suitable for use in pharmaceutical compositions and dosage forms include corn starch, potato starch or other starches, gelatin, natural and synthetic gums such as *acacia*, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone, methyl cellulose, pre-gelatinized starch, hydroxypropyl methyl cellulose, (e.g., Nos. 2208, 2906, 2910), microcrystalline cellulose and mixtures thereof.

Non-limiting examples of suitable forms of microcrystalline cellulose include, but are not limited to, the materials sold as AVICEL® (microcrystalline cellulose) PH-101, AVICEL® (microcrystalline cellulose) PH-103, AVICEL RC-5810 (crystalline cellulose and carboxymethylcellulose sodium), AVICEL® (microcrystalline cellulose) PH-105 (available from FMC Corporation, American Viscose Division, Avicel Sales, Marcus Hook, Pa.), and mixtures thereof. A specific binder is a mixture of microcrystalline cellulose and sodium carboxymethyl cellulose sold as AVICEL RC-581® (crystalline cellulose and carboxymethylcellulose sodium). Suitable anhydrous or low moisture excipients or additives include AVICEL-PH-103® (microcrystalline cellulose) PH-103 and Starch 1500® LM (pre gelatinized starch).

Non-limiting examples of fillers suitable for use in the pharmaceutical compositions and dosage forms disclosed herein include talc, calcium carbonate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch and mixtures thereof. The binder or filler in pharmaceutical compositions is typically present in from about 50 to about 99 weight percent of the pharmaceutical composition or dosage form.

Disintegrants are used in the compositions to provide tablets that disintegrate when exposed to an aqueous environment. Tablets that contain too much disintegrant may disintegrate in storage, while those that contain too little may not disintegrate at a desired rate or under the desired conditions. Thus, a sufficient amount of disintegrant that is neither too much nor too little to detrimentally alter the release of the active ingredients should be used to form solid oral dosage forms. The amount of disintegrant used varies based upon the type of formulation and is readily discernible to those of ordinary skill in the art. Typical pharmaceutical compositions comprise from about 0.5 to about 15 weight percent of disintegrant, preferably from about 1 to about 5 weight percent of disintegrant.

Non-limiting examples of disintegrants that can be used in pharmaceutical compositions and dosage forms include agar-agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrilin potassium, sodium starch glycolate, potato or tapioca starch, other starches, pre-gelatinized starch, other starches, clays, other algins, other celluloses, gums and mixtures thereof.

Non-limiting examples of lubricants that can be used in pharmaceutical compositions and dosage forms include calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar and mixtures thereof. Additional lubricants include, for example, a syloid silica gel (AEROSIL200® (silica), manufactured by W.R. Grace Co. of Baltimore, Md.), a coagulated aerosol of synthetic silica (marketed by Degussa Co. of Plano, Tex.), CAB-O-SIL® (fumed silica) (a pyrogenic silicon dioxide product sold by Cabot Co. of Boston, Mass.) and mixtures thereof. If used at all, lubricants are typically used in an amount of less than about 1 weight percent of the pharmaceutical compositions or dosage forms into which they are incorporated.

Non-limiting examples of dosage forms include tablets; caplets; capsules, such as soft elastic gelatin capsules; cachets; troches; lozenges; dispersions; suppositories; powders; aerosols (e.g., nasal sprays or inhalers); gels; liquid dosage forms suitable for oral or mucosal administration to a patient, including suspensions (e.g., aqueous or non-aqueous liquid suspensions, oil-in-water emulsions or a water-in-oil liquid emulsions), solutions and elixirs.

The composition, shape and type of dosage forms will typically vary depending on their use. For example, a dosage form used in the acute treatment of a disease may contain larger amounts of one or more of the active ingredients it comprises than a dosage form used in the chronic treatment of the same disease. These and other ways in which specific dosage forms will vary from one another will be readily

apparent to those skilled in the art. See, e.g., *Remington's Pharmaceutical Sciences,* 20th ed., Mack Publishing, Easton Pa. (2,000).

Typical pharmaceutical compositions and dosage forms comprise one or more excipients. Suitable excipients are well known to those skilled in the art of pharmacy and non-limiting examples of suitable excipients are provided herein. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a patient. The suitability of a particular excipient may also depend on the specific active ingredients in the dosage form. For example, the decomposition of some active ingredients can be accelerated by some excipients such as lactose or when exposed to water. Active ingredients that comprise primary or secondary amines are particularly susceptible to such accelerated decomposition.

In certain embodiments, provided herein are anhydrous pharmaceutical compositions and dosage forms comprising active ingredients, since water can facilitate the degradation of some compounds. For example, the addition of water (e.g., 5%) is widely accepted in the pharmaceutical arts as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. See, e.g., Jens T. Carstensen, *Drug Stability: Principles & Practice,* 2d. Ed., Marcel Dekker, NY, N.Y., 1995, pp. 379-80. In effect, water and heat accelerate the decomposition of some compounds. Thus, the effect of water on a formulation can be of great significance since moisture and/or humidity are commonly encountered during manufacture, handling, packaging, storage, shipment and use of formulations.

Anhydrous pharmaceutical compositions and dosage forms can be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Pharmaceutical compositions and dosage forms that comprise lactose and at least one active ingredient that comprises a primary or secondary amine are preferably anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging and/or storage is expected.

An anhydrous pharmaceutical composition should be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions are preferably packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits. Non-limiting examples of suitable packaging include hermetically sealed foils, plastics, unit dose containers (e.g., vials), blister packs and strip packs.

Also provided herein are pharmaceutical compositions and dosage forms that comprise one or more compounds that reduce the rate by which an active ingredient will decompose. Such compounds, which are referred to herein as "stabilizers," include, but are not limited to, antioxidants such as ascorbic acid, pH buffers or salt buffers. Like the amounts and types of excipients, the amounts and specific types of active ingredients in a dosage form may differ depending on factors such as, but not limited to, the route by which it is to be administered to patients. However, typical oral dosage forms comprise apremilast in an amount of 10 mg, 20 mg or 30 mg. In a particular embodiments, the oral dosage forms are 10 mg, 20 mg or 30 mg tablets. Each tablet contains apremilast as the active ingredient and the following inactive ingredients: lactose monohydrate, microcrystalline cellulose, croscarmellose sodium, magnesium stearate, polyvinyl alcohol, titanium dioxide, polyethylene glycol, talc, iron oxide red, iron oxide yellow (20 and 30 mg only), and iron oxide black (30 mg only).

Delayed Release Dosage Forms

In certain embodiments, active ingredients can be administered by controlled release means or by delivery devices that are well known to those of ordinary skill in the art. Non-limiting examples of controlled release means or delivery devices include those described in U.S. Pat. Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; and 4,008,719, 5,674,533, 5,059,595, 5,591,767, 5,120,548, 5,073,543, 5,639,476, 5,354,556 and 5,733,566, each of which is incorporated herein by reference. Such dosage forms can be used to provide slow or controlled-release of one or more active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the active ingredients. In certain embodiments, provided herein are single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps and caplets that are adapted for controlled-release.

All controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency and increased patient compliance. In addition, controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the drug and can thus affect the occurrence of side (e.g., adverse) effects.

Most controlled-release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect and gradually and continually release of other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled-release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, temperature, enzymes, water or other physiological conditions or compounds.

5. EXAMPLES

Some embodiments are illustrated by the following non-limiting examples. The examples should not be construed as a limitation in the scope thereof.

The recommended dose of apremilast is 30 mg twice daily taken orally for treating patients with psoriasis, ankylosing spondylitis, Behcet's disease, rheumatoid arthritis, atopic dermatitis, Crohn's disease or ulcerative colitis. The recommended dosage titration schedule is described below.

(i) 10 mg in the morning on the first day of administration;

(ii) 10 mg in the morning and 10 mg after noon on the second day of administration;

(iii) 10 mg in the morning and 20 mg after noon on the third day of administration;

(iv) 20 mg in the morning and 20 mg after noon on the fourth day of administration;

(v) 20 mg in the morning and 30 mg after noon on the fifth day of administration; and

(vi) 30 mg in the morning and 30 mg after noon on the sixth and every subsequent day of administration.

Other titration schedules with a target dose of 20 mg BID and 40 mg BID are also described herein. The dosage titration schedule can be used for patients with psoriasis, ankylosing spondylitis, Behcet's disease, rheumatoid arthritis, atopic dermatitis, or ulcerative colitis.

This dosage titration is intended to reduce the gastrointestinal symptoms that may be associated with initial treatment. Apremilast can be administered without regard to meals.

5.1. Clinical Activity of Apremilast in Patients with Psoriasis in a Clinical Study (Esteem 1)

A randomized, double-blind, placebo controlled, multicenter clinical study was performed in patients with moderate to severe plaque psoriasis who had a body surface area (BSA) involvement of ≥10%, static Physician Global Assessment (sPGA) of ≥3 (moderate or severe disease), Psoriasis Area and Severity Index (PASI) score≥12, and who were candidates for phototherapy or systemic therapy. In the study 844 patients were enrolled; patients ranged in age from 18 to 83 years, with an overall median age of 46 years. The mean baseline body surface area (BSA) involvement was 25.19% (median 21.0%), the mean baseline PASI score was 19.07 (median 16.80), and the proportion of patients with sPGA score of 3 (moderate) and 4 (severe) at Baseline were 70.0% and 29.8%, respectively. Patients were randomized to Apremilast, in an amount of 30 mg twice per day (after the initial dose titration schedule) or placebo for the first 16 weeks, and from Weeks 16 to 32, all patients received Apremilast in an amount of 30 mg twice per day.

Dose Titration Schedule:

Target Dose: 30 mg (po) BID
Day 1: 10 mg in the morning
Day 2: 10 mg morning; 10 mg after noon
Day 3: 10 mg morning; 20 mg after noon
Day 4: 20 mg morning; 20 mg after noon
Day 5: 20 mg morning; 30 mg after noon
Day 6 onward: 30 mg BID

During the Randomized Treatment Withdrawal Phase (Weeks 32-52), patients originally randomized to Apremilast who achieved at least a 75% reduction in PASI score (PASI-75) at Week 32 were re-randomized to either placebo or Apremilast 30 mg BID. Patients who were re-randomized to placebo and who lost PASI-75 response at Week 32 compared to Baseline, were retreated with Apremilast 30 mg BID. Patients who did not achieve the designated PASI response by Week 32, or who were initially randomized to placebo, remained on Apremilast until Week 52.

Results:

The primary endpoint, PASI 75 at Week 16, was achieved by a significantly greater proportion of patients randomized to apremilast (186 [33.1%]) than placebo (15 [5.3%]; 95% CI 23.1-32.5; p<0.0001; Table 1). An sPGA score of 0 or 1 at Week 16, the major secondary endpoint, was achieved by a significantly greater proportion of patients randomized to apremilast than placebo (122 [21.7%] versus 11 [3.9%]; 95% CI 13.7-21.9; p<0.0001). Similar results were obtained in sensitivity analyses including nonresponder imputation for PASI 75 (p<0.0001) and sPGA (p<0.0001) responses. Non-overlapping confidence intervals (representing a clinically meaningful difference) between apremilast and placebo in the mean percentage improvement in PASI from baseline were detected as early as Week 2. Other endpoints at Week 16, including PASI-50 and PASI-90 response, mean percentage change in PASI score, NAPSI score, mean change from baseline in DLQI score, and DLQI response (decrease of at least five points in DLQI total score), were all significantly greater with apremilast than placebo (all p<0.0001). For patients with nail and scalp psoriasis at baseline, at least a 50% improvement in baseline NAPSI (NAPSI 50) response and ScPGA response (score of 0 [clear] or 1 [minimal]) were achieved by a significantly greater proportion of patients randomized to apremilast than placebo at Week 16 (both NAPSI-50 and ScPGA p<0.0001).

Responses to apremilast were generally maintained from Weeks 16 to 32. At Week 32, patients initially randomized to placebo at baseline who were switched to apremilast at Week 16 had similar response rates as those receiving apremilast from baseline. PASI 50 responses and DLQI scores at Week 32 showed similar time-response profiles.

In the randomized treatment withdrawal phase, 154 patients randomized to apremilast at baseline who achieved PASI 75 responses at Week 32 were re-randomized to continue apremilast (n=77) or switch to placebo (n=77). Of the 77 patients re-randomized to apremilast at Week 32, 47 (61.0%) had PASI-75 response at Week 52, and 58 (75.3%) had at least 70% improvement in PASI score from baseline. Mean PASI responses were generally maintained through Week 52 in patients re-randomized to apremilast. Mean percentage change from baseline in PASI score at Week 52 was 80.5%. Among patients re-randomized to placebo, 13 (16.9%) did not resume apremilast before Week 52. At Week 52, mean percentage change in PASI score from baseline in these 13 patients was 88.1%; nine (11.7%) patients re-randomized to placebo achieved PASI 75 response at Week 52. 64 patients re-initiated apremilast before Week 52 after losing PASI 75 responses; of these, 45 (70.3%) regained PASI 75 response after re-treatment (re-treatment time ranged from 3.4 to 22.1 weeks). Median time to first loss of PASI 75 after re-randomization was 5.1 weeks for patients re-randomized to placebo and 17.7 weeks for patients re-randomized to apremilast.

The 5 most common adverse events (AEs) were diarrhea, nausea, upper respiratory tract infection, tension headache, and headache. Discontinuations due to AEs were nausea (1.2%), diarrhea (0.8%), headache (0.4%), and tension headache (0.2%). No patient discontinued due to upper respiratory tract infection.

Conclusion

The study results showed that Apremilast significantly improved signs and symptoms of psoriasis. The majority of adverse events was mild to moderate and did not lead to discontinuation. The results were very promising with respect to efficacy and safety of Apremilast in psoriasis patients.

TABLE 1

Clinical response across efficacy endpoints

	Placebo-controlled phase (Week 16)	
	Placebo n = 282	Apremilast 30 mg twice daily n = 562
Primary endpoint		
PASI-75	15 (5.3%)	186 (33.1%)*
Major secondary endpoint		
sPGA respons[§]	11 (3.9%)	122 (21.7%)*
Other endpoints		
PASI-50	48 (17.0%)	330 (58.7%)*
PASI-90	1 (0.4%)	55 (9.8%)*
Percentage change in PASI score, mean (SD)	−16.7 (31.52)	−52.1 (32.81)*
Percentage change in PASI score, median (range)	−14.0 (−91 to 72)	−59.0 (−100 to 86)
Mean Change in DLQI score	−2.1 (5.69)	−6.6 (6.66)*
Patients with baseline DLQI >5	n = 236	n = 459
DLQI response[‡]	79 (33.5%)	322 (70.2%)*
DLQI + PASI-50 response[‖]	26 (11.0%)	221 (48.1%)*
Patients with nail psoriasis at baseline	n = 195	n = 363
Percentage change in NAPSI score[¶]	6.5 (60.57)	−22.5 (54.86)*
NAPSI-50[¶]	29 (14.9%)	121 (33.3%)*
Patients with scalp psoriasis at baseline	n = 189	n = 374
ScPGA score 0-1[#]	33 (17.5%)	174 (46.5%)*

Data are n (%), mean (SD), or median (range).
PASI-75 = 75% reduction from baseline psoriasis area and severity index score;
sPGA = static physician global assessment;
PASI-50 = 50% reduction from baseline psoriasis area and severity index score;
PASI-90 = 90% reduction from baseline psoriasis area and severity index score;
DLQI = dermatology life quality index; NAPSI = nail psoriasis severity index;
NAPSI-50 = at least a 50% improvement from baseline nail psoriasis severity index score;
ScPGA = scalp physician global assessment.
Week 16 missing data were handled with last-observation-carried-forward methodology.
*P < 0.0001 versus placebo.
[§]sPGA score of clear (0) or almost clear (1) with at least a two-point reduction from baseline.
[‡]Decrease of at least five points in DLQI total score in patients with baseline total DLQI scor greater than 5. A reduction in score indicated improvement.
[‖]Decrease of at least five points in DLQI total score and PASI-50 achievement in patients with baseline total DLQI score greater than 5.
[¶]Patients with nail psoriasis (score of at least 1) at baseline. A reduction in the NAPSI score indicated improvement.
[#]Patients with ScPGA score of at least 3 at baseline.

5.2. Clinical Activity of Apremilast in Patients with Psoriasis in a Clinical Study (Esteem 2)

A randomized, double-blind, placebo controlled, multicenter clinical study was performed in patients with moderate to severe plaque psoriasis who had a body surface area (BSA) involvement of ≥10%, static Physician Global Assessment (sPGA) of ≥3 (moderate or severe disease), Psoriasis Area and Severity Index (PASI) score≥12, and who were candidates for phototherapy or systemic therapy. In the study 413 patients were enrolled; patients ranged in age from 18 to 83 years, with an overall median age of 46 years. The mean baseline body surface area (BSA) involvement was 25.19% (median 21.0%), the mean baseline PASI score was 19.07 (median 16.80), and the proportion of patients with sPGA score of 3 (moderate) and 4 (severe) at Baseline were 70.0% and 29.8%, respectively. Patients were randomized to Apremilast in an amount of 30 mg twice per day (after the initial dose titration schedule) or placebo for the first 16 weeks, and from Weeks 16 to 32, all patients received Apremilast in an amount of 30 mg twice per day.

Dose Titration Schedule:
Target Dose: 30 mg (po) BID
Day 1: 10 mg in the morning
Day 2: 10 mg morning; 10 mg after noon
Day 3: 10 mg morning; 20 mg after noon
Day 4: 20 mg morning; 20 mg after noon
Day 5: 20 mg morning; 30 mg after noon
Day 6 onward: 30 mg BID

During the Randomized Treatment Withdrawal Phase (Weeks 32-52), patients originally randomized to Apremilast who achieved at least a 50% reduction in PASI score (PASI-50) were re-randomized to either placebo or Apremilast 30 mg BID. Patients who were re-randomized to placebo and who lost 50% of the improvement in PASI at Week 32 compared to Baseline, were retreated with Apremilast 30 mg BID. Patients who did not achieve the designated PASI response by Week 32, or who were initially randomized to placebo, remained on Apremilast until Week 52.

Results:

Placebo-controlled phase (Weeks 0 to 16). At Week 16, significantly more patients receiving apremilast achieved a PASI-75 response (primary endpoint) vs. placebo (28.8% vs. 5.8%; P<0.0001; Table 2). The major secondary endpoint, sPGA score of 0 or 1 at Week 16, was also achieved by significantly more patients receiving apremilast vs. placebo (20.4% vs. 4.4%; P<0.0001). Significantly more patients receiving apremilast achieved PASI-50 and PASI-90 responses vs. placebo (55.5% vs. 19.7%; P<0.0001 and 8.8% vs. 1.5%; P=0.0042) at Week 16. Results of the NRI sensitivity analysis were similar to the primary analysis (Table 2). The mean/median percent change from baseline in PASI score was −50.9%/−56.0% for apremilast vs. −15.8%/−18.0% for placebo (P<0.0001, mean change) at Week 16. Non-overlapping confidence intervals (representing a clinically meaningful difference) between apremilast and placebo in the mean percentage improvement in PASI from baseline were detected as early as Week 2, the first post-baseline visit.

Significant improvements at Week 16 were seen with Apremilast vs. placebo based on other efficacy endpoints, including PASI response and Dermatology Life Quality Index (DLQI) score and response (all P<0.0001; Table 2). At Week 16, among patients with nail psoriasis (Nail Psoriasis Severity Index [NAPSI]≥1), NAPSI-50 response was achieved by significantly more patients receiving apremilast vs. placebo (44.6% vs. 18.7%; P<0.0001; Table 2). Similarly, among patients with scalp psoriasis (Scalp Physician Global Assessment [ScPGA]≥3) at baseline, ScPGA score of 0 (clear) or 1 (minimal) was achieved by significantly more patients receiving apremilast vs. placebo (40.9% vs. 17.2%; P<0.0001; Table 2) and among patients with palmoplantar psoriasis at baseline (Palmoplantar Psoriasis Physician Global Assessment [PPPGA]≥3), a score of 0 (clear) or 1 (almost clear) was achieved by significantly more patients receiving apremilast vs. placebo (65.4% vs. 31.3%; P=0.0315; Table 2).

At Week 16, mean improvements from baseline in pruritus visual analog scale (VAS; mm) scores were significantly greater with apremilast vs. placebo (−33.5 vs. −12.2; P<0.0001). Mean changes from baseline with apremilast represented a decrease of nearly 50% in pruritus severity. At

Week 16, mean improvements in skin discomfort/pain VAS (mm) scores were also significantly greater with apremilast vs. placebo (−28.5 vs. −9.5; P<0.0001; Table 2), which also represented a decrease of nearly 50% in severity from baseline. Non-overlapping confidence intervals (representing a clinically meaningful difference) between apremilast and placebo in improvement in pruritus and skin discomfort/pain from baseline were detected as early as Week 2.

Maintenance Phase (Weeks 16 to 32).

PASI-50, PASI-75, and PASI-90 responses were generally maintained from Weeks 16 to 32 in patients treated with apremilast from baseline. PASI, sPGA, and DLQI responses were also generally maintained from Weeks 16 to 32 in patients treated with apremilast from baseline and placebo patients who switched to apremilast at Week 16 had response rates similar to those in patients receiving apremilast in both treatment periods.

Randomized Treatment Withdrawal Phase (Weeks 32 to 52).

For the randomized treatment withdrawal phase, among the 123 patients initially randomized to apremilast who achieved≥PASI-50 at Week 32, 61 and 62 patients were re-randomized to apremilast and placebo, respectively. Of patients re-randomized to apremilast, 80.3% had a PASI-50 response and 60.7% had ≥70% improvement from baseline in PASI score at Week 52; mean percent change in PASI at Week 52 was −74.4%. Among the 36 patients re-randomized to apremilast who were also PASI-75 responders at Week 32, 66.7% had PASI-75 at Week 52. Among patients initially randomized to placebo at baseline who switched to apremilast at Week 16 and achieved PASI-50 at Week 32, 83.1% had PASI-50 at Week 52; mean percent change in PASI from baseline was −71.8%.

Of the 62 patients re-randomized to placebo at Week 32, 30 did not resume treatment with apremilast before Week 52 (these patients did not lose 50% of their PASI improvement prior to Week 52). Among patients re-randomized to placebo, 24.2% had a PASI-50 response at Week 52. Thirty-two (32) patients re-randomized to placebo lost 50% of their PASI improvement and re-initiated treatment with apremilast before Week 52; 65.6% of these patients regained PASI-50 response after re-treatment (re-treatment time ranged from 2.6 to 18.3 weeks). The median time to first loss of 50% of the PASI improvement obtained at Week 32 was 12.4 weeks among patients re-randomized to placebo and 21.9 weeks among patients re-randomized to apremilast (P<0.0001).

Most patients initially randomized to apremilast who did not achieve PASI-50 at Week 32 continued to experience mean PASI improvements through Week 52. At Week 52, PASI-50 was achieved by 32.8% of these patients; mean percent change from baseline in PASI score was −45.7%. Among patients randomized to placebo at baseline who switched to apremilast at Week 16 and who did not achieve PASI-50 at Week 32, 16.0% achieved PASI-50 at Week 52; mean percent change from baseline was −24.7%.

The 5 most common adverse events (AEs) were diarrhea, nausea, upper respiratory tract infection, tension headache, and headache. Discontinuations due to AEs were nausea (1.2%), diarrhea (0.8%), headache (0.4%), and tension headache (0.2%). No patient discontinued due to upper respiratory tract infection.

Conclusion

The study results showed that Apremilast significantly improved signs and symptoms of psoriasis. The majority of adverse events was mild to moderate and did not lead to discontinuation. The results were very promising and consistent with efficacy and safety of Apremilast in psoriasis patients.

TABLE 2

Clinical response across efficacy endpoints at Week 16 (placebo-controlled phase)

	Placebo n = 137	Apremilast 30 mg BID n = 274
Primary endpoint		
PASI-75, % (LOCF)	5.8	28.8*
PASI-75, % (NRI)	5.1	28.1*
Major secondary endpoint		
sPGA score 0 (clear) or 1 (almost clear)[§], % (LOCF)	4.4	20.4*
sPGA score 0 (clear) or 1 (almost clear), % (NRI)	3.6	19.7*
Other endpoints		
PASI-50, % (LOCF)	19.7	55.5*
PASI-50, % (NRI)	17.5	53.6*
PASI-90, % (LOCF)	1.5	8.8[‡]
Mean % change in PASI score	−15.8	−50.9*
Median % change in PASI score	−18.0	−56.0
Mean change in DLQI score	−2.8	−6.7*
Mean change in pruritus VAS score, mm	−12.2	−33.5*
Mean change in skin discomfort/pain VAS score, mm	−9.5	−28.5*
Patients with baseline DLQI >5	n = 119	n = 226
DLQI response (decrease of ≥5 points)[†], %	42.9	70.8*
DLQI (decrease of ≥5 points) + PASI-50 responsep[#], %	13.4	49.1*
Patients with nail psoriasis	n = 91	n = 175
Mean % change in NAPSI score[¶]	−7.1	−29.0**
NAPSI-50[¶], %	18.7	44.6*
Patients with scalp psoriasis	n = 93	n = 176
ScPGA score 0 (clear) or 1 (minimal)[§§], %	17.2	40.9*
Patients with palmoplantar psoriasis	n = 16	n = 26
PPPGA score 0 (clear) or 1 (almost clear)[‡‡], %	31.3	65.4[||]

Note:
Week 16 missing data were handled with last-observation-carried-forward methodology, except where noted for non-responder imputation. Decreases in DLQI score, pruritus VAS score, skin discomfort/pain VAS score, and NAPSI score indicate improvement.
*P < 0.0001 vs. placebo.
[§]sPGA score of 0 (clear) or 1 (almost clear) with a ≥2-point reduction from baseline.
[‡]P = 0.0042 vs. placebo.
[†]Decrease of ≥5 points in DLQI total score in patients with a baseline total DLQI score >5.
[#]Decrease of ≥5 points in DLQI total score and PASI-50 achievement in patients with baseline total DLQI score >5.
[¶]Patients with nail psoriasis (score ≥1) at baseline.
**P = 0.0052 vs. placebo.
[§§]Patients with ScPGA score of ≥3 (moderate to very severe) at baseline.
[‡‡]Patients with PPPGA score ≥3 (moderate to severe) at baseline.
[||]P = 0.0315 vs. placebo.
DLQI, Dermatology Life Quality Index;
LOCF, last observation carried forward;
NAPSI, Nail Psoriasis Severity Index;
NRI, non-responder imputation;
PASI, Psoriasis Area and Severity Index;
PPPGA, Palmoplantar Psoriasis Physician Global Assessment;
scPGA, Scalp Physician Global Assessment;
sPGA, static Physician Global Assessment;
VAS = visual analog scale.

5.3. Clinical Activity of Apremilast in Patients with Atopic Dermatitis in a Clinical Study

An open-label pilot study examining 2 doses of apremilast was performed in patients with adult atopic dermatitis. A total of 16 patients with moderate to severe atopic dermatitis were treated with apremilast in 2 different cohorts. Cohort 1 consisted of 6 adult patients treated with apremilast according to the dosing schedule below, 20 mg twice a day, for a total of 3 months. At the conclusion of this cohort, the US Food and Drug Administration (FDA) approved a higher dose and longer treatment course for apremilast. Thus, a second cohort was initiated. Cohort 2 consisted of 10 adult patients treated with apremilast according to the dosing schedule below, 30 mg twice a day, for a total of 6 months. A diagnosis of atopic dermatitis was determined by the Hanifin-Rajka criteria.

Dose Titration Schedule:

Target Dose: 20 mg (po) BID

Day 1: 10 mg in the morning
Day 2: 10 mg BID
Day 3: 10 mg morning; 20 mg after noon
Day 4 onward: 20 mg BID

Dose Titration Schedule:

Target Dose: 30 mg (po) BID

Day 1: 10 mg in the morning
Day 2: 10 mg BID
Day 3: 10 mg morning; 20 mg after noon
Day 4: 20 mg BID
Day 5: 20 mg morning; 30 mg after noon
Day 6 onward: 30 mg BID

In certain patients, a dosing schedule with an amount up to 40 mg BID is used.

Efficacy of apremilast was assessed at each study visit using the Eczema Area and Severity Index (EASI), Dermatology Life Quality Index (DLQI), investigator global assessment (IGA), and the visual analog scale (VAS) for pruritus. Patients were monitored for adverse events (AEs) and improvement in eczema as determined by the EASI, DLQI, and VAS for pruritus at 1 week, 2 weeks, 4 weeks, and every 4 weeks thereafter in cohort 1 and at 2 weeks, 4 weeks, and every 4 weeks thereafter in cohort 2. After the last dose of medication, patients in both cohorts were asked to return for a 4-week follow-up visit.

To participate in the study, patients must have met the following inclusion criteria: age of at least 18 years at time of consent, disease severity of at least 6 on the Rajka-Langeland severity scoring system, EASI score of at least 11, and be a candidate for or previously receiving systemic therapy. In addition, patients were required to remain on a stable regimen of triamcinolone acetonide ointment, 0.1%, for 2 weeks prior to the start of the study and throughout the trial. Most patients applied the ointment twice a day 2 times a week. No other topical therapy except emollients was allowed.

Patients were excluded if they had a history of active mycobacterial infection with any species (including *Mycobacterium tuberculosis*) within 3 years prior to the screening visit, latent or incompletely treated *M tuberculosis* infection, as indicated by a positive purified protein derivative skin test. Patients were not allowed to participate in the trial if they had had at least 3 major bacterial infections resulting in hospitalization and/or requiring intravenous antibiotic treatment within the past 2 years; clinically significant abnormality on chest radiography at screening; use of any investigational medication or systemic medication within 4 weeks prior to the start of the study drug or 5 pharmacokinetic/pharmacodynamic half-lives (whichever was longer); any clinically significant abnormality on 12-lead electrocardiogram at screening; a history of congenital or acquired immunodeficiency; positive results at screening for antinuclear antibody, hepatitis B surface antigen or hepatitis B core antibody, or antibodies to hepatitis C; a history of human immunodeficiency virus infection; malignant disease or a history of malignant disease (except for treated [i.e., cured] basal cell skin carcinomas>3 years prior to screening); systemic corticosteroid—dependent asthma; or active infection of any type at the time of enrollment.

As an exploratory end point to potentially identify immune pathways affected by apremilast, peripheral whole blood was obtained for differential gene expression analyses at baseline and after 3 (cohort 1) and 6 (cohort 2) months of treatment to determine apremilast's potential mechanism of action in patients with atopic dermatitis. RNA isolation and microarray analyses were performed in the Oregon Health and Science University Gene Microarray Shared Resource. Total RNA was isolated from PAXGene tubes using the PAXGene Blood RNA Isolation kit (QIAGEN Inc). RNA quantity was measured by spectrophotometric analysis; RNA quality was evaluated by size analysis on the Bioanalyzer 2100 (Agilent Technologies Inc). All samples passed RNA quality assessment review.

RNA samples were labeled using the OvationWTAPico Amplification and Labeling System (NuGEN Technologies Inc). Fifty nanograms of each sample were amplified with the Ovation WTA Pico kit, converted to sense complementary DNA (cDNA) with the WT-Ovation Exon Module, version 1, kit, and labeled with the Encore Biotin Module kit. Hybridization and array processing were performed as described in the NuGEN Encore Biotin Module User Guide http://www.nugeninc.com/tasks/sites/nugen/assets/File/user_guides/userguide_encore_biotin.pdf). Two micrograms of each labeled cDNA target werehybridized with the GeneChip Human Gene 1.0 ST array (Affymetrix) and scanned on the Affymetrix GeneChip 3000 Scanner. The array image was processed with Affymetrix Command Console (version 3.1.1). Data were normalized using the robust multichip average method.

Differential expression analyses were performed on 16 paired samples. All putatively differentially expressed genes were based on false discovery rate-adjusted P values<0.05. Based on the putative differentially expressed gene list, both enriched pathways and functional gene ontologic characteristics were identified (P<0.05 for hypergeometric test) in the GoStats package within the Bioconductor statistical programming environment (http://www.bioconductor.org).

In both cohorts 1 and 2, a trend toward improvement was seen in all outcomes. Intent-to-treat analyses performed at 3 months revealed significant reduction of itch from baseline (VAS) and improvement in quality of life (DLQI) in cohort 1 (P=0.02 and P=0.003, respectively). Disease severity (EASI) and quality of life (DLQI) improved in cohort 2 (P=0.008 and P=0.01, respectively). Statistically significant clinical improvement in atopic dermatitis was noted within the first 2 weeks of study drug in cohort 2 (P=0.03). Patients experienced an average reduction in itch of 49% using a VAS, from a mean baseline of 62.3 mm to 30.5 mm in cohort 1 and a 25% reduction in cohort 2, from 45.8 mm to 32.4 mm. The EASI scores reduced an average of 19% in cohort 1 from a mean baseline of 30.9 to 22.1 and an average of 39% in cohort 2, from a mean baseline of 21.4 to 13.2 at 3 months. The DLQI scores reduced an average of 55% in cohort 1, from a mean baseline of 14.2 to 6.2 and an average of 58% in cohort 2, from a mean baseline of 10.1 to 3.8. In

cohort 1, patients reported a statistically significant decline in pruritus within the first 2 weeks of use (P=0.045) with a trend for a decline in pruritus in cohort 2 (P=0.06). In cohort 1, 1 of 6 patients reduced their IGA score by 1 U (e.g., from very severe to severe). Two of 10 patients in cohort 2 reduced their IGA score by 1 U. No patient in either cohort reached an IGA score of clear or almost clear at the 3-month time point. One patient achieved an IGA score of mild in cohort 2.

Evaluation of cohort 1 was concluded at 3 months; consequently, no 6-month data were available for that cohort. Statistically significant improvement was seen in all outcomes at 6 months in cohort 2. Intent to treat analyses revealed significant reduction in EASI, from 21.1 to 11.6 (P=0.002); VAS, from 45.8 mm to 25.3 mm (P=0.03); and the DLQI, from 10.1 mm to 4.2 mm (P=0.03). Per protocol, EASI reduced from 21.1 to 10.4 (P=0.001), VAS from 45.8 to 22.7 (P=0.01), and DLQI from 10.1 to 4.0 (P=0.02). Five patients (50%) improved at least 1 U in the IGA at 6 months. Four of these 5 reached an IGA of mild, and 1 achieved an IGA of almost clear.

Post hoc intent-to-treat analyses performed on combined data from both cohorts were performed to improve the power of our analyses. The data from both cohorts combined showed statistically significant improvement in all outcomes. The EASI score was reduced from a mean baseline of 24.8 to 16.2 (P=0.002), the VAS was reduced from a mean baseline of 52.0 mm to 31.7 mm (P=0.003), and the DLQI was reduced from a mean baseline of 11.6 to 4.7 (P=0.001). Post hoc per-protocol analyses, which included data from all patients who were able to finish the study, also revealed significance in all outcomes (EASI, P=0.001; VAS, P=0.007; DLQI, P=0.001).

In cohort 1, gene expression data revealed significant differential expression of the cAMP response element binding (CREB) pathway ($P=3.19\times10^{4}$) and BAD (bcl-2 antagonist of cell death) phosphorylation pathway ($P=2.54\times10^{3}$). In addition, gene ontologic analyses of biological processes revealed significant differential expression of chemokine-mediated signaling ($P=9.5\times10^{6}$), IL-12 signaling ($P<0.05$), cytoskeleton remodeling ($P<0.05$), and regulation of immune complex clearing by monocytes and macrophages ($P=1.9\times10^{6}$). In cohort 2, there was significant differential expression of CCR3 signaling in eosinophils ($P=5\ 0.497\times 10^{2}$).

5.4. Clinical Activity of Apremilast in Patients with Ankylosing Spondylitis in a Clinical Study

A 24-week open-label clinical study of adult subjects with moderate to severe ankylosing spondylitis is conducted to assess the ability of an oral formulation comprising apremilast to treat patients having ankylosing spondylitis. All subjects will receive apremilast at a dose of 30 mg twice daily for a total of 24 weeks after the initial dosing schedule. The dose may be reduced to 30 mg per day if significant adverse events develop that lead to poor tolerability. After the last dose of apremilast, there will be a 4-week follow-up period. Subjects will be evaluated on Days 169 and 197.

Adult male and female subjects 18 years of age or older will participate in this study after the objectives, methods, and potential hazards of the study have been fully explained, and after they have signed the informed consent form. The Investigator is responsible for keeping a record of all subjects who sign an informed consent form for entry into this study. Screening procedures will be followed. To be enrolled into the study, subjects must meet the inclusion/exclusion criteria, which includes a diagnosis of ankylosing spondylitis.

Dose Titration Schedule:

Target Dose: 30 mg (po) BID

Day 1: 10 mg in the morning
Day 2: 10 mg BID
Day 3: 10 mg morning; 20 mg after noon
Day 4: 20 mg BID
Day 5: 20 mg morning; 30 mg after noon
Day 6 onward: 30 mg BID

In certain patients, a dosing schedule with an amount up to 20 mg BID is used.

Subjects will take apremilast tablets twice daily (BID). If at any time during the study a subject encounters overt study medication-related adverse effects, dose reduction will be allowed following discussions between the subject and the investigator.

Study medication should be taken at approximately the same time every day, 12 hours apart, once in the morning and once in the after noon. If a subject reports GI side effects when taking the study medication prior to meals, the subject will be advised to switch to the postprandial dosing schedule.

5.5. Clinical Activity of Apremilast in Patients with Rheumatoid Arthritis in a Clinical Study

A 24-week open-label clinical study of adult subjects with moderate to severe rheumatoid arthritis is conducted to assess the ability of an oral formulation comprising apremilast to treat patients having rheumatoid arthritis. All subjects will receive apremilast at a dose of 30 mg twice daily for a total of 24 weeks after the initial dosing schedule. The dose may be reduced to 30 mg per day if significant adverse events develop that lead to poor tolerability. After the last dose of apremilast, there will be a 4-week follow-up period. Subjects will be evaluated on Days 169 and 197.

Adult male and female subjects 18 years of age or older will participate in this study after the objectives, methods, and potential hazards of the study have been fully explained, and after they have signed the informed consent form. The Investigator is responsible for keeping a record of all subjects who sign an informed consent form for entry into this study. Screening procedures will be followed. To be enrolled into the study, subjects must meet the inclusion/exclusion criteria, which includes a diagnosis of rheumatoid arthritis.

Dose Titration Schedule:

Target Dose: 30 mg (po) BID

Day 1: 10 mg in the morning
Day 2: 10 mg BID
Day 3: 10 mg morning; 20 mg after noon
Day 4: 20 mg BID
Day 5: 20 mg morning; 30 mg after noon
Day 6 onward: 30 mg BID

In certain patients, a dosing schedule with an amount up to 20 mg BID is used.

Subjects will take apremilast tablets twice daily (BID). If at any time during the study a subject encounters overt study medication-related adverse effects, dose reduction will be allowed following discussions between the subject and the investigator.

Study medication should be taken at approximately the same time every day, 12 hours apart, once in the morning and once in the after noon. If a subject reports GI side effects

when taking the study medication prior to meals, the subject will be advised to switch to the postprandial dosing schedule.

5.6. Clinical Activity of Apremilast in Patients with Behcet's Disease in a Clinical Study

A 24-week open-label clinical study of adult subjects with moderate to severe Behcet's disease is conducted to assess the ability of an oral formulation comprising apremilast to treat patients having Behcet's disease. All subjects will receive apremilast at a dose of 30 mg twice daily for a total of 24 weeks after the initial dosing schedule. The dose may be reduced to 30 mg per day if significant adverse events develop that lead to poor tolerability. After the last dose of apremilast, there will be a 4-week follow-up period. Subjects will be evaluated on Days 169 and 197.

Adult male and female subjects 18 years of age or older will participate in this study after the objectives, methods, and potential hazards of the study have been fully explained, and after they have signed the informed consent form. The Investigator is responsible for keeping a record of all subjects who sign an informed consent form for entry into this study. Screening procedures will be followed. To be enrolled into the study, subjects must meet the inclusion/exclusion criteria, which includes a diagnosis of Behcet's disease.

Dose Titration Schedule:

Target Dose: 30 mg (po) BID

Days 1-2: 10 mg in the morning and 10 mg after noon
Days 3-4: 20 mg in the morning and 20 mg after noon
Days 5-onwards: 30 mg in the morning and 30 mg after noon.

Other dose titration schedules described herein, for example the titration schedules for patients with psoriasis in Example 5.1, can also be used. Subjects will take apremilast tablets twice daily (BID). If at any time during the study a subject encounters overt study medication-related adverse effects, dose reduction will be allowed following discussions between the subject and the investigator.

Study medication should be taken at approximately the same time every day, 12 hours apart, once in the morning and once in the after noon. If a subject reports GI side effects when taking the study medication prior to meals, the subject will be advised to switch to the postprandial dosing schedule.

5.7. Clinical Activity of Apremilast in Patients with Ulcerative Colitis in a Clinical Study

A 24-week open-label clinical study of adult subjects with moderate to severe ulcerative colitis is conducted to assess the ability of an oral formulation comprising apremilast to treat patients having ulcerative colitis. All subjects will receive apremilast at a dose of 30 mg or 40 mg twice daily for a total of 24 weeks after the initial dosing schedule. The dose may be reduced by half if significant adverse events develop that lead to poor tolerability. After the last dose of apremilast, there will be a 4-week follow-up period. Subjects will be evaluated on Days 169 and 197.

Adult male and female subjects 18 years of age or older will participate in this study after the objectives, methods, and potential hazards of the study have been fully explained, and after they have signed the informed consent form. The Investigator is responsible for keeping a record of all subjects who sign an informed consent form for entry into this study. Screening procedures will be followed. To be enrolled into the study, subjects must meet the inclusion/exclusion criteria, which includes a diagnosis of ulcerative colitis.

Dose Titration Schedule:

Target Dose: 30 mg (po) BID

Day 1: 10 mg in the morning
Day 2: 10 mg BID
Day 3: 10 mg morning; 20 mg after noon
Day 4: 20 mg BID
Day 5: 20 mg morning; 30 mg after noon
Day 6 onward: 30 mg BID

Dose Titration Schedule:

Target Dose: 40 mg (po) BID

Day 1: 10 mg in the morning
Day 2: 10 mg BID
Day 3: 10 mg morning; 20 mg after noon
Day 4: 20 mg BID
Day 5: 20 mg morning; 30 mg after noon
Day 6: 30 mg BID
Day 7: 30 mg morning; 40 mg after noon
Day 8 onward: 40 mg BID

Subjects will take apremilast tablets twice daily (BID). If at any time during the study a subject encounters overt study medication-related adverse effects, dose reduction will be allowed following discussions between the subject and the investigator.

Study medication should be taken at approximately the same time every day, 12 hours apart, once in the morning and once in the after noon. If a subject reports GI side effects when taking the study medication prior to meals, the subject will be advised to switch to the postprandial dosing schedule.

All of the references cited herein are incorporated by reference in their entirety. While the methods provided herein have been described with respect to the particular embodiments, it will be apparent to those skilled in the art that various changes and modifications can be made without departing from the spirit and scope as recited by the appended claims.

The embodiments described above are intended to be merely exemplary and those skilled in the art will recognize or will be able to ascertain using no more than routine experimentation, numerous equivalents of specific compounds, materials and procedures. All such equivalents are considered to be within the scope and are encompassed by the appended claims.

What is claimed is:

1. A method for treating a patient with stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, wherein the patient is suffering from psoriasis, the method consisting of:

(a) administering to the patient stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione in an initial titration dosing schedule consisting of

(i) 10 mg in the morning on the first day of administration;

(ii) 10 mg in the morning and 10 mg after noon on the second day of administration;

(iii) 10 mg in the morning and 20 mg after noon on the third day of administration;

(iv) 20 mg in the morning and 20 mg after noon on the fourth day of administration;

(v) 20 mg in the morning and 30 mg after noon on the fifth day of administration; and

(b) on the sixth and every subsequent day, administering to the patient stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acety-

laminoisoindoline-1,3-dione at a dose of between about 40 mg/day and about 100 mg/day.

2. A method for treating a patient with stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, wherein the patient is suffering from psoriasis, the method consisting of:

(a) administering to the patient stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione in an initial titration dosing schedule consisting of

(i) 10 mg in the morning on the first day of administration;

(ii) 10 mg in the morning and 10 mg after noon on the second day of administration;

(iii) 10 mg in the morning and 20 mg after noon on the third day of administration;

(iv) 20 mg in the morning and 20 mg after noon on the fourth day of administration;

(v) 20 mg in the morning and 30 mg after noon on the fifth day of administration; and

(b) on the sixth and every subsequent day, administering to the patient 30 mg in the morning and 30 mg after noon of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

3. A method for treating a patient with stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, wherein the patient is suffering from ankylosing spondylitis, the method consisting of:

(a) administering to the patient stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione in an initial titration dosing schedule consisting of

(i) 10 mg in the morning on the first day of administration;

(ii) 10 mg in the morning and 10 mg after noon on the second day of administration;

(iii) 10 mg in the morning and 20 mg after noon on the third day of administration;

(iv) 20 mg in the morning and 20 mg after noon on the fourth day of administration;

(v) 20 mg in the morning and 30 mg after noon on the fifth day of administration; and

(b) on the sixth and every subsequent day, administering to the patient stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione at a dose of between about 40 mg/day and about 100 mg/day.

4. A method for treating a patient with stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, wherein the patient is suffering from ankylosing spondylitis, the method consisting of:

(a) administering to the patient stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione in an initial titration dosing schedule consisting of

(i) 10 mg in the morning on the first day of administration;

(ii) 10 mg in the morning and 10 mg after noon on the second day of administration;

(iii) 10 mg in the morning and 20 mg after noon on the third day of administration;

(iv) 20 mg in the morning and 20 mg after noon on the fourth day of administration;

(v) 20 mg in the morning and 30 mg after noon on the fifth day of administration; and

(b) on the sixth and every subsequent day, administering to the patient 30 mg in the morning and 30 mg after noon of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

5. A method for treating a patient with stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, wherein the patient is suffering from Behcet's disease, the method consisting of:

(a) administering to the patient stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione in an initial titration dosing schedule consisting of

(i) 10 mg in the morning on the first day of administration;

(ii) 10 mg in the morning and 10 mg after noon on the second day of administration;

(iii) 10 mg in the morning and 20 mg after noon on the third day of administration;

(iv) 20 mg in the morning and 20 mg after noon on the fourth day of administration;

(v) 20 mg in the morning and 30 mg after noon on the fifth day of administration; and

(b) on the sixth and every subsequent day, administering to the patient stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione at a dose of between about 40 mg/day and about 100 mg/day.

6. A method for treating a patient with stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, wherein the patient is suffering from Behcet's disease, the method consisting of:

(a) administering to the patient stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione in an initial titration dosing schedule consisting of

(i) 10 mg in the morning on the first day of administration;

(ii) 10 mg in the morning and 10 mg after noon on the second day of administration;

(iii) 10 mg in the morning and 20 mg after noon on the third day of administration;

(iv) 20 mg in the morning and 20 mg after noon on the fourth day of administration;

(v) 20 mg in the morning and 30 mg after noon on the fifth day of administration; and

(b) on the sixth and every subsequent day, administering to the patient 30 mg in the morning and 30 mg after noon of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

7. A method for treating a patient with stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, wherein the patient is suffering from rheumatoid arthritis, the method consisting of:

(a) administering to the patient stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione in an initial titration dosing schedule consisting of

(i) 10 mg in the morning on the first day of administration;

(ii) 10 mg in the morning and 10 mg after noon on the second day of administration;

(iii) 10 mg in the morning and 20 mg after noon on the third day of administration;

(iv) 20 mg in the morning and 20 mg after noon on the fourth day of administration;

(v) 20 mg in the morning and 30 mg after noon on the fifth day of administration; and

(b) on the sixth and every subsequent day, administering to the patient stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione at a dose of between about 40 mg/day and about 100 mg/day.

8. A method for treating a patient with stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, wherein the patient is suffering from rheumatoid arthritis, the method consisting of:

(a) administering to the patient stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione in an initial titration dosing schedule consisting of

(i) 10 mg in the morning on the first day of administration;

(ii) 10 mg in the morning and 10 mg after noon on the second day of administration;

(iii) 10 mg in the morning and 20 mg after noon on the third day of administration;

(iv) 20 mg in the morning and 20 mg after noon on the fourth day of administration;

(v) 20 mg in the morning and 30 mg after noon on the fifth day of administration; and

(b) on the sixth and every subsequent day, administering to the patient 30 mg in the morning and 30 mg after noon of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

9. A method for treating a patient with stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, wherein the patient is suffering from atopic dermatitis, the method consisting of:

(a) administering to the patient stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione in an initial titration dosing schedule consisting of

(i) 10 mg in the morning on the first day of administration;

(ii) 10 mg in the morning and 10 mg after noon on the second day of administration;

(iii) 10 mg in the morning and 20 mg after noon on the third day of administration;

(iv) 20 mg in the morning and 20 mg after noon on the fourth day of administration;

(v) 20 mg in the morning and 30 mg after noon on the fifth day of administration; and

(b) on the sixth and every subsequent day, administering to the patient stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione at a dose of between about 40 mg/day and about 100 mg/day.

10. A method for treating a patient with stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, wherein the patient is suffering from atopic dermatitis, the method consisting of:

(a) administering to the patient of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione in an initial titration dosing schedule consisting of

(i) 10 mg in the morning on the first day of administration;

(ii) 10 mg in the morning and 10 mg after noon on the second day of administration;

(iii) 10 mg in the morning and 20 mg after noon on the third day of administration;

(iv) 20 mg in the morning and 20 mg after noon on the fourth day of administration;

(v) 20 mg in the morning and 30 mg after noon on the fifth day of administration; and

(b) on the sixth and every subsequent day, administering to the patient 30 mg in the morning and 30 mg after noon of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

11. A method for treating a patient with stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, wherein the patient is suffering from Crohn's disease, the method consisting of:

(a) administering to the patient stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione in an initial titration dosing schedule consisting of

(i) 10 mg in the morning on the first day of administration;

(ii) 10 mg in the morning and 10 mg after noon on the second day of administration;

(iii) 10 mg in the morning and 20 mg after noon on the third day of administration;

(iv) 20 mg in the morning and 20 mg after noon on the fourth day of administration;

(v) 20 mg in the morning and 30 mg after noon on the fifth day of administration; and

(b) on the sixth and every subsequent day, administering to the patient stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione at a dose of between about 40 mg/day and about 100 mg/day.

12. A method for treating a patient with stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, wherein the patient is suffering from Crohn's disease, the method consisting of:

(a) administering to the patient stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione in an initial titration dosing schedule consisting of

(i) 10 mg in the morning on the first day of administration;

(ii) 10 mg in the morning and 10 mg after noon on the second day of administration;

(iii) 10 mg in the morning and 20 mg after noon on the third day of administration;

(iv) 20 mg in the morning and 20 mg after noon on the fourth day of administration;

(v) 20 mg in the morning and 30 mg after noon on the fifth day of administration; and

(b) on the sixth and every subsequent day, administering to the patient 30 mg in the morning and 30 mg after noon of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

13. A method for treating a patient with stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, wherein the patient is suffering from ulcerative colitis, the method consisting of:

(a) administering to the patient stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione in an initial titration dosing schedule consisting of

(i) 10 mg in the morning on the first day of administration;

(ii) 10 mg in the morning and 10 mg after noon on the second day of administration;

(iii) 10 mg in the morning and 20 mg after noon on the third day of administration;

(iv) 20 mg in the morning and 20 mg after noon on the fourth day of administration;

(v) 20 mg in the morning and 30 mg after noon on the fifth day of administration; and

(b) on the sixth and every subsequent day, administering to the patient stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione at a dose of between about 40 mg/day and about 100 mg/day.

14. A method for treating a patient with stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, wherein the patient is suffering from ulcerative colitis, the method consisting of:

(a) administering to the patient stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione in an initial titration dosing schedule consisting of

(i) 10 mg in the morning on the first day of administration;

(ii) 10 mg in the morning and 10 mg after noon on the second day of administration;

(iii) 10 mg in the morning and 20 mg after noon on the third day of administration;

(iv) 20 mg in the morning and 20 mg after noon on the fourth day of administration;

(v) 20 mg in the morning and 30 mg after noon on the fifth day of administration; and

(b) on the sixth and every subsequent day, administering to the patient 30 mg in the morning and 30 mg after noon of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

15. A method as in any one of claims **1-14**, wherein the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione comprises greater than about 90% by weight of the (+) isomer of 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione based on the total weight percent of 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

16. A method as in any one of claims **1-14**, wherein the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione comprises greater than about 95% by weight of the (+) isomer of 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione based on the total weight percent of 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

17. A method as in any one of claims **1-14**, wherein the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione comprises greater than about 96% by weight of the (+) isomer of 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione based on the total weight percent of 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

18. A method as in any one of claims **1-14**, wherein the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione comprises greater than about 97% by weight of the (+) isomer of 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione based on the total weight percent of 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

19. A method as in any one of claims **1-14**, wherein the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione comprises greater than about 98% by weight of the (+) isomer of 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione based on the total weight percent of 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

20. A method as in any one of claims **1-14**, wherein the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione comprises greater than about 99% by weight of the (+) isomer of 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione based on the total weight percent of 2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

21. A method as in any one of claims **1-14**, wherein the stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione is administered in tablet form.

22. A method as in any one of claims **1-14**, which comprises administering stereomerically pure (+)-2-[1-(3-ethoxy-4-m ethoxyphenyl)-2-methyl sulfonylethyl]-4-acetylaminoisoindoline-1,3-dione, substantially free of any salt, or solvate forms of (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

23. A method as in any one of claims **1-14**, which comprises administering a pharmaceutically acceptable salt of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methyl sulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

24. A method as in any one of claims **1-14**, which comprises administering a pharmaceutically acceptable solvate of stereomerically pure (+)-2-[1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-4-acetylaminoisoindoline-1,3-dione.

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