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IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF NEW JERSEY

GILEAD SCIENCES, INC. and EMORY UNIVERSITY, Plaintiffs, v. STRIDES PHARMA, INC. and STRIDES PHARMA GLOBAL PTE LIMITED, Defendants.	Civil Action No.: *Electronically Filed*

COMPLAINT FOR PATENT INFRINGEMENT

Plaintiffs Gilead Sciences, Inc. ("Gilead") and Emory University ("Emory") (collectively, "Plaintiffs"), for their complaint against Defendants Strides Pharma, Inc. ("Strides Inc.") and Strides Pharma Global PTE Limited ("Strides Global") (Strides Pharma and Strides Global are referred to collectively as "Strides"), hereby allege as follows:

Nature of Action

1. This is an action for patent infringement under the patent laws of the United States, Title 35 of the United States Code.

The Parties

2. Gilead is a corporation organized and existing under the laws of the State of

Delaware, having a principal place of business at 333 Lakeside Drive, Foster City, California 94404.

3. Emory is a non-profit corporation of the State of Georgia, having an office at 201 Dowman Drive, Atlanta, Georgia 30322.

4. On information and belief, Defendant Strides Inc. is a New Jersey corporation, having a principal place of business at 2 Tower Center Blvd., Suite 1102, East Brunswick, NJ 08816 and is an agent or affiliate of Defendant Strides Global and is acting as the agent of Strides Global with respect to Abbreviated New Drug Application ("ANDA") No. 091055.

5. On information and belief, Defendant Strides Global is a Singapore corporation, having a principal place of business at No. 8 Eu Tong Sen Street, #15-93, The Central, Singapore—059818.

Jurisdiction and Venue

6. This action arises under the Patent Laws of the United States and the Food and Drug Laws of the United States, Titles 35 and 21 of the United States Code. Jurisdiction is based on 28 U.S.C. §§ 1331 and 1338(a).

7. On information and belief, this Court has personal jurisdiction over Strides Inc. and Strides Global.

8. On information and belief, Strides Inc. and Strides Global are generic pharmaceutical companies in the business of marketing and distributing generic drug products, and derive substantial revenue from selling various pharmaceutical drug products and doing business throughout the United States, including in New Jersey.

9. On information and belief, Strides Inc. and Strides Global, themselves or through their wholly-owned subsidiaries or affiliates, manufacture pharmaceutical drug products that are sold and used throughout the United States, including in New Jersey.

10. On information and belief, residents of New Jersey purchase pharmaceutical drug products from Strides in New Jersey.

11. On information and belief, Strides Inc. is registered with the State of New Jersey to do business as a domestic corporation in New Jersey, is an agent or affiliate of Strides Global, and is acting as the agent of Strides Global with respect to ANDA No. 091055.

12. On information and belief, Strides Inc. is registered with the State of New Jersey Department of Health as a drug manufacturer and wholesaler under registration number 5004572.

13. On information and belief, Strides' submission of ANDA No. 091055, discussed below, indicates Strides' intention to engage in the commercial manufacture, use, sale and/or importation of products that will compete directly with Gilead's Truvada® product, which is currently being sold throughout the United States, including in New Jersey. On information and belief, Strides will sell tablets containing 200 mg of emtricitabine and 300 mg of tenofovir disoproxil fumarate for the use for which Strides seeks approval in ANDA No. 091055, if approved, throughout the United States, including in New Jersey.

14. On information and belief, Strides Inc. and Strides Global have previously consented to personal jurisdiction in this District.

15. Venue is proper in this District under 28 U.S.C. § 1391(b), (c), (d), and 28 U.S.C. § 1400(b). Specifically, venue is proper in New Jersey because Strides Inc. is incorporated in New Jersey.

Background

16. Gilead is the holder of New Drug Application ("NDA") No. 21-752 which relates to tablets containing 200 mg of emtricitabine and 300 mg of tenofovir disoproxil fumarate. On August 2, 2004, the United States Food and Drug Administration ("FDA") approved the use of the tablets described in NDA No. 21-752 for the treatment of HIV-1 infection in adults. These tablets are prescribed and sold in the United States under the trademark Truvada®.

17. United States Patent No. 6,642,245 ("the '245 Patent," copy attached as Exhibit A), entitled "Antiviral Activity and Resolution of 2-Hydroxymethyl-5-(5-fluorocytosin-1-yl) -1,3-oxathiolane," was duly and legally issued by the United States Patent and Trademark Office on November 4, 2003. The '245 Patent claims, *inter alia*, methods for treating HIV infection in humans with emtricitabine (one of the active ingredients in Truvada®), and is listed in the *Approved Drug Products with Therapeutic Equivalence Evaluations* ("FDA Orange Book") for Truvada®.

18. United States Patent No. 6,703,396 ("the '396 Patent," copy attached as Exhibit B), entitled "Method of Resolution and Antiviral Activity of 1,3-Oxathiolane Nucleoside Enantiomers," was duly and legally issued by the United States Patent and Trademark Office on March 9, 2004. The '396 Patent claims, *inter alia*, emtricitabine (one of the active ingredients in Truvada®), and is listed in the FDA Orange Book for Truvada®.

19. United States Patent No. 8,592,397 ("the '397 Patent," copy attached as Exhibit C), entitled "Compositions and Methods for Combination Antiviral Therapy" was duly and legally issued by the United States Patent and Trademark Office on November 26, 2013. The '397 Patent claims, *inter alia*, a pharmaceutical combination tablet containing emtricitabine and tenofovir disoproxil fumarate (the two active ingredients in Truvada®) and methods for treating HIV

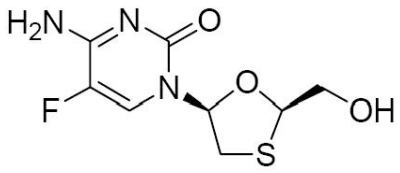
infection in humans with the emtricitabine and tenofovir disoproxil fumarate combination. The '397 Patent is also listed in the FDA Orange Book for Truvada®.

20. United States Patent No. 8,716,264 ("the '264 Patent," copy attached as Exhibit D), entitled "Compositions and Methods for Combination Antiviral Therapy," was duly and legally issued by the United States Patent and Trademark Office on May 6, 2014. The '264 Patent claims, *inter alia*, a pharmaceutical combination tablet containing emtricitabine and tenofovir disoproxil fumarate (the two active ingredients in Truvada®) and methods for treating HIV infection in humans with the emtricitabine and tenofovir disoproxil fumarate combination. The '264 Patent is also listed in the FDA Orange Book for Truvada®.

21. United States Patent No. 9,457,036 ("the '036 Patent," copy attached as Exhibit E), entitled "Compositions and Methods for Combination Antiviral Therapy," was duly and legally issued by the United States Patent and Trademark Office on October 4, 2016. The '036 Patent claims, *inter alia*, a pharmaceutical combination tablet containing emtricitabine and tenofovir disoproxil fumarate (the two active ingredients in Truvada®) and methods for treating HIV infection in humans with the emtricitabine and tenofovir disoproxil fumarate combination. The '036 Patent is also listed in the FDA Orange Book for Truvada®.

22. United States Patent No. 9,744,181 ("the '181 Patent," copy attached as Exhibit F), entitled Compositions and Methods for Combination Antiviral Therapy," was duly and legally issued by the United States Patent and Trademark Office on August 29, 2017. The '181 Patent claims, *inter alia*, a fixed-dose pharmaceutical combination tablet containing emtricitabine and tenofovir disoproxil fumarate (the two active ingredients in Truvada®) and methods for treating HIV infection in humans with the emtricitabine and tenofovir disoproxil fumarate combination. The '181 Patent is also listed in the FDA Orange Book for Truvada®.

23. Emtricitabine is a compound that has a molecular formula of $C_8H_{10}FN_3O_3S$, and which has the following chemical structure:



24. Emtricitabine can be referred to by any of several chemical names. The chemical name given to emtricitabine in the Emtriva® label is "5-fluoro-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine." Two chemical names recited for emtricitabine in the '245 Patent are "(–)-β-L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl) -1,3-oxathiolane" and "β-L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane." Two chemical names recited for emtricitabine in the '396 Patent are "(–)-cis-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolane-5-yl)-(1H)-pyrimidin-2-one" and "(–)-enantiomer of cis-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolane-5-yl)-(1H)-pyrimidin-2-one."

25. The named inventors on the '245 and '396 Patents are Dennis C. Liotta, Raymond F. Schinazi, and Woo-Baeg Choi.

26. Dennis C. Liotta, Raymond F. Schinazi, and Woo-Baeg Choi assigned the '245 and '396 Patents to Emory.

27. Pursuant to an agreement entered into between Gilead and Emory, Gilead has substantial rights in the '245 and '396 Patents, including, but not limited to, rights associated with being a licensee of the '245 and '396 Patents, and the right to sue for infringement of the '245 and '396 Patents.

28. The named inventors on the '397, '264, '036, and '181 Patents are Terrence C. Dahl, Mark M. Menning, and Reza Oliyai.

29. Terrence C. Dahl, Mark M. Menning and Reza Oliyai assigned the '397, '264, '036, and '181 Patents to Gilead.

COUNT 1
Infringement of U.S. Patent No. 6,642,245

30. Plaintiffs repeat and reallege paragraphs 1-29 above as if set forth herein.

31. On information and belief, Strides submitted or caused to be submitted ANDA No. 091055 to the FDA seeking approval to engage in the commercial manufacture, use, sale and/or importation of tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate for the purpose of treating HIV infection.

32. By letter dated May 15, 2018, pursuant to 21 U.S.C. § 355(j)(2)(B)(ii) (the "May 18, 2018 Notice Letter"), Strides notified Plaintiffs that it had submitted ANDA No. 091055 to the FDA seeking approval to engage in the commercial manufacture, use, sale and/or importation of capsules containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate prior to the expiration of the '245 Patent. This complaint has been filed within 45 days of Plaintiffs' receipt of the May 15, 2018 Notice Letter.

33. In its May 15, 2018 Notice Letter, Strides notified Plaintiffs that, as a part of ANDA No. 091055, it had filed a certification of the type described in 21 U.S.C. § 355(j)(2)(A)(vii)(IV) ("Paragraph IV") with respect to the '245 Patent. Paragraph IV requires, *inter alia*, certification by the ANDA applicant, in its opinion and to the best of its knowledge, that the subject patent, here the '245 Patent, "is invalid or will not be infringed by the manufacture, use or sale of the new drug for which the application is submitted . . . ." The statute (21 U.S.C. § 355(j)(2)(B)(iv)(II)) also requires a Paragraph IV Notice Letter to "include a detailed statement of the factual and legal basis of the opinion of the applicant that the patent is invalid or will not be infringed." The FDA Rules and Regulations (21 C.F.R. § 314.95(c)(6)) further require that the detailed statement include

"(i) [f]or each claim of a patent alleged not to be infringed, a full and detailed explanation of why the claim is not infringed" and "(ii) [f]or each claim of a patent alleged to be invalid or unenforceable, a full and detailed explanation of the grounds supporting the allegations."

34. Strides alleged in its May 15, 2018 Notice Letter that Claims 1, 2, 4, 6, 7 and 8 of the '245 Patent are invalid and that Claims 3, 5, and 9-22 of the '245 Patent would not be infringed by the commercial manufacture, use, sale and/or importation of its proposed product that is the subject of ANDA No. 091055.

35. The May 15, 2018 Notice Letter does not allege non-infringement of Claims 1, 2, 4, 6, 7 and 8 of the '245 Patent.

36. By filing ANDA 091055 under 21 U.S.C. § 355(j) for the purposes of obtaining approval to engage in the commercial manufacture, use, sale and/or importation of tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate before the '245 Patent's expiration, Strides has committed an act of infringement of the '245 Patent under 35 U.S.C. § 271(e)(2).

37. Strides' submission of ANDA No. 091055 and service of the May 15, 2018 Notice Letter indicates a refusal to change its current course of action.

38. On information and belief, the commercial manufacture, use, sale and/or importation of tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate for which Strides seeks approval in ANDA No. 091055, if approved, will infringe one or more claims of the '245 Patent.

39. On information and belief, Strides will directly or indirectly infringe at least Claim 1 of the '245 Patent. Claim 1 recites a "method for treating HIV infection in humans comprising administering an effective amount of [emtricitabine], or its physiologically acceptable salt,

optionally in a pharmaceutically acceptable carrier." On information and belief, Strides will infringe Claim 1 of the '245 Patent because the product for which it seeks approval in ANDA No. 091055 will be labeled for and used to treat HIV infection in humans with an effective amount of emtricitabine. In its May 15, 2018 Notice Letter, Strides does not allege that Claim 1 would not be infringed by the commercial manufacture, use, sale, offer for sale and/or importation of its proposed product that is the subject of ANDA No. 091055. For the same reasons, on information and belief, Strides will likewise infringe Claims 2, 4, 6, 7 and 8 of the '245 Patent.

40. On information and belief, the tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate for the use for which Strides seeks approval in ANDA No. 091055, if approved, will be administered to human patients in an effective amount for treating HIV infection. Such administration will infringe at least one claim of the '245 Patent, as described in the preceding paragraph. On information and belief, this administration will occur at Strides' active behest and with its intent, knowledge, and encouragement. On information and belief, Strides will actively encourage, aid, and abet this administration with knowledge that it is in contravention of Plaintiffs' rights under the '245 Patent. Further, by filing ANDA No. 091055 with a Paragraph IV certification, Strides admits that it has knowledge of the '245 Patent.

COUNT 2
Infringement of U.S. Patent No. 6,703,396

41. Plaintiffs repeat and reallege paragraphs 1-40 above as if set forth herein.

42. On information and belief, Strides submitted or caused to be submitted ANDA No. 091055 to the FDA seeking approval to engage in the commercial manufacture, use, sale and/or importation of tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate for the purpose of treating HIV infection.

43. In its May 15, 2018 Notice Letter, Strides notified Plaintiffs that it had submitted ANDA No. 091055 to the FDA seeking approval to engage in the commercial manufacture, use, sale and/or importation of tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate prior to the expiration of the '396 Patent. This complaint has been filed within 45 days of Plaintiffs' receipt of the May 15, 2018 Notice Letter.

44. In its May 15, 2018 Notice Letter, Strides notified Plaintiffs that, as a part of its ANDA No. 091055, it had filed a Paragraph IV certification with respect to the '396 Patent. Paragraph IV requires, *inter alia*, certification by the ANDA applicant, in its opinion and to the best of its knowledge, that the subject patent, here the '396 Patent, "is invalid or will not be infringed by the manufacture, use or sale of the new drug for which the application is submitted . . ."

45. Strides alleged in its May 15, 2018 Notice Letter that Claims 1-7, 11-16, 22, and 23 of the '396 Patent are invalid and that Claims 8-10, 17-21, and 24-28 would not be infringed by the commercial manufacture, use, sale and/or importation of its proposed product that is the subject of ANDA No. 091055.

46. The May 15, 2018 Notice Letter does not allege non-infringement of Claims 1-7, 11-16, 22 and 23 of the '396 Patent.

47. By filing ANDA No. 091055 under 21 U.S.C. § 355(j) for the purposes of obtaining approval to engage in the commercial manufacture, use, sale and/or importation of tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate before the '396 Patent's expiration, Strides has committed an act of infringement of the '396 Patent under 35 U.S.C. § 271(e)(2).

48. Strides' submission of ANDA No. 091055 and service of the May 15, 2018 Notice Letter indicates a refusal to change its current course of action.

49. On information and belief, the commercial manufacture, use, sale and/or importation of tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate for which Strides seeks approval in ANDA No. 091055, if approved, will infringe one or more claims of the '396 Patent.

50. On information and belief, Strides will directly or indirectly infringe at least Claim 2 of the '396 Patent. Claim 2 recites "[emtricitabine] or a pharmaceutically acceptable salt, ester or salt of an ester thereof." On information and belief, Strides will infringe Claim 2 of the '396 Patent because the product for which it seeks approval in ANDA No. 091055 will contain emtricitabine as the active ingredient. In its May 15, 2018 Notice Letter, Strides does not allege that Claim 2 would not be infringed by the commercial manufacture, use, sale, offer for sale, and/or importation of its proposed product that is the subject of ANDA No. 091055. For the same reasons, on information and belief, Strides will also infringe Claims 1, 3-7, 11-16, 22, and 23 of the '396 Patent.

51. On information and belief, the tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate for the use for which Strides seeks approval in ANDA No. 091055, if approved, will infringe at least one claim of the '396 Patent, as described in the preceding paragraph. On information and belief, the manufacture of these tablets will occur at Strides' active behest and with its intent, knowledge, and encouragement. On information and belief, Strides will actively encourage, aid, and abet the manufacture of these tablets with knowledge that it is in contravention of Plaintiffs' rights under the '396 Patent. Further, by filing

ANDA No. 091055 with a Paragraph IV certification, Strides admits that it has knowledge of the '396 Patent.

COUNT 3
Infringement of U.S. Patent No. 8,592,397

52. Plaintiffs repeat and reallege paragraphs 1-51 above as if set forth herein.

53. On information and belief, Strides submitted or caused to be submitted ANDA No. 091055 to the FDA seeking approval to engage in the commercial manufacture, use, sale and/or importation of tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate for the purpose of treating HIV infection.

54. In its May 15, 2018 Notice Letter, Strides notified Plaintiffs that it had submitted ANDA No. 091055 to the FDA seeking approval to engage in the commercial manufacture, use, sale and/or importation of tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate prior to the expiration of the '397 Patent. This complaint has been filed within 45 days of Plaintiffs' receipt of the May 15, 2018 Notice Letter.

55. In its May 15, 2018 Notice Letter, Strides notified Plaintiffs that, as a part of ANDA No. 091055, it had filed a Paragraph IV certification with respect to the '397 Patent. Paragraph IV requires, *inter alia*, certification by the ANDA applicant, in its opinion and to the best of its knowledge, that the subject patent, here the '397 Patent, "is invalid or will not be infringed by the manufacture, use or sale of the new drug for which the application is submitted . . . ."

56. Strides alleged in its May 15, 2018 Notice Letter that Claims 1-7, 14-16, and 18-26 of the '397 Patent are invalid and that Claims 8-13 and 17 would not be infringed by the commercial manufacture, use, sale, and/or importation of its proposed product that is the subject of ANDA No. 091055.

57. Strides did not allege in its May 15, 2018 Notice Letter that Claims 1-7, 14-16, and 18-26 of the '397 Patent would not be infringed by the commercial manufacture, use, sale, and/or importation of its proposed product that is the subject of ANDA No. 203442.

58. By filing ANDA No. 091055 under 21 U.S.C. § 355(j) for the purposes of obtaining approval to engage in the commercial manufacture, use, sale, and/or importation of tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate before the '397 Patent's expiration, Strides has committed an act of infringement of the '397 Patent under 35 U.S.C. § 271(e)(2).

59. Strides' submission of ANDA No. 091055 and service of the May 15, 2018 Notice Letter indicates a refusal to change its current course of action.

60. On information and belief, the commercial manufacture, use, sale, and/or importation of tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate for which Strides seeks approval in ANDA No. 091055, if approved, will infringe one or more claims of the '397 Patent.

61. On information and belief, Strides will directly or indirectly infringe at least Claim 1 of the '397 Patent. Claim 1 recites a "chemically stable fixed dose combination pharmaceutical dosage form comprising 300 mg tenofovir disoproxil fumarate and 200 mg emtricitabine; a binder selected from the group consisting of povidone, gelatin, hydroxypropyl methylcellulose, cellulose, microcrystalline cellulose, starch, and acacia; a disintegrant selected from sodium starch glycolate, crosslinked-povidone, cross-linked sodium carboxymethylcellulose, and alginic acid; and a lubricant selected from the group consisting of magnesium stearate, stearic acid, and talc; wherein said pharmaceutical dosage form exhibits less than 10% degradation of the tenofovir disoproxil fumarate or emtricitabine after 6 months when packaged and stored with silica gel dessicant at 40°

C./75% relative humidity." On information and belief, Strides will infringe Claim 1 of the '397 Patent because the product for which it seeks approval in ANDA No. 091055 will be a chemically stable, fixed-dose tablet containing 300 mg tenofovir disoproxil fumarate and 200 mg emtricitabine, and at least one of each enumerated binder, disintegrant, and lubricant, or an equivalent thereof, and will exhibit less than 10% degradation of the tenofovir disoproxil fumarate or emtricitabine after six months when packaged and stored with silica gel dessicant at 40° C./75% relative humidity. In its May 15, 2018 Notice Letter, Strides does not allege that Claim 1 would not be infringed by the commercial manufacture, use, sale, offer for sale, and/or importation of its proposed product that is the subject of ANDA No. 091055. For the same reasons, on information and belief, Strides will also infringe other claims of the '397 Patent.

62. On information and belief, the tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate for the use for which Strides seeks approval in ANDA No. 091055, if approved, will infringe at least one claim of the '397 Patent, as described in the preceding paragraph. On information and belief, the manufacture of these tablets and use of these tablets to treat HIV infection will occur at Strides' active behest and with its intent, knowledge, and encouragement. On information and belief, Strides will actively encourage, aid, and abet the manufacture of these tablets and use of these tablets to treat HIV infection with knowledge that it is in contravention of Gilead's rights under the '397 Patent. Further, by filing ANDA No. 091055 with a Paragraph IV certification, Strides admits that it has knowledge of the '397 Patent.

COUNT 4
Infringement of U.S. Patent No. 8,716,264

63. Plaintiffs repeat and reallege paragraphs 1-62 above as if set forth herein.

64. On information and belief, Strides submitted or caused to be submitted ANDA No. 091055 to the FDA seeking approval to engage in the commercial manufacture, use, sale, and/or

importation of tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate for the purpose of treating HIV infection.

65. In its May 15, 2018 Notice Letter, Strides notified Plaintiffs that it had submitted ANDA No. 091055 to the FDA seeking approval to engage in the commercial manufacture, use, sale, and/or importation of tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate prior to the expiration of the '264 Patent. This complaint has been filed within 45 days of Plaintiffs' receipt of the May 15, 2018 Notice Letter.

66. In its May 15, 2018 Notice Letter, Strides notified Plaintiffs that, as a part of its ANDA No. 091055, it had filed a Paragraph IV certification with respect to the '264 Patent. Paragraph IV requires, *inter alia*, certification by the ANDA applicant, in its opinion and to the best of its knowledge, that the subject patent, here the '264 Patent, "is invalid or will not be infringed by the manufacture, use or sale of the new drug for which the application is submitted . . ."

67. Strides alleged in its May 15, 2018 Notice Letter that Claims 1-18, 25, 33, and 34 of the '264 Patent are invalid and that Claims 19-24, 26-32, and 35-38 would not be infringed by the commercial manufacture, use, sale, and/or importation of its proposed product that is the subject of ANDA No. 091055.

68. The May 15, 2018 Notice Letter does not allege non-infringement of Claims 1-18, 25, 33, and 34 of the '264 Patent.

69. By filing ANDA No. 091055 under 21 U.S.C. § 355(j) for the purposes of obtaining approval to engage in the commercial manufacture, use, sale, and/or importation of tablets containing 200 mg of emtricitabine and 300 mg of tenofovir disoproxil fumarate before the '264

Patent's expiration, Strides has committed an act of infringement of the '264 Patent under 35 U.S.C. § 271(e)(2).

70. Strides' submission of ANDA No. 091055 and service of the May 15, 2018 Notice Letter indicates a refusal to change its current course of action.

71. On information and belief, the commercial manufacture, use, sale and/or importation of tablets containing 200 mg of emtricitabine and 300 mg of tenofovir disoproxil fumarate for which Strides seeks approval in ANDA No. 091055, if approved, will infringe one or more claims of the '264 Patent.

72. On information and belief, Strides will directly or indirectly infringe at least Claim 1 of the '264 Patent. Claim 1 recites a "chemically stable fixed-dose combination comprising 300 mg of tenofovir disoproxil fumarate and 200 mg of emtricitabine wherein the combination exhibits less than 10% degradation of tenofovir disoproxil fumarate and emtricitabine after six months at 40° C./75% relative humidity when packaged and stored with silica gel dessicant at 40° C./70% relative humidity."[1] On information and belief, Strides will infringe Claim 1 of the '264 Patent because the product for which it seeks approval in ANDA No. 091055 will be a chemically stable, fixed-dose tablet containing 300 mg of tenofovir disoproxil fumarate and 200 mg of emtricitabine and will exhibit less than 10% degradation of tenofovir disoproxil fumarate and emtricitabine after six months at 40° C./75% relative humidity when packaged and stored with silica gel dessicant at 40° C./70% relative humidity. In its May 15, 2018 Notice Letter, Strides does not allege that Claim 1 would not be infringed by the commercial manufacture, use, sale, offer for sale and/or importation of its proposed product that is the subject of ANDA No. 091055. For the same reasons,

[1] Claim 1 contains a clear typographical error in stating "70% relative humidity" in the last clause rather than "75% relative humidity." Plaintiff Gilead will request that the Court correct this error.

on information and belief, Strides will also infringe at least Claims 2-18, 25, 33, and 34 of the '264 Patent.

73. On information and belief, the tablets containing 200 mg of emtricitabine and 300 mg of tenofovir disoproxil fumarate for the use for which Strides seeks approval in ANDA No. 091055, if approved, will infringe at least one claim of the '264 Patent, as described in the preceding paragraph. On information and belief, the manufacture of these tablets and use of these tablets to treat HIV infection will occur at Strides' active behest and with its intent, knowledge, and encouragement. On information and belief, Strides will actively encourage, aid, and abet the manufacture of these tablets and use of these tablets to treat HIV infection with knowledge that it is in contravention of Gilead's rights under the '264 Patent. Further, by filing ANDA No. 091055 with a Paragraph IV certification, Strides admits that it has knowledge of the '264 Patent.

COUNT 5
Infringement of U.S. Patent No. 9,457,036

74. Plaintiffs repeat and reallege paragraphs 1-73 above as if set forth herein.

75. On information and belief, Strides submitted or caused to be submitted ANDA No. 091055 to the FDA seeking approval to engage in the commercial manufacture, use, sale, and/or importation of tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate for the purpose of treating HIV infection.

76. In its May 15, 2018 Notice Letter, Strides notified Plaintiffs that it had submitted ANDA No. 091055 to the FDA seeking approval to engage in the commercial manufacture, use, sale, and/or importation of tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate prior to the expiration of the '036 Patent. This complaint has been filed within 45 days of Plaintiffs' receipt of the May 15, 2018 Notice Letter.

77. In its May 15, 2018 Notice Letter, Strides notified Plaintiffs that, as a part of ANDA No. 091055, it had filed a Paragraph IV certification with respect to the '036 Patent. Paragraph IV requires, *inter alia*, certification by the ANDA applicant, in its opinion and to the best of its knowledge, that the subject patent, here the '036 Patent, "is invalid or will not be infringed by the manufacture, use or sale of the new drug for which the application is submitted . . . ."

78. Strides alleged in its May 15, 2018 Notice Letter that Claims 1-5, 12-13, and 15-16 of the '036 Patent are invalid and that Claims 6-11 and 14 would not be infringed by the commercial manufacture, use, sale, and/or importation of its proposed product that is the subject of ANDA No. 091055.

79. Strides did not allege in its May 15, 2018 Notice Letter that claims 1-5, 12-13, 15 or 16 of the '036 Patent would not be infringed by the commercial manufacture, use, sale, and/or importation of its proposed product that is the subject of ANDA No. 091055.

80. By filing ANDA No. 091055 under 21 U.S.C. § 355(j) for the purposes of obtaining approval to engage in the commercial manufacture, use, sale, and/or importation of tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate before the '036 Patent's expiration, Strides has committed an act of infringement of the '036 Patent under 35 U.S.C. § 271(e)(2).

81. Strides' submission of ANDA No. 091055 and service of the May 15, 2018 Notice Letter indicates a refusal to change its current course of action.

82. On information and belief, the commercial manufacture, use, sale, and/or importation of tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate for which Strides seeks approval in ANDA No. 091055, if approved, will infringe one or more claims of the '036 Patent.

83. On information and belief, Strides will directly or indirectly infringe at least Claim 1 of the '036 Patent. Claim 1 recites a "fixed dose combination pharmaceutical dosage form comprising 300 mg tenofovir disoproxil fumarate and 200 mg emtricitabine; a binder selected from the group consisting of povidone, gelatin, hydroxypropyl methylcellulose, cellulose, microcrystalline cellulose, starch, and acacia; a disintegrant selected from sodium starch glycolate, crosslinked-povidone, cross-linked sodium carboxymethylcellulose, and alginic acid; and a lubricant selected from the group consisting of magnesium stearate, stearic acid, and talc; wherein said pharmaceutical dosage form exhibits equal to or less than 5% degradation of the tenofovir disoproxil fumarate or emtricitabine after 6 months when packaged and stored with silica gel dessicant at 40° C./75% relative humidity; and wherein said pharmaceutical dosage form is a tablet." On information and belief, Strides will infringe Claim 1 of the '036 Patent because the product for which it seeks approval in ANDA No. 091055 will be a fixed-dose tablet containing 300 mg tenofovir disoproxil fumarate and 200 mg emtricitabine and at least one of each enumerated binder, disintegrant, and lubricant, or an equivalent thereof, and will exhibit equal to or less than 5% degradation of the tenofovir disoproxil fumarate or emtricitabine after six months when packaged and stored with silica gel dessicant at 40° C./75% relative humidity. In its May 15, 2018 Notice Letter, Strides does not allege that Claim 1 would not be infringed by the commercial manufacture, use, sale, offer for sale and/or importation of its proposed product that is the subject of ANDA No. 091055. For the same reasons, on information and belief, Strides will also infringe other claims of the '036 Patent.

84. On information and belief, the tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate for the use for which Strides seeks approval in ANDA No. 091055, if approved, will infringe at least one claim of the '036 Patent, as described in the

preceding paragraph. On information and belief, the manufacture of these tablets and use of these tablets to treat HIV infection will occur at Strides' active behest and with its intent, knowledge, and encouragement. On information and belief, Strides will actively encourage, aid, and abet the manufacture of these tablets and use of these tablets to treat HIV infection with knowledge that it is in contravention of Gilead's rights under the '036 Patent. Further, by filing ANDA No. 091055 with a Paragraph IV certification, Strides admits that it has knowledge of the '036 Patent.

COUNT 6
Infringement of U.S. Patent No. 9,744,181

85. Plaintiffs repeat and reallege paragraphs 1-84 above as if set forth herein.

86. On information and belief, Strides submitted or caused to be submitted ANDA No. 091055 to the FDA seeking approval to engage in the commercial manufacture, use, sale, and/or importation of tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate for the purpose of treating HIV infection.

87. In its May 15, 2018 Notice Letter, Strides notified Plaintiffs that it had submitted ANDA No. 091055 to the FDA seeking approval to engage in the commercial manufacture, use, sale, and/or importation of tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate prior to the expiration of the '181 Patent. This complaint has been filed within 45 days of Plaintiffs' receipt of the May 15, 2018 Notice Letter.

88. In its May 15, 2018 Notice Letter, Strides notified Plaintiffs that, as a part of ANDA No. 091055, it had filed a Paragraph IV certification with respect to the '181 Patent. Paragraph IV requires, *inter alia*, certification by the ANDA applicant, in its opinion and to the best of its knowledge, that the subject patent, here the '036 Patent, "is invalid or will not be infringed by the manufacture, use or sale of the new drug for which the application is submitted . . . ."

89. Strides alleged in its May 15, 2018 Notice Letter that Claims 1-5, 7, 11, 13-18, 21, 25, and 29 of the '181 Patent are invalid and that Claims 6, 8-10, 12, 19, 20, 22-24, 26-28, and 30 would not be infringed by the commercial manufacture, use, sale, and/or importation of its proposed product that is the subject of ANDA No. 091055.

90. Strides did not allege in its May 15, 2018 Notice Letter that claims 1-5, 7, 11, 13-18, 21, 25 or 29 of the '181 Patent would not be infringed by the commercial manufacture, use, sale, and/or importation of its proposed product that is the subject of ANDA No. 091055.

91. By filing ANDA No. 091055 under 21 U.S.C. § 355(j) for the purposes of obtaining approval to engage in the commercial manufacture, use, sale, and/or importation of tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate before the '181 Patent's expiration, Strides has committed an act of infringement of the '181 Patent under 35 U.S.C. § 271(e)(2).

92. Strides' submission of ANDA No. 091055 and service of the May 15, 2018 Notice Letter indicates a refusal to change its current course of action.

93. On information and belief, the commercial manufacture, use, sale, and/or importation of tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate for which Strides seeks approval in ANDA No. 091055, if approved, will infringe one or more claims of the '181 Patent.

94. On information and belief, Strides will directly or indirectly infringe at least Claim 1 of the '181 Patent. Claim 1 recites a "fixed dose combination comprising 300 mg tenofovir disoproxil fumarate and 200 mg emtricitabine wherein the combination exhibits equal to or less than 5% degradation of the tenofovir disoproxil fumarate and emtricitabine after six months at 40° C./75% relative humidity when packaged and stored with silica gel dessicant, and wherein the

fixed-dose combination is a tablet." On information and belief, Strides will infringe Claim 1 of the '181 Patent because the product for which it seeks approval in ANDA No. 091055 will be a fixed-dose tablet containing 300 mg tenofovir disoproxil fumarate and 200 mg emtricitabine and will exhibit equal to or less than 5% degradation of the tenofovir disoproxil fumarate and emtricitabine after six months when packaged and stored with silica gel dessicant at 40° C./75% relative humidity. In its May 15, 2018 Notice Letter, Strides does not allege that Claim 1 would not be infringed by the commercial manufacture, use, sale, offer for sale, and/or importation of its proposed product that is the subject of ANDA No. 091055. For the same reasons, on information and belief, Strides will also infringe other claims of the '181 Patent.

95. On information and belief, the tablets containing 200 mg of emtricitabine and 300 mg tenofovir disoproxil fumarate for the use for which Strides seeks approval in ANDA No. 091055, if approved, will infringe at least one claim of the '181 Patent, as described in the preceding paragraph. On information and belief, the manufacture of these tablets and use of these tablets to treat HIV infection will occur at Strides' active behest and with its intent, knowledge, and encouragement. On information and belief, Strides will actively encourage, aid, and abet the manufacture of these tablets and use of these tablets to treat HIV infection with knowledge that it is in contravention of Gilead's rights under the '181 Patent. Further, by filing ANDA No. 091055 with a Paragraph IV certification, Strides admits that it has knowledge of the '181 Patent.

PRAYER FOR RELIEF

WHEREFORE, Plaintiffs respectfully request the following relief:

(a) A judgment declaring that the effective date of any approval of Strides' ANDA No. 091055 under Section 505(j) of the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 355(j)) be a date which is not earlier than the expiration of the '245 Patent or any later date of exclusivity to which Plaintiffs are or become entitled;

(b) A judgment declaring that the effective date of any approval of Strides' ANDA No. 091055 under Section 505(j) of the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 355(j)) be a date which is not earlier than the expiration of the '396 Patent or any later date of exclusivity to which Plaintiffs are or become entitled;

(c) A judgment declaring that the effective date of any approval of Strides' ANDA No. 091055 under Section 505(j) of the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 355(j)) be a date which is not earlier than the expiration of the '397 Patent or any later date of exclusivity to which Plaintiffs are or become entitled;

(d) A judgment declaring that the effective date of any approval of Strides' ANDA No. 091055 under Section 505(j) of the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 355(j)) be a date which is not earlier than the expiration of the '264 Patent or any later date of exclusivity to which Plaintiffs are or become entitled;

(e) A judgment declaring that the effective date of any approval of Strides' ANDA No. 091055 under Section 505(j) of the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 355(j)) be a date which is not earlier than the expiration of the '036 Patent or any later date of exclusivity to which Plaintiffs are or become entitled;

(f) A judgment declaring that the effective date of any approval of Strides' ANDA No. 091055 under Section 505(j) of the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 355(j)) be a date which is not earlier than the expiration of the '181 Patent or any later date of exclusivity to which Plaintiffs are or become entitled;

(g) A judgment declaring that the '245 Patent remains valid and enforceable, and that one or more claims have been infringed by Strides;

(h) A judgment declaring that the ’396 Patent remains valid and enforceable, and that one or more claims have been infringed by Strides;

(i) A judgment declaring that the ’397 Patent remains valid and enforceable, and that one or more claims have been infringed by Strides;

(j) A judgment declaring that the ’264 Patent remains valid and enforceable, and that one or more claims have been infringed by Strides;

(k) A judgment declaring that the ’036 Patent remains valid and enforceable, and that one or more claims have been infringed by Strides;

(l) A judgment declaring that the ’181 Patent remains valid and enforceable, and that one or more claims have been infringed by Strides;

(m) A permanent injunction against any infringement of the ’245 Patent by Strides, their officers, agents, attorneys and employees, and those acting in privity or contract with them;

(n) A permanent injunction against any infringement of the ’396 Patent by Strides, their officers, agents, attorneys and employees, and those acting in privity or contract with them;

(o) A permanent injunction against any infringement of the ’397 Patent by Strides, their officers, agents, attorneys and employees, and those acting in privity or contract with them;

(p) A permanent injunction against any infringement of the ’264 Patent by Strides, their officers, agents, attorneys and employees, and those acting in privity or contract with them;

(q) A permanent injunction against any infringement of the ’036 Patent by Strides, their officers, agents, attorneys and employees, and those acting in privity or contract with them;

(r) A permanent injunction against any infringement of the ’181 Patent by Strides, their officers, agents, attorneys and employees, and those acting in privity or contract with them;

(s) To the extent that Strides has committed any acts with respect to the subject matter claimed in the ’245 Patent, other than those acts expressly exempted by 35 U.S.C. § 271(e)(1), an award of damages for such acts, which should be trebled pursuant to 35 U.S.C. § 284;

(t) To the extent that Strides has committed any acts with respect to the subject matter claimed in the ’396 Patent, other than those acts expressly exempted by 35 U.S.C. § 271(e)(1), an award of damages for such acts, which should be trebled pursuant to 35 U.S.C. § 284;

(u) To the extent that Strides has committed any acts with respect to the subject matter claimed in the ’397 Patent, other than those acts expressly exempted by 35 U.S.C. § 271(e)(1), an award of damages for such acts, which should be trebled pursuant to 35 U.S.C. § 284;

(v) To the extent that Strides has committed any acts with respect to the subject matter claimed in the ’264 Patent, other than those acts expressly exempted by 35 U.S.C. § 271(e)(1), an award of damages for such acts, which should be trebled pursuant to 35 U.S.C. § 284;

(w) To the extent that Strides has committed any acts with respect to the subject matter claimed in the ’036 Patent, other than those acts expressly exempted by 35 U.S.C. § 271(e)(1), an award of damages for such acts, which should be trebled pursuant to 35 U.S.C. § 284;

(x) To the extent that Strides has committed any acts with respect to the subject matter claimed in the ’181 Patent, other than those acts expressly exempted by 35 U.S.C. § 271(e)(1), an award of damages for such acts, which should be trebled pursuant to 35 U.S.C. § 284;

(y) Costs and expenses in this action; and

(z) Such other relief as this Court may deem proper.

Dated: June 27, 2018

Respectfully submitted,

s/ Liza M. Walsh
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EXHIBIT A

US006642245B1

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

(10) **Patent No.: US 6,642,245 B1**
(45) **Date of Patent: *Nov. 4, 2003**

(54) **ANTIVIRAL ACTIVITY AND RESOLUTION OF 2-HYDROXYMETHYL-5-(5-FLUOROCYTOSIN-1-YL)-1,3-OXATHIOLANE**

(75) Inventors: **Dennis C. Liotta**, Stone Mountain, GA (US); **Raymond F. Schinazi**, Decatur, GA (US); **Woo-Baeg Choi**, North Brunswick, NJ (US)

(73) Assignee: **Emory University**, Atlanta, GA (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.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **08/475,339**

(22) Filed: **Jun. 7, 1995**

Related U.S. Application Data

(63) Continuation of application No. 07/831,153, filed on Feb. 12, 1992, now abandoned, and a continuation-in-part of application No. 07/736,089, filed on Jul. 26, 1991, now abandoned, which is a continuation-in-part of application No. 07/659,760, filed on Feb. 22, 1991, now Pat. No. 5,210,085, which is a continuation-in-part of application No. 07/473,318, filed on Feb. 1, 1990, now Pat. No. 5,204,466.

(51) **Int. Cl.**[7] **A61K 31/506**; C07D 411/04

(52) **U.S. Cl.** **514/274**; 514/86; 544/243; 544/317

(58) **Field of Search** 514/86, 274

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,000,137	A	12/1976	Dvonch et al.	260/252
4,336,381	A	6/1982	Nagata et al.	544/313
4,861,759	A	8/1989	Hiroaki et al.	514/46
4,879,277	A	11/1989	Mitsuya et al.	514/49
4,900,828	A	2/1990	Belica et al.	544/317
4,916,122	A	4/1990	Chu et al.	514/50
4,963,533	A	10/1990	de Clercq et al.	514/49
5,011,774	A	4/1991	Farina et al.	435/87
5,041,449	A	8/1991	Belleau et al.	514/274
5,047,407	A	9/1991	Belleau et al.	514/274
5,059,690	A	10/1991	Zahler et al.	544/276
5,071,983	A	12/1991	Koszalka et al.	544/317
5,179,104	A	1/1993	Chu et al.	544/310
5,185,437	A	2/1993	Koszalka et al.	536/24
5,204,466	A	4/1993	Liotta et al.	544/317
5,210,085	A	5/1993	Liotta et al.	514/274
5,234,913	A	8/1993	Furman, Jr. et al.	514/49
5,248,776	A	9/1993	Chu et al.	544/310
5,270,315	A	12/1993	Belleau et al.	514/262
5,276,151	A	1/1994	Liotta	544/317
5,444,063	A	8/1995	Schinazi	514/262
5,466,806	A	11/1995	Belleau et al.	544/310
5,486,520	A	1/1996	Belleau et al.	514/274
5,532,246	A	7/1996	Belleau et al.	514/274
5,539,116	A	7/1996	Liotta et al.	544/317
5,587,480	A	12/1996	Belleau et al.	544/310
5,618,820	A *	4/1997	Dionne	514/274

FOREIGN PATENT DOCUMENTS

AU	73004/91	8/1991
AU	665187	2/1992
AU	630913	9/1992
EP	0 217 580	4/1987
EP	0 337 713	10/1988
EP	350 811	1/1990
EP	0 375 329	1/1990
EP	357 009	3/1990
EP	0 361 831	4/1990
EP	0 382 526	6/1990
EP	0 433 898	8/1990
EP	421 636	4/1991
EP	0 494 119	7/1992
EP	0 515 144	11/1992
EP	0 515 156	11/1992
EP	0 515 157	11/1992
EP	0 526 253	2/1993
JP	2-69469	3/1990
JP	2-69476	3/1990
JP	07109221	4/1995
NL	8901258	12/1990
NL	238017	6/1994
WO	WO88/07532	10/1988
WO	WO90/12023	10/1990
WO	WO91/11186	8/1991
WO	WO91/17159	11/1991
WO	WO92/08727	5/1992
WO	WO92/10496	6/1992
WO	WO92/10497	6/1992
WO	WO92/14729	9/1992
WO	WO92/14743	9/1992
WO	WO92/15308	9/1992
WO	WO92/15309	9/1992
WO	WO92/18517	10/1992
WO	WO92/21676	12/1992
WO	WO94/04154	3/1994
WO	WO94/09793	5/1994
WO	WO94/14802	7/1994

OTHER PUBLICATIONS

Abobo, et al., "Pharmacokinetics of 2',3'–Dideoxy–5–fluoro–3'–thiacytidine in Rats," *J. of Pharmaceutical Sciences*, 83(1):96–99 (1994).

(List continued on next page.)

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(57) **ABSTRACT**

A method and composition for the treatment of HIV and HBV infections in humans is disclosed that includes administering an effective amount of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane, a pharmaceutically acceptable derivative thereof, including a 5' or N^4 alkylated or acylated derivative, or a pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable carrier.

A process for the resolution of a racemic mixture of nucleoside enantiomers is also disclosed that includes the step of exposing the racemic mixture to an enzyme that preferentially catalyzes a reaction in one of the enantiomers.

22 Claims, 6 Drawing Sheets

OTHER PUBLICATIONS

Agranat and Biedermann, "Intellectual Property and Chirality: Patentability of Enantiomers of Racemic Drugs in a Racemic Switch Scenario," *8th Chirality Conference, Edinburgh*, UK (Jul. 2, 1996).

Balzarini, J., et al., "Potent and Selective Anti–HTLV–III/LAV Activity of 2,', 3'–Dideoxycytidine, the 2', 3'–Unsaturated Derivative of 2', 3'–Dideoxycytidine," *Biochemical and Biophysical Research Communications*, 140(2): 735–742 (1986).

Baschang, et al., "The enantiomers of 1.beta.–adenyl–2.alpha.–hydroxy–3.beta.–(hydroxymethyl) cyclobutane," *Tetrahedron:Asymmetry*, 3(2): 193–6 (1992).

Belleau, B., et al., "Design and Activity of a Novel Class of Nucleoside Analogs Effective Against HIV–1,", *International Conference on AIDS*, Montreal, Quebec, Canada, Jun. 4–9, 1989.

Borthwick, et al., "Synthesis and Enyzmatic Resolution of Carbocyclic 2'–Ara–Fluoro–Guanosine: A Potent New Anti– Herpetic Agent," *J. Chem. Soc. Commun.*, vol. 10, pp. 656–658 (1988).

Carter, et al., "Activities of (–)–Carbovir and 3'–Azido–3'–Deoxythymidine Against Human Immunodeficiency Virus In Vitro," *Antimicrobial Agents and Chemotherapy*, 34(6):1297–1300 (1990).

Chang, Chien–Neng, et al., "Deoxycytidine Deaminase–resistant Steroisomer Is the Active Form of (+)–2', '–Dideoxy–3'–thiacytidine in the Inhibition of Hepatitis B Virus Replication," *The Journal of Biological Chemistry*, 267(20):13938–13942 (1992).

Chu, C.K., et al., "An Efficient Total Synthesis of 3'–Azido–3'–Deoxythiymidine (AZT) and 3'–Azido–2', 3'–Dideoxyuridine (AZDDU, CS–87) from D–Mannitol," *Tetrahedron Lett.*, 29(42):5349–5352 (1988).

Chu, et al., "Comparative Activity of 2', 3'–Saturated and Unsaturated Pyrimidine and Purine Nucleosides Against Human Immunodeficiency Virus Type 1 in Peripheral Blood Mononuclear Cells," *Biochem. Pharm.*, 37(19):3543–3548 (1988).

Chu, et al., "Structure–Activity Relationships of Pyrimidine Nucleosides as Antiviral Agents for Human Immunodeficiency Virus Type 1 in Peripheral Blood Mononuclear Cells," *J. Med. Chem.*, 32:612(1989).

Condreay, et al., "Evaluation of the Potent Anti–Hepatitis B Virus Agent(–) *cis*–5–Fluoro–1–[2–(Hydroxymethyl)–1, 3–Oxathiolan–5–yl]Cytosine in a Novel in Vivo Model," *Antimicrobial Agents and Chemotherapy*, 616–619 (1992).

Connolly and Hammer, "Minireview: Antiretroviral Therapy: Reverse Transcriptase Inhibition," *Antimicrobial Agents and Chemotherapy*, 36(2):245–254 (1992).

Cretton, E., et al., "Catabolism of 3'–Azido–3'–Dexoythymidine in Hepatocytes and Liver Microsomes, with Evidence of Formation of 3'–Amino–3'–Deoxythymidine, a Highly Toxic Catabolite for Human Bone Marrow Cells," *Molecuilar Pharmacology*, 39:258–266 (1991).

Cretton, E., et al., "Pharmokinetics of 3'–Azido–3'–Deoxythymidine and its Catabolites and Interactions with Probenecid in Rhesus Monkeys," *Antimicrobial Agents and Chemotherapy*, 35(5):801–807 (1991).

Doong, Shin–Lian., et al., "Inhibition of the Replication of Hepatitis B Virus in vitro by 2',3'–Dideoxy–3'–Thiacytidine and Related Analogues," *Natl. Acad. Sci. USA*, 88:8495–8499 (1991).

Feorino, et al., "Prevention of activation of HIV–1 by antiviral agents in OM 10.1 cells," *Antiviral Chem. & Chemotherapy*, 4(1):55–63 (1993).

Frick, et al., "Pharmacokinetics, Oral Bioavailability, and Metabolic Disposition in Rats of (–)–cis–5–Fluoro–1–[2–(Hydroxymethyl)–1,3–Oxathiolan–5–yl] Cytosine, a Nucleoside Analog Active against Human Immunodeficiency Virus and Hepatitis B Virus," *Antimicrobial Agents and Chemotherapy*, 37(11):2285–2292 (1993).

Furman, et al., "The Anti–Hepatitis B Virus Activities, Cytotoxicities, and Anabolic Profiles of the (–) and (+) Enantiomers of cis–5–Fluoro–1–[2–(Hydromethyl)–1, 3–Oxthiolane–5–yl]Cytosine," *antimicrobial Agents and Chemotherapy*, 36(12):2686–2692 (1992).

Herdewijn, et al., "Resolution of Aristeromycin Enantiomers," *J. Med. Chem.*, 1985, vol. 28, 1385–1386.

Hoong, et al., "Enzyme–Mediated Enatioselective Preparation of Pure Enantiomers of the Antiviral Agent 2', 3'–Dideoxy–5–fluoro–thiacytidine (FTC) and Related Compounds," *J. Org. Chem.*, 57:5563–5565 (1992).

Ito, et al., "Chirally Selective Synthesis of Sugar Moiety of Nucleosides by Chemicoenzymatic Approach: L–and D–Riboses, Showdomycin, and Cordycepin," *J. Am. Chem. Soc.*, 103:6739–6741 (1981).

Jansen, et al., "High–Capacity In Vitro Assessment of Anti–Hepatitis B Virus Compound Selectivity by a Virion–Specific Polymerase Chain Reaction Assay," *Antimicrobial Agents and Chemotherapy*, 441–447 (1993).

Jeong, L., et al., "Asymmetric Synthesis and Biological Evaluation of β–L–(2R,5S)–and a–L(2R–5R)–1,3–Oxathiolane–Pyrimidine and –Purine Nucleosides and Potential Anti–HIV Agents," *J. Med. Chem.*, 36(2):181–195 (1993).

Krenitsky, T.A., et al., "3'–Amino–2',3'–Dideoxyribonucleosides of Some Pyrimidines: Synthesis and Biological Activities," *J. Med. Chem.*, vol. 26 (1983).

Krenitsky, et al., "An Enzymic Synthesis of Purine D–arabinonucleosides," *Carbohydrate Research*, 97:139–146 (1981).

Lin, et al., "Potent and Selective In Vitro Activity of 3–Deoxythmindine–2–Ene–(3'–Deoxy–2',3'–Didehydrothymidine) Against Human Immunodeficiency Virus," *Biochem. Pharm.*, 36(17):2713–2718 (1987).

Mahmoudian, et al., "Enzymatic Production of Optically Pure (2'R–cis)–2'–deoxy–3'–thiacytidine (3TC, Lamivudine): A Potent Anti–HIV Agent," *Enzyme Microb. Technol.*, Sep. 1993, vol. 15, 749–755, published by the Glaxo Group Research.

Mitsuya, H., et al., 3'–Azido–3'–Deoxythymidine (BW A 509U): An Antiviral Agent that Inhibits the Infectivity and Cytopathic Effect of Human T–Lymphotropic Virus Type III/Lymphadenopathy–Associated Virus *In Vitro, Proc. Natl. Acad. Sci., USA*, 82:7096–7100 (1985).

Mitsuya, H., et al., "Molecular Targets for AIDS Therapy," Science, vol. 249, pp. 1533–1544 (1990).

Mitsuya, H., et al., "Rapid in Vitro Systems for Assessing Activity of Agents Against HTLV–III/LAV," *AIDS: Modern Concepts and Therapeutic Challenges*, S. Broder, Ed. pp. 303–333, Marcel Dekker: New York, (1987).

Norbeck, D., et al., "A New 2',3'–Dideoxynucleoside Prototype with In Vitro Activity Against HIV," *Tetrahedron Lett.*, 30(46):6263–6266 (1989).

Ohno, et al., "Synthetic Studies on Biologically Active Natural Products by a Chemicoenzymatic Approach," *Tet. Letters*, 40:145–152 (1984).

Okabe, M., et al., "Synthesis of the Dideoxynucleosides ddC and CNT from Glutamic Acid, Ribonolactone, and Pyrimidine Bases," *J. Org. Chem.*, 53(20):4780–4786 (1988).

Paff, et al., "Intracellular Metabolism of (–)–and (+)–cis–5–Fluoro–1–[2–Hydroxymethyl)–1, 3–Oxathiolan–5–yl]Cytosine in HepG2 Derivative 2.2.15 (Subclone P5A)Cells," *Antimicrobial Agents and Chemotherapy*, 1230–1238 (1994).

Pirkle and Pochansky, "Chiral Stationary Phases for the Direct LC Separation of Enantiomers," *Advances in Chromatography*, Giddings, J.C., Grushka, E., Brown, P.R., eds.: Marcel Dekker: New York, 1987; vol. 27, Chap. 3, pp. 73–127.

Richman, D. D., et al., "The Toxicity of Azidothymidine (AZT) in the Treatment of Patients with AIDS and AIDS–Related Complex," *N. Eng. J. Med.*, 317(4):192–197 (1987).

Roberts, et al., "Enzymic Resolution of cis–and trans–4–hydroxycyclopent–2–enylmethanol..." *J. Chem. Soc.*, Perkin Trans. 1, (10):2605–7 (1991).

Saari, et al., "Synthesis and Evaluation of 2–Pyridinone Derivatives as HIV–1–Specific Reverse Transcriptase Inhibitors, 2. Analogues of 3–Aminopyridin–2(1H)–one," *J. Med. Chem.*, 35:3792–3802 (1992).

Satsumabayashi, S. et al., "The Synthesis of 1,3–Oxathiolane–5–one Derivatives," *Bull, Chem. Soc. Japan*, 45:913–915 (1972).

Saunders, "Non–Nucleoside Inhibitors of HIV Reverse Transcriptase: Screening Successes–Clinical Failures," *Drug Design and Discovery*, 8:255–263 (1992).

Schinazi, R.F., et al., "Actvities of the Four Optical Isomers of 2',3'–Dideoxy–3'–Thiacytidine (BCH–189) against Human Immunodeficiency Virus Type 1 in Human Lymphocytes," *Antimicrobial Agents and Chemotherapy* 36(3):672–676 (1992).

Schinazi, R.F., et al., "Insights into HIV Chemotherapy, "*AIDS Research and Human Retroviruses* 8(6):963–990 (1992).

Schinazi, R.F., et al., "Pharmacokinetics and Metabolism of Racemic 2', 3'–Dideoxy–5–Fluoro–3'–Thiacytidine in Rhesus Monkeys," *Antimicrobial Agents and Chemotherapy* 36(11):2432–2438 (1992).

Schinazi, R.F., et al., "Selective Inhibition of Human Immunodeficiency Viruses by Racemates and Enantiomers of *cis*–5–Fluoro–1–[2–(Hydroxymethyl)–1, 3–Oxathiolan–5–yl]Cytosine," *Antimicrobial Agents and Chemotherapy* 36(11):2423–2431 (1992).

Schinazi, R.F., et al., "Substrate Specificity of *Escherichia Coli* Thymidine Phosphorylase for Pyrimidine Nucleoside with an Anti–Human Immunodeficiency Virus Activity," *Biochemical Pharmacology* 44(2):199–204 (1992).

Secrist, et al., "Resolution of Racemic Carbocyclic Analogues of Purine Nucleosides Through the Action of Adenosine Deaminase Antiviral Activity of the Carbocyclic 2'–Deoxyguanosine Enantiomers," *J. Med. Chem.* , vol. 30, pp. 746–749 (1987).

Shewach, et al., "Affinity of the antiviral enantiomers of oxathiolane cytosine nucleosides for human 2'–deoxycytidine kinase," *Biochem. Pharmacol.*, 45(7):1540–1543 (1993).

Sterzycki, R.Z., et al., "Synthesis and anti–HIV activity of several 2'–fluoro–containing pyrimidine nucleosides," *J. Med. Chem.*, 33(8):2150–2157 (1990).

Storer, R., et al., "The Resolution and Absolute Stereochemistry of the Enantiomeris of *cis*–1–2–(Hydromethyl)–1, 3–Oxathiolan–5–yl)cytosine (BCH198): Equipotent Anti–HIV Agents," *Nucleosides & Nucleotides*, 12(2):225–236 (1993).

van Roey, et al., "Solid–State Conformation of Anti–Human Immnosudeficiency Virus Type–1 Agents: Crystal Structures of Three 3'–Azido–3'–deoxythymidine Analogues," *J. Am. Chem. Soc.*, 110:2277–2782 (1988).

Vorbrüggen, et al., "Nucleoside Synthesis with Trimethylsiyl Triflate and Perchlorate as Catalysts," *Chem. Ber.*, 114:1234–1255 (1981).

Wilson, et al., "The 5'–Triphosphates of the (1) and (+) Enantiomers of *cis*–5–Fluoro–1–[2–(Hydroxymethyl)–1, 3–Oxathiolane–5–yl]Cytosine Equally Inhibit Human Immunodeficiency Virus Type 1 Reverse Transcriptase," *Antimicrob. Agents and Chemother.*, 37(8):1720–1722 (1993).

Wilson, L.J., et al., "A General Method for Controlling Glycosylation Stereochemistry in the Synthesis of 2'–Deoxyribose Nucleosides," *Tetrahedron Lett.*, 31(13):1815–1818 (1990).

Wilson, L.J., et al., "The Synthesis and Anti–HIV Activity of Pyrimidine Dioxolanyl Nucleosides," *Bioorganic & Medicinal Chemistry Letters*, 3(2):169–174 (1993).

Winslow, et al., "*In vitro* susceptibility of clinical isolates of HIV–1 to XM323, a non–peptidyl HIV protease inhibitor," *AIDS*, 8:753–756 (1994).

Zhu, Zhou, et al., "Cellular Metabolism of 3'–Azido–2', 3'–Dideoxyuridine with Formation of 5'–O–Diphophoshexase Derivatives by Previously Unrecognized Metabolic Pathways of 2'–Deoxyuridine Analogs," *Molecular Pharmacology*, 0:929–938 (1990).

Journal of Acquired Immune Deficiency Syndromes, (Raven Press, Pubisher), vol. 6 (1993).

* cited by examiner

FIGURE 1

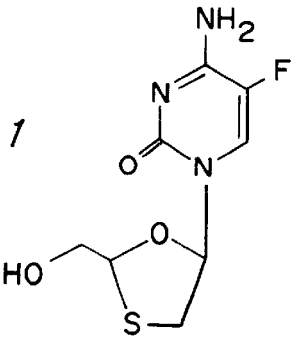
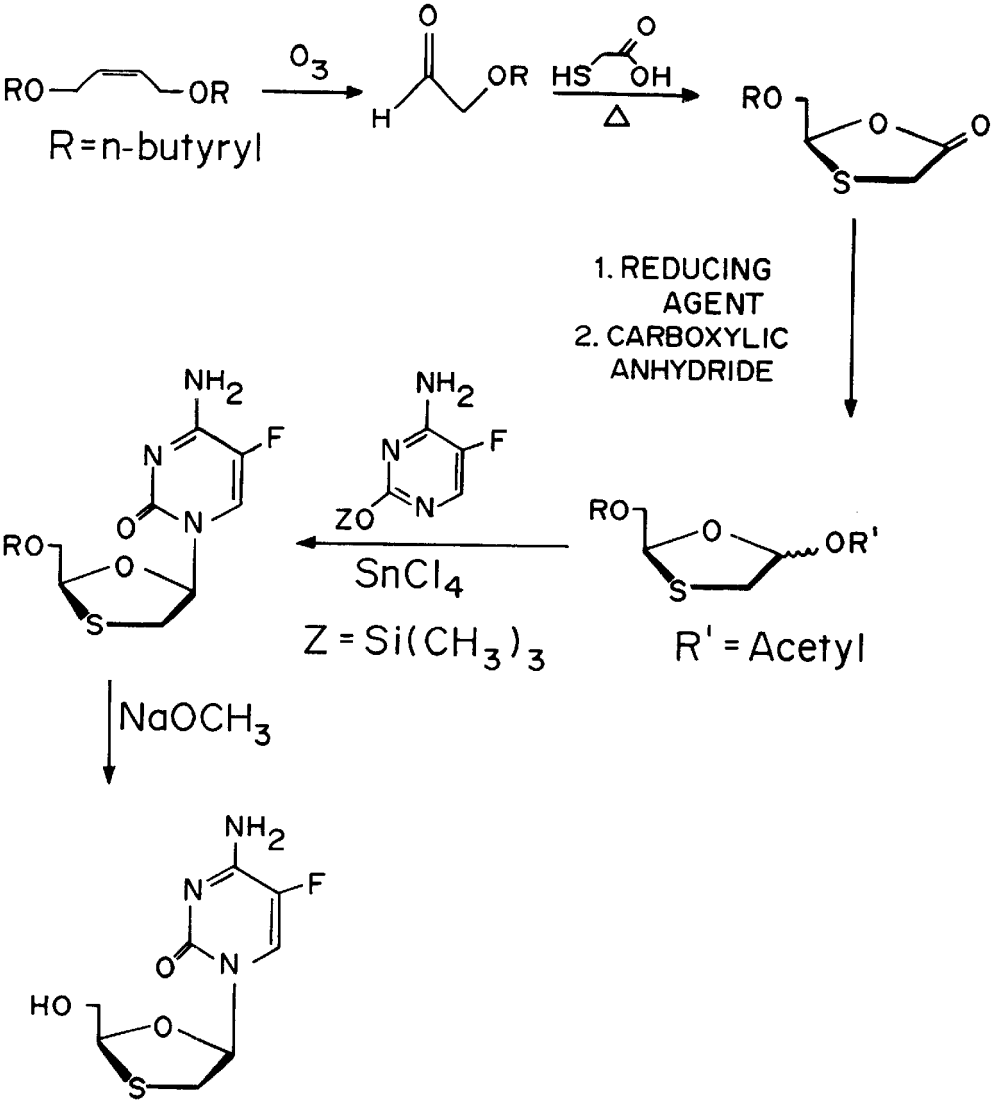


FIGURE 2



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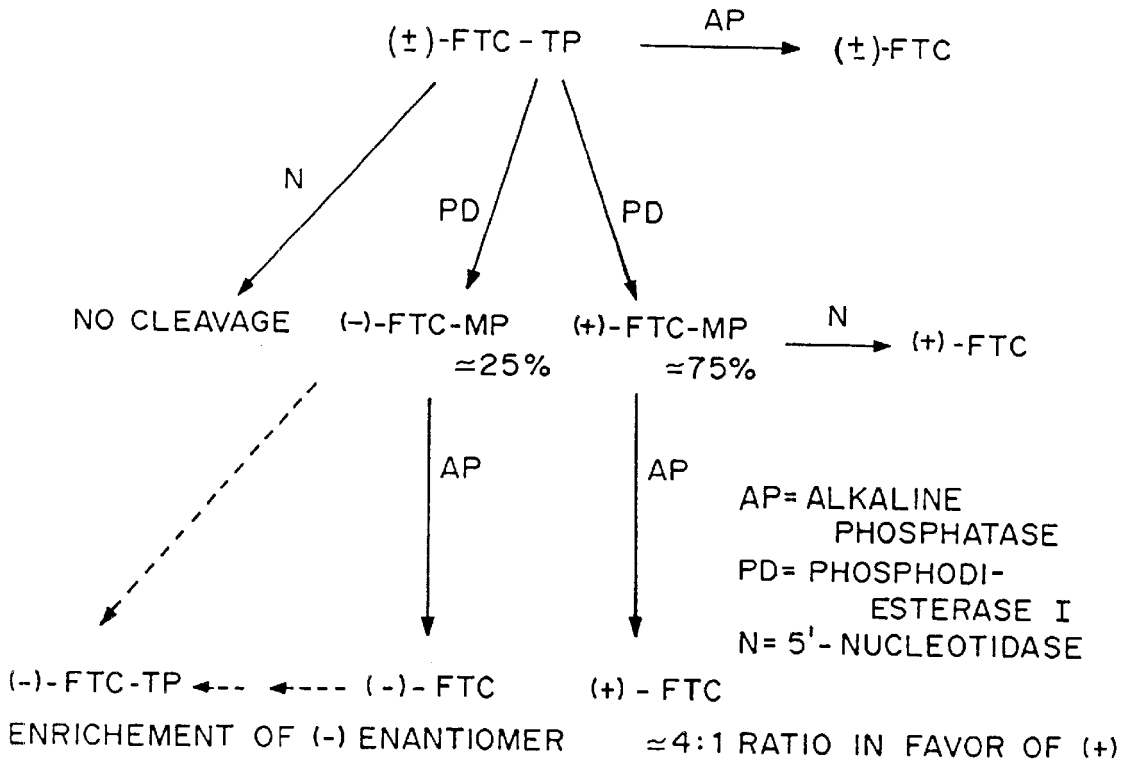

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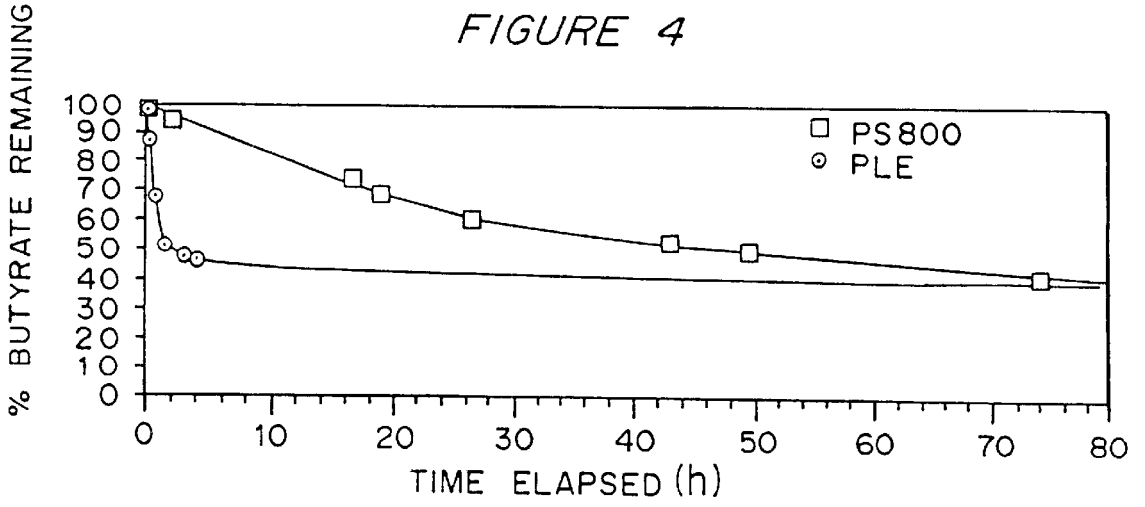

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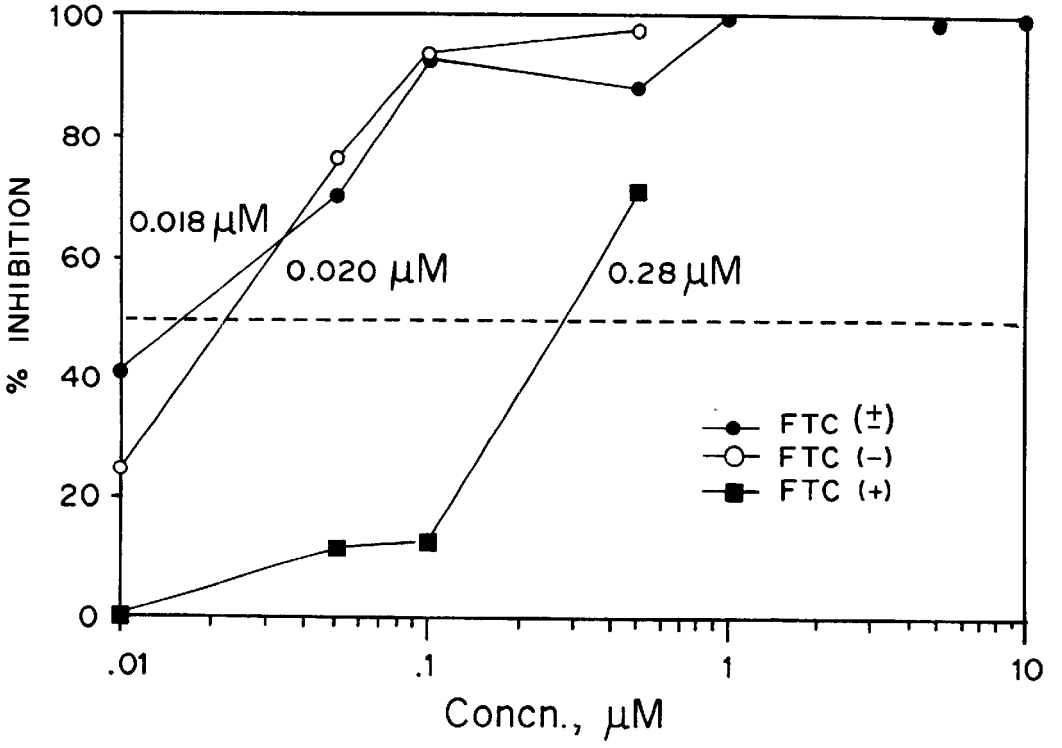

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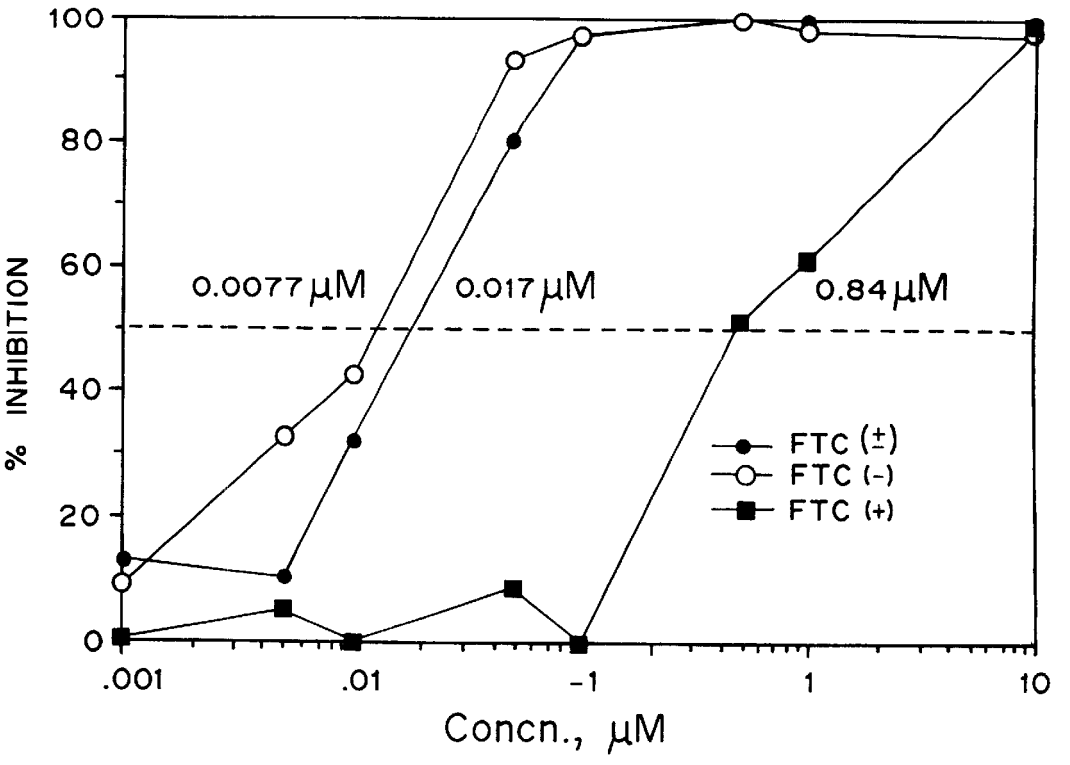

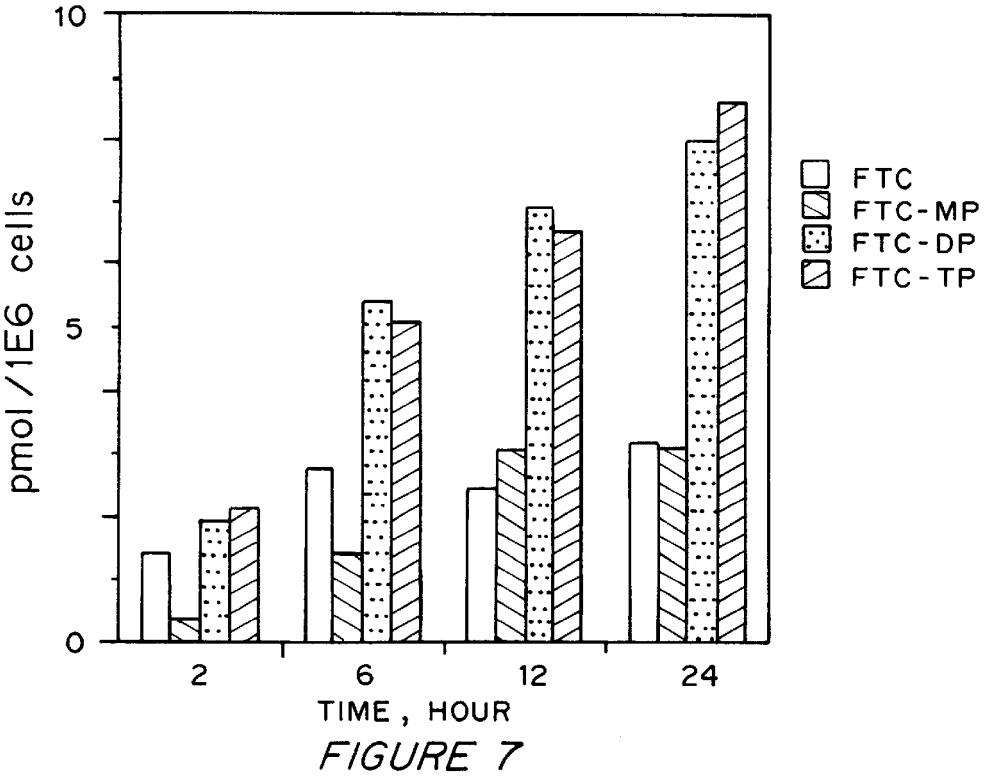

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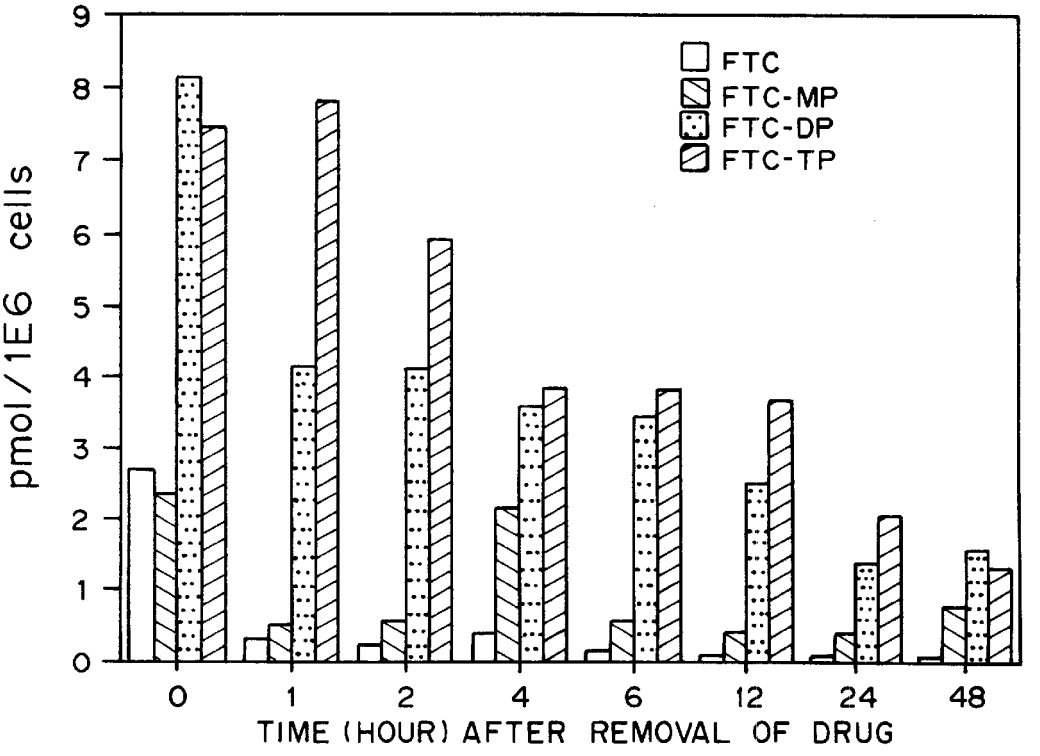

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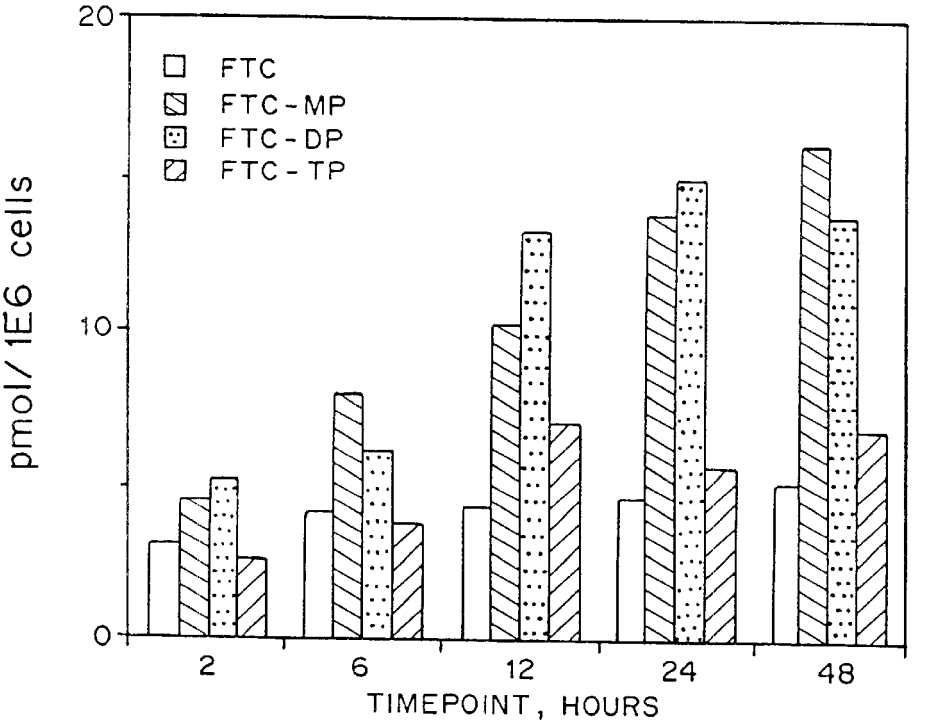

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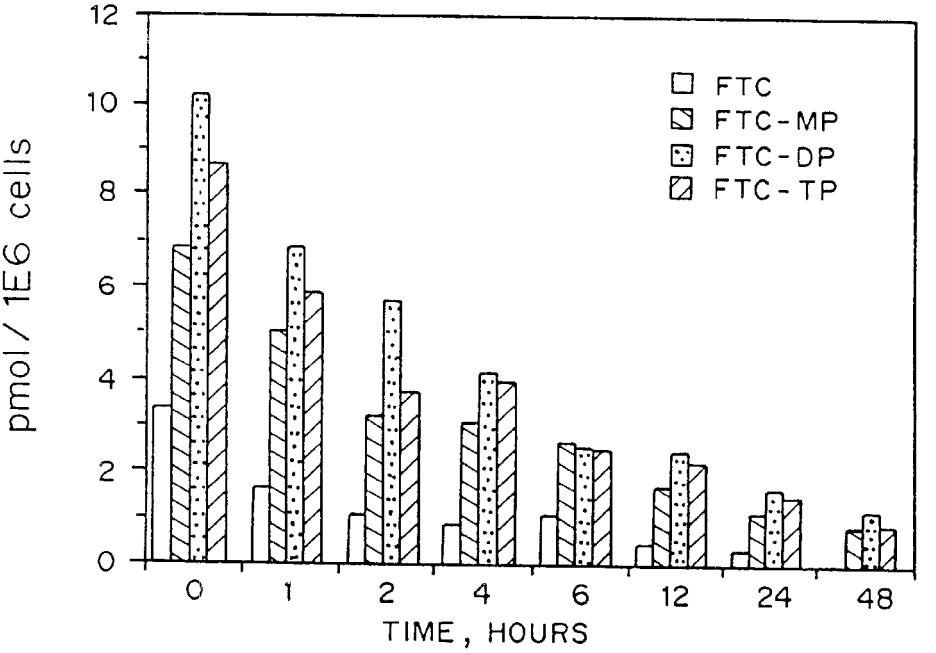

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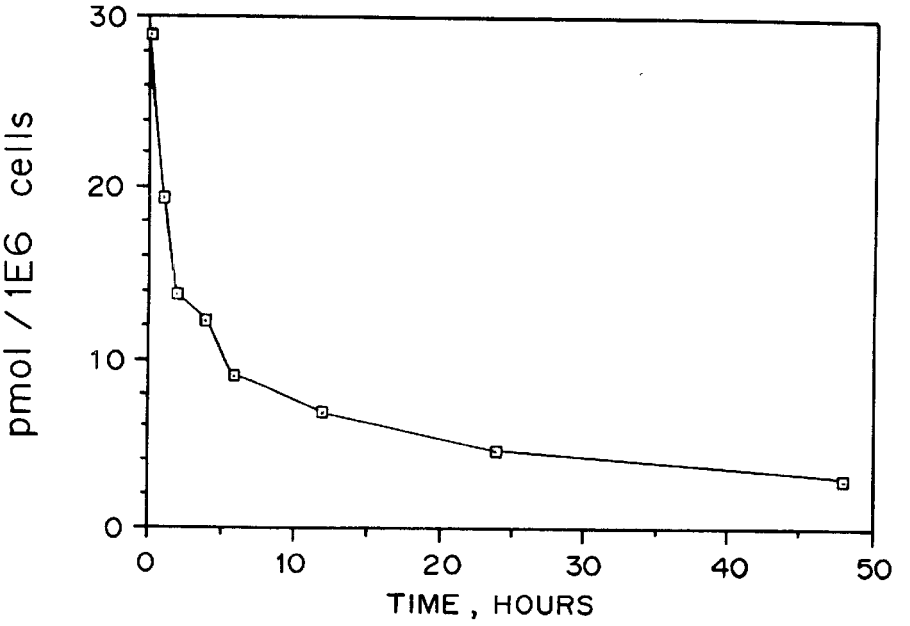


FIGURE 11

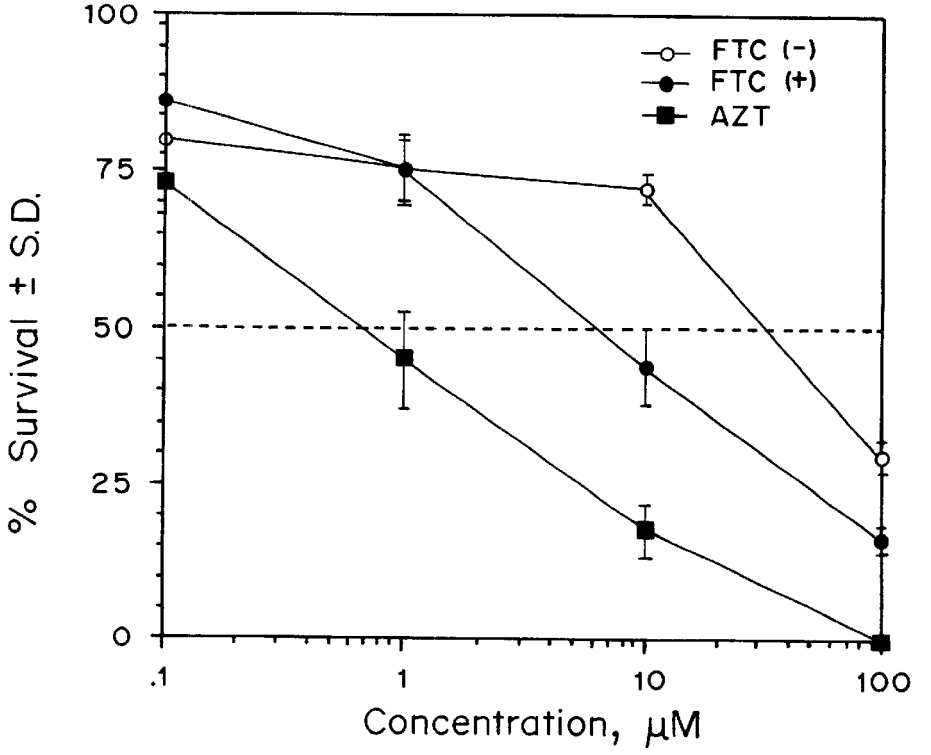


FIGURE 12

ANTIVIRAL ACTIVITY AND RESOLUTION OF 2-HYDROXYMETHYL-5-(5-FLUOROCYTOSIN-1-YL)-1,3-OXATHIOLANE

This application is a Continuation application of U.S. Ser. No. 07/831,153, filed on Feb. 12, 1992 now abandoned, by Dennis C. Liotta, Raymond F. Schinazi, and Woo-Baeg Choi for "Antiviral Activity and Resolution of 2-Hydroxymethyl-5(5-Fluorocytosin-1-yl)-1,3-Oxathiolane" which is a continuation-in-part application of (1) U.S. Ser. No. 07/659,760, now U.S. Pat. No. 5,210,085, entitled "Method for the Synthesis, Compositions and Use of 2'-Deoxy-5-Fluoro-3'-Thiacytidine and Related Compounds", filed on Feb. 22, 1991, by Dennis C. Liotta, Raymond F. Schinazi, and Woo-Baeg Choi, which is a continuation in part application of U.S. Ser. No. 07/473,318, now U.S. Pat. No. 5,204,466, entitled "Method and Compositions for the Synthesis of BCH-189 and Related Compounds", filed on Feb. 1, 1990, by Dennis C. Liotta and Woo-Baeg Choi and, (2) a continuation-in-part of U.S. Ser. No. 07/736,089, now abandoned, entitled "Method of Resolution and Antiviral Activity of 1,3-Oxathiolane Nucleoside Enantiomers" filed on Jul. 26, 1991, by Dennis C. Liotta, Raymond F. Schinazi, and Woo-Baeg Choi, which is a continuation-in-part of U.S. Ser. No. 07/659,760, now U.S. Pat. No. 5,210,085, referenced above.

The U.S. Government has rights in this invention arising out of the partial funding of work leading to this invention through the National Institutes of Health Grant Nos. AI-26055, AI-28731, NIH 5-21935, as well as a Veteran's Administration Merit Review Award.

BACKGROUND OF THE INVENTION

This invention is in the area of biologically active nucleosides, and specifically includes antiviral compositions that include 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane ("FTC"), its physiologically acceptable derivative, or physiologically acceptable salt, and a method for the resolution and use of the (−)-β-L and (+)-β-D enantiomers of FTC.

In 1981, acquired immune deficiency syndrome (AIDS) was identified as a disease that severely compromises the human immune system, and that almost without exception leads to death. In 1983, the etiological cause of AIDS was determined to be the human immunodeficiency virus (HIV). By December of 1990, the World Health Organization estimated that between 8 and 10 million people worldwide were infected with HIV, and of that number, between 1,000,000 and 1,400,000 were in the U.S.

In 1985, it was reported that the synthetic nucleoside 3'-azido-3'-deoxythymidine (AZT) inhibits the replication of human immunodeficiency virus. Since then, a number of other synthetic nucleosides, including 2',3'-dideoxyinosine (DDI), 2',3'-dideoxycytidine (DDC), 3'-fluoro-3'-deoxythymidine (FLT), and 2',3'-dideoxy-2',3'-didehydrothymidine (D4T), have been proven to be effective against HIV. A number of other 2',3'-dideoxynucleosides have been demonstrated to inhibit the growth of a variety of viruses in vitro. It appears that, after cellular phosphorylation to the 5'-triphosphate by cellular kinases, these synthetic nucleosides are incorporated into a growing strand of viral DNA, causing chain termination due to the absence of the 3'-hydroxyl group.

The success of various 2',3'-dideoxynucleosides in inhibiting the replication of HIV in vivo or in vitro has led a number of researchers to design and test nucleosides that

substitute a heteroatom for the carbon atom at the 3'-position of the nucleoside. Norbeck, et al., disclose that (±)-1-[(2β, 4β)-2-(hydroxymethyl)-4-dioxolanyl]thymine (referred to as (±)-dioxolane-T) exhibits a modest activity against HIV (EC_{50} of 20 μm in ATH8 cells), and is not toxic to uninfected control cells at a concentration of 200 μM. *Tetrahedron Letters* 30 (46), 6246, (1989). European Patent Application Publication No. 0 337 713 and U.S. Pat. No. 5,041,449, assigned to IAF BioChem International, Inc., disclose 2-substituted-4-substituted-1,3-dioxolanes that exhibit antiviral activity.

U.S. Pat. No. 5,047,407 and European Patent Application Publication No. 0 382 526, also assigned to IAF Biochem International, Inc. disclose a number of 2-substituted-5-substituted-1,3-oxathiolane nucleosides with antiviral activity, and specifically report that the racemic mixture (about the C4'-position) of the C1'-β isomer of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane (referred to below as (±)-BCH-189) has approximately the same activity against HIV as AZT, and no cellular toxicity at the tested levels. (±)-BCH-189 has also been found to inhibit the replication of AZT-resistant HIV isolates in vitro from patients who have been treated with AZT for longer than 36 weeks.

Another virus that causes a serious human health problem is the hepatitis B virus (referred to below as "HBV"). HBV is second only to tobacco as a cause of human cancer. The mechanism by which HBV induces cancer is unknown, although it is postulated that it may directly trigger tumor development, or indirectly trigger tumor development through chronic inflammation, cirrhosis, and cell regeneration associated with the infection.

After a two to six month incubation period in which the host is unaware of the infection, HBV infection can lead to acute hepatitis and liver damage, that causes abdominal pain, jaundice, and elevated blood levels of certain enzymes. HBV can cause fulminant hepatitis, a rapidly progressive, often fatal form of the disease in which massive sections of the liver are destroyed.

Patients typically recover from acute hepatitis. In some patients, however, high levels of viral antigen persist in the blood for an extended, or indefinite, period, causing a chronic infection. Chronic infections can lead to chronic persistent hepatitis. Patients infected with chronic persistent HBV are most common in developing countries. By mid-1991, there were approximately 225 million chronic carriers of HBV in Asia alone, and worldwide, almost 300 million carriers. Chronic persistent hepatitis can cause fatigue, cirrhosis of the liver, and hepatocellular carcinoma, a primary liver cancer.

In western industrialized countries, high risk groups for HBV infection include those in contact with HBV carriers or their blood samples. The epidemiology of HBV is very similar to that of acquired immune deficiency syndrome, which accounts for why HBV infection is common among patients with AIDS or AIDS-related complex. However, HBV is more contagious than HIV.

A human serum-derived vaccine has been developed to immunize patients against HBV. While it has been found effective, production of the vaccine is troublesome because the supply of human serum from chronic carriers is limited, and the purification procedure is long and expensive. Further, each batch of vaccine prepared from different serum must be tested in chimpanzees to ensure safety. Vaccines have also been produced through genetic engineering. Daily treatments with a-interferon, a genetically engineered

protein, has also shown promise. However, to date there is no known pharmaceutical agent that effectively inhibits the replication of the virus.

To market a nucleoside for pharmaceutical purposes, it must not only be efficacious with low toxicity, it must also be cost effective to manufacture. An extensive amount of research and development has been directed toward new, low cost processes for large scale nucleoside production. 2',3'-Dideoxynucleosides are currently prepared by either of two routes: derivatization of an intact nucleoside or condensation of a derivatized sugar moiety with a heterocyclic base. Although there are numerous disadvantages associated with obtaining new nucleoside analogues by modifying intact nucleosides, a major advantage of this approach is that the appropriate absolute stereochemistry has already been set by nature. However, this approach cannot be used in the production of nucleosides that contain either nonnaturally occurring bases or nonnaturally occurring carbohydrate moieties (and which therefore are not prepared from intact nucleosides), such as 1,3-oxathiolane nucleosides and 1,3-dioxolane nucleosides.

When condensing a carbohydrate or carbohydrate-like moiety with a heterocyclic base-to form a synthetic nucleoside, a nucleoside is produced that has two chiral centers (at the C1'and C4'-positions), and thus exists as a diastereomeric pair. Each diastereomer exists as a set of enantiomers. Therefore, the product is a mixture of four enantiomers.

It is often found that nucleosides with nonnaturally-occurring stereochemistry in either the C1'or the C4'-positions are less active than the same nucleoside with the stereochemistry as set by nature. For example, Carter, et al., have reported that the concentration of the (−)-enantiomer of carbovir (2',3'-didehydro-2',3'-dideoxyguanosine) in cell culture required to reduce the reverse transcriptase activity by 50% (EC_{50}) is 0.8 μM, whereas the EC_{50} for the (+)-enantiomer of carbovir is greater than 60 μM. *Antimicrobial Agents and Chemotherapy*, 34:6, 1297–1300 (June 1990).

PCT International Publication No. WO 91/11186 discloses that 1,3-oxathiolane nucleosides can be prepared with high diastereoselectivity (high percentage of nucleoside with a β configuration of the bond from the C1'-carbon to the heterocyclic base) by careful selection of the Lewis acid used in the condensation process. It was discovered that condensation of a 1,3-oxathiolane nucleoside with a base occurs with almost complete β-stereospecificity when stannic chloride is used as the condensation catalyst. Other Lewis acids provide low (or no) C1'-β selectivity or simply fail to catalyze the reactions.

In light of the fact that acquired immune deficiency syndrome, AIDS-related complex, and hepatitis B virus have reached epidemic levels worldwide, and have tragic effects on the infected patient, there remains a strong need to provide new effective pharmaceutical agents to treat these diseases that have low toxicity to the host.

There is also a need to provide a cost effective, commercially viable method to produce pharmaceutically important nucleosides, and specifically attain β-stereospecificity in the C4'-position of synthetic nucleosides prepared by condensing a carbohydrate-like moiety with a base.

Therefore, it is an object of the present invention to provide a method and composition for the treatment of human patients infected with HIV.

It is another object of the present invention to provide a method and composition for the treatment of human patients or other host animals infected with HBV.

It is still another object of the present invention to provide enantiomerically enriched 1,3-oxathiolane nucleosides.

It is still another object of the present invention to provide a method for the resolution of C4'-enantiomers of 1,3-oxathiolane nucleosides.

SUMMARY OF THE INVENTION

A method and composition for the treatment of HIV and HBV infections in humans and other host animals is disclosed that includes administering an effective amount of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane, a pharmaceutically acceptable derivative thereof, including a 5'or N^4 alkylated or acylated derivative, or a pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable carrier.

It has been discovered that 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane ("FTC"), exhibits surprisingly high activity against human immunodeficiency virus with very low host cell toxicity. It has also been discovered that FTC exhibits very significant activity against HBV, and therefore can be used to treat patients who have a variety of illnesses associated with HBV infection.

Toxicity and pharmacokinetic studies confirm the usefulness of FTC as an antiviral agent for pharmaceutical administration. FTC and its enantiomers are nontoxic to peripheral human bone marrow cells at concentrations up to 50 μM and other cell lines at concentrations up to 200 μM. FTC-TP is a major intracellular metabolite in PBMC and HepG2 cells. FTC-TP competitively inhibits HIV-1 reverse transcriptase (RT) with a K_1, of 0.2 μM using a poly(I)oligo(dC) template-primer. Using sequencing analysis, FTC-TP can be shown to be a potent DNA chain terminator when HIV-RT is used (C-stops).

Chronic treatment with FTC is not toxic to rodents, even at oral doses of 85 mg/kg per day for at least two months. The pharmacokinetics of FTC in rhesus monkeys indicates high oral bioavailability (approximately 73±6%) and a plasma terminal half life of approximately 1.34±0.18 (mean of oral and I.V. administration).

A process for the resolution of a racemic mixture of nucleoside enantiomers, including the racemic mixture of FTC, is also disclosed that includes the step of exposing the racemic mixture to an enzyme that preferentially catalyzes a reaction in one of the enantiomers. The process can be used to resolve a wide variety of nucleosides, including pyrimidine and purine nucleosides that are optionally substituted in the carbohydrate moiety or base moiety. The process can also be used to resolve nucleoside derivatives that contain additional heteroatoms in the carbohydrate moiety, for example, (±)-FTC and (±)-BCH-189. The resolution of nucleosides can be performed on large scale at moderate cost.

Using methods described herein, FTC was resolved into its (+)-β-D and (−)-β-L enantiomers. The (−)-β-L-enantiomer appears to be more potent that the (+)-β-D-enantiomer against HIV, HBV, and SIV. The (+)-enantiomer of FTC is also active against HIV, HBV, and SIV.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** is an illustration of the chemical structure of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane ("FTC").

FIG. **2** is an illustration of a method for the preparation of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane.

FIG. **3** is a flow chart of the specificity of alkaline phosphatase and snake venom phosphodiesterase for the (+) and (−) enantiomers of FTC.

FIG. **4** is a graph indicating the progress of lipase-catalyzed hydrolysis of the 5'-butyryl ester of FTC over time using the enzymes Amano PS-800® (-open square-) and PLE (-open circle with dot-).

FIG. **5** is a graph of the effect of concentration (μM) of racemic and enantiomerically enriched FTC (prepared by the method of Example 4) versus the percent inhibition of human PBM cells infected with HIV-1. ((-darkened circle-, (±)-FTC), (-open circle-,(−)-FTC), (-darkened square-, (+)-FTC).

FIG. **6** is a graph of the effect of concentration (μM) of racemic and enantiomerically enriched FTC (prepared by method of Example 3) on the percent inhibition of human PBM cells infected with HIV-1. ((-darkened circle-, (±)-FTC), (-open circle-,(−)-FTC), (-darkened square-, (+)-FTC).

FIG. **7** is a graph of the uptake of tritiated (±)-FTC in human PBM cells (average of two determinations) in time (hours) versus pmol/10^6 cells.

FIG. **8** is a graph of the egress of radiolabeled (±)-FTC from human PBM cells, measured in hours versus pmol/10^6 cells.

FIG. **9** illustrates the presence of [^{3}H]-(±)-FTC and its phosphorylated derivatives in human HepG-2 cells (average of two determinations) incubated in media containing 10 μM [^{3}H]-(±)-FTC, measured in pmol/10^6 cells over time.

FIG. **10** illustrates the egress of [^{3}H]-(±)-FTC and its phosphorylated derivatives in human HepG2 in pmol/10^6 cells over time cells after pulsing cells with 10 μM [^{3}H]-(±)-FTC (700 DPM/pmole) for 24 hours, and evaluating the concentration of compound 24 hours after removal.

FIG. **11** illustrates the decrease in the combined concentration of [^{3}H]-(±)-FTC and its phosphorylated derivatives from human HepG2 cells after incubation with 10 μM [^{3}H]-(±)-FTC (700 DPM/pmole) for 24 hours, in pmol/10^6 cells over time.

FIG. **12** is a graph of the effect of the enantiomers of FTC on colony formation of granulocyte-macrophage precursor cells, as measured in percent survival versus concentration in μM ((−)-FTC, open circle; (+)-FTC, darkened circle; AZT, darkened square.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term "enantiomerically enriched nucleoside" refers to a nucleoside composition that includes at least 95% of a single enantiomer of that nucleoside.

As used herein, the term FTC refers to 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane (the racemic form or enantiomers), also referred to as 2'-deoxy-5-fluoro-3'-thiacytidine.

As used herein, the term (±)-FTC refers to (±)-β-D,L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1, 3-oxathiolane.

As used herein, the term (−)-FTC refers to (−)-β-L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1, 3-oxathiolane.

As used herein, the term (+)-FTC refers to (+)-β-D-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1, 3-oxathiolane.

As used herein, the terms FTC-MP, FTC-DP, and FTC-TP refer to the monophosphate, diphosphate, and triphosphate of FTC, respectively.

As used herein, the term BCH-189 refers to 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane.

As used herein, the term "preferential enzyme catalysis" refers to catalysis by an enzyme that favors one substrate over another.

As used herein, a leaving group means a functional group that forms an incipient carbonation when it separates from the molecule that it is attached to.

The invention as disclosed herein is a method and composition for the treatment of HIV and HBV infections, and other viruses replicating in like manner, in humans or other host animals, that includes administering an effective amount of the (±)-β-D,L, the (−)-β-Lor (+)-β-Denantiomer of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane, a pharmaceutically acceptable derivative, including a 5' or N^4 alkylated or acylated derivative, or a pharmaceutically acceptable salt thereof, in a pharmaceutically acceptable carrier. As shown below, the compounds of this invention either possess antiretroviral activity, such as anti-HIV-1, anti-HIV-2 and anti-simian immunodeficiency virus (anti-SIV) activity, themselves or are metabolized to a compound that exhibits antiretroviral activity.

FTC and its pharmaceutically acceptable derivatives or salts or pharmaceutically acceptable formulations containing these compounds are useful in the prevention and treatment of HIV infections and other related conditions such as AIDS-related complex (ARC), persistent generalized lymphadenopathy (PGL), AIDS-related neurological conditions, anti-HIV antibody positive and HIV-positive conditions, Kaposi's sarcoma, thrombocytopenia purpurea and opportunistic infections. In addition, these compounds or formulations can be used prophylactically to prevent or retard the progression of clinical illness in individuals who are anti-HIV antibody or HIV-antigen positive or who have been exposed to HIV.

FTC and its pharmaceutically acceptable derivatives or pharmaceutically acceptable formulations containing these compounds are also useful in the prevention and treatment of HBV infections and other related conditions such as anti-HBV antibody positive and HBV-positive conditions, chronic liver inflammation caused by HBV, cirrhosis, acute hepatitis, fulminant hepatitis, chronic persistant hepatitis, and fatigue. These compounds or formulations can also be used prophylactically to prevent or retard the progression of clinical illness in individuals who are anti-HBV antibody or HBV-antigen positive or who have been exposed to HBV.

FTC can be converted into a pharmaceutically acceptable ester by reaction with an appropriate esterifying agent, for example, an acid halide or anhydride. FTC or its pharmaceutically acceptable derivative can be converted into a pharmaceutically acceptable salt thereof in a conventional manner, for example, by treatment with an appropriate base. The ester or salt of FTC can be converted into FTC, for example, by hydrolysis.

In summary, the present invention includes the following features:

(a) (±)-β-D,L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1, 3-oxathioiane and pharmaceutically acceptable derivatives and salts thereof;

(b) (−)-β-L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1, 3-oxathiolane and pharmaceutically acceptable derivatives and salts thereof;

(c) (+)-βD-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1, 3-oxathiolane and pharmaceutically acceptable derivatives and salts thereof;

(d) (±)-β-D,L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1, 3-oxathiolanei its (−) and (+) enantiomers, and pharmaceutically acceptable derivatives and salts thereof for use in medical therapy, for example for the treatment or prophylaxis of a HIV or HBV infection;

(e) use of (±)-β-D,L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1, 3-oxathiolane, its (−) and (+)

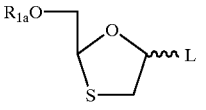
enantiomers, and pharmaceutically acceptable derivatives and salts thereof in the manufacture of a medicament for treatment of a HIV or HBV infection;

(f) pharmaceutical formulations comprising (±)-β-D,L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1, 3-oxathiolane, its (−) or (+) enantiomer, or a pharmaceutically acceptable derivative or salt thereof together with a pharmaceutically acceptable carrier or diluent;

(g) a process for the preparation of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane which comprises:

(i) reacting optionally protected 5-fluorocytosine with a 1,3-oxathiolane of formula A

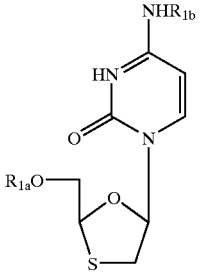
A



wherein R_{1a} is hydrogen or a hydroxyl protecting group, including an acyl group, and L is a leaving group; and optionally removing any hydroxyl protecting group.

(ii) reacting a compound of formula B

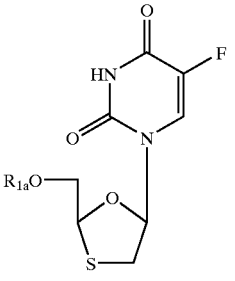
B



(wherein R_{1a} is as defined above and R_{1b} is an amino protecting group) with a fluorinating agent serving to introduce a fluorine atom in the 5-position of the cytosine ring; or

(iii) reacting a compound of formula C

C



(wherein R_{1a} is as defined above) with an agent serving to convert the oxo group in the 4-position of the uracil ring to an amino group; any remaining protecting groups being removed to produce the desired product.

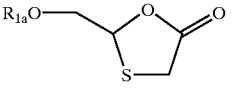
f) a process for the preparation of a (−) or (+) enantiomer of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1, 3-oxathiolane which comprises subjecting the compound or derivative (e.g. 5'-ester) thereof in the form of a mixture of (−) and (+) enantiomers to conditions or reacting with reagents serving to separate the enantiomers and if necessary converting the resulting derivative to the parent compound.

With regard to process e) (i), the hydroxy protecting group includes protecting groups described in detail below, including acyl (e.g. acetyl), arylacyl (e.g. benzoyl or substituted benzoyl), trityl or monomethoxytrityl, benzyl or substituted benzyl, trisubstituted silyl, including trialkylsilyl (e.g. dimethyl-t-butylsilyl) or diphenylmethylsilyl. The 5-fluorocytosine compound can be optionally protected with silyl, e.g., trisubstituted silyl groups. The protecting groups can be removed in a conventional manner. The leaving group L is a leaving group typical of those known in the art of nucleoside chemistry, e.g. halogen such as chlorine or bromine, alkoxy such as methoxy or ethoxy, or acyl such as acetyl or benzoyl.

The reaction in process e) (i) can be carried out in an organic solvent (e.g., 1,2-dichloroethane or acetonitrile) in the presence of a Lewis acid, preferably stannic chloride, or trimethylsilyl triflate.

Compounds of formula A (wherein L represents an acyl group, e.g., an acetyl group) can be obtained by reaction of a compound of formula D

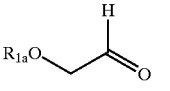
D



(wherein R_1 is defined above) with a reducing agent, e.g., a lithium aluminum hydride compound, following by treatment with the appropriate conventional reagent for the desired intermediate, for example, a carboxylic acid anhydride, e.g. acetic anhydride, for acylation, chlorinating or brominating reagents for halogenation, or alkylating reagents.

The compound of formula D can be prepared by reaction of a compound of formula E

E



with $HSCH_2CO_2H$ at an elevated temperature.

The compound of formula E can be prepared by ozonolysis of an allyl ether or ester having the formula CH_2═CH—CH_2—OR or a diether or diester of 2-butene-1,3-diol having the formula $ROCH_2$—CH═CH—CH_2OR, in which R is a protecting group, such as an alkyl, silyl, or acyl group.

With regard to process e) (ii), the 5-fluoro substituent can be introduced by methods known in the art (M. J. Robins, et al., in Nucleic Acid Chemistry, Part 2, L. B. Townsend and R. S. Tipson, editors,. J. Wiley and Sons, New York, 895–900 (19/8) and references therein; R. Duschinsky in Nucleic Acid Chemistry, Part 1, L. B. Townsend and R. S. Tipson, editors, J. Wiley and Sons, New York 43–46 (1978) and references therein). The fluorinating agent may be, for example, trimethylhypofluorite in fluorotrichloromethane.

With regard to process e) iii), the compound of formula C can be treated with 1,2,4-triazole, together with 4-chlorophenyl dichlorophosphate, to form the corresponding 4-(1,2,4-triazoylyl) compound which is then converted to the desired 4-amino (cytidine) compound by reaction with for example methanol.

The starting materials of formulas B and C can be prepared for example by reaction of an appropriate

(optionally protected) base with a compound of formula A in an analogous manner to that described in process e) i). 5-Fluorouracil and 5-fluorocytosine are commercially available from Aldrich Chemical Co., Milwaukee, Wis. 53233, USA.

Resolution of the (±)-enantiomers can be accomplished as specified in detail in Section III. below.

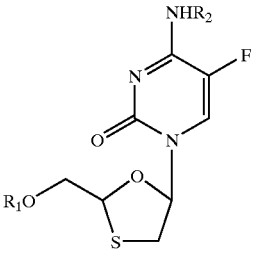
FTC can be converted into a pharmaceutically acceptable ester by reaction with an appropriate esterifying agent, for example, an acid halide or anhydride. FTC or its pharmaceutically acceptable derivative can be converted into a pharmaceutically acceptable salt thereof in a conventional manner, for example, by treatment with an appropriate base. The ester or salt of FTC can be converted into FTC, for example, by hydrolysis.

I. Active Compound, and Physiologically Acceptable Derivatives and Salts Thereof

The antivirally active compound disclosed herein is 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane (see FIG. **1**), in the racemic form or as an isolated enantiomer.

The active compound can be administered as any derivative that upon administration to the recipient, is capable of providing directly or indirectly, the parent FTC compound, or that exhibits activity itself. Nonlimiting examples are the pharmaceutically acceptable salts (alternatively referred to as "physiologically acceptable salts"), and the 5' and N^4 acylated or alkylated derivatives of the active compound (alternatively referred to as "physiologically active derivatives"). In one embodiment, the acyl group is a carboxylic acid ester in which the non-carbonyl moiety of the ester group is selected from straight, branched, or cyclic alkyl, alkoxyalkyl including methoxymethyl, aralkyl including benzyl, aryloxyalkyl such as phenoxymethyl, aryl including phenyl optionally substituted with halogen, C_1 to C_4 alkyl or C_1 to C_4 alkoxy, sulfonate esters such as alkyl or aralkyl sulphonyl including methanesulfonyl, the mono, di or triphosphate ester, trityl or monomethoxytrityl, substituted benzyl, trialkylsilyl (e.g. dimethyl-t-butylsilyl) or diphenylmethylsilyl. Aryl groups in the esters optimally comprise a phenyl group. The alkyl group can be straight, branched, or cyclic, and is optimally a C_1 to C_{18} group.

Specific examples of pharmaceutically acceptable derivatives of FTC include, but are not limited to:



wherein R_1 and R_2 are independently selected from the group consisting of alkyl and acyl, specifically including but not limited to methyl, ethyl, propyl, butyl, pentyl, hexyl, isopropyl, isobutyl, sec-butyl, t-butyl, isopentyl, amyl, t-pentyl, 3-methylbutyryl, hydrogen succinate, 3-chlorobenzoate, cyclopentyl, cyclohexyl, benzoyl, acetyl, pivaloyl, mesylate, propionyl, butyryl, valeryl, caproic, caprylic, capric, lauric, myristic, palmitic, stearic, oleic, amino acids including but not limited to alanyl, valinyl, leucinyl, isoleucinyl, prolinyl, phenylalaninyl, tryptophanyl, methioninyl, glycinyl, serinyl, threoninyl, cysteinyl, tyrosinyl, asparaginyl, glutaminyl, aspartoyl, glutaoyl, lysinyl, argininyl, and histidinyl, and wherein one of R_1 and R_2 can be H.

FTC or its derivatives can be provided in the form of pharmaceutically acceptable salts. As used herein, the term pharmaceutically acceptable salts or complexes refers to salts or complexes of FTC that retain the desired biological activity of the parent compound and exhibit minimal, if any, undesired toxicological effects. Nonlimiting examples of such salts are (a) acid addition salts formed with inorganic acids (for example, hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid, and the like), and salts formed with organic acids such as acetic acid, oxalic acid, tartaric acid, succinic acid, malic acid, ascorbic acid, benzoic acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acids, naphthalenedisulfonic acids, and polygalacturonic acid; (b) base addition salts formed with polyvalent metal cations such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium, sodium, potassium, and the like, or with an organic cation formed from N,N-dibenzylethylenediamine, ammonium, or ethylenediamine; or (c) combinations of (a) and (b); e.g., a zinc tannate salt or the like.

Modifications of the active compound, specifically at the N^4 and 5'-O positions, can affect the bioavailability and rate of metabolism of the active species, thus providing control over the delivery of the active species. Further, the modifications can affect the antiviral activity of the compound, in some cases increasing the activity over the parent compound. This can easily be assessed by preparing the derivative and testing its antiviral activity according to the methods described herein, or other method known to those skilled in the art.

II. Preparation of the Active Compounds

The racemic mixture of FTC can be prepared according to the method disclosed in detail in PCT International Publication No. WO 91/11186, published on Aug. 8, 1991, and filed by Emory University, or by the method disclosed in Example 1. In general, the method includes ozonizing either an allyl ether or ester having the formula CH_2═CH—CH_2—OR or a diether or diester of 2-butene-1,3-diol having the formula ROCH$_2$—CH═CH—CH_2OR, in which R is a protecting group, such as an alkyl, silyl, or acyl group, to form a glycoaldehyde having the formula OHC—CH_2—OR; adding thioglycolic acid to the glycoaldehyde to form a lactone of the formula 2-(R-oxy)-methyl-5-oxo-1,3-oxathiolane; reducing the lactone to various compounds containing a leaving group at the 5 position of the oxathiolane ring; coupling these compounds with silyated 5-fluorocytosine in the presence of $SnCl_4$ to form the β-isomer of FTC; and optionally removing the protecting groups.

EXAMPLE 1

Preparation of (±)-β-D,L-2-Hydroxymethyl-5-(5-fluorocytosin-1-yl)-1, 3-oxathiolane

A method for the preparation of the racemic mixture of FTC is illustrated in FIG. **2**, and described in detail below.

Protection of 2-Butene-1,4-diol

In a dry, 2L, 3-neck flask under inert atmosphere, 100 grams (93.5 ml=1.135 mol=1.00 eq.) of 2-butene-1,4-diol and 15 grams (approx. 0.1 eq.) of DMAP (4-dimethylaminopyridine) were dissolved in 800 ml of dry pyridine and stirred while cooling to 0° C. Butyryl chloride

(260 ml=2.2 eq) was then added slowly to prevent overheating and allowed to stir for one hour. The reaction was quenched with a small amount of ice water. The liquid was decanted off from the salt and evaporated in vacuo. The remaining salt was dissolved in water and the aqueous solution was extracted twice with ethyl ether. The combined other layers were washed once with saturated $CuSO_4$, twice with saturated $NaHCO_3$ containing Norit®, and then vacuum filtered through a celite® plug.

The concentrated reaction mixture was dissolved in ether and washed following the same procedure as above for the salt solution. The combined organic layers were concentrated by rotary evaporation, then placed under vacuum. This reaction is typically very close to quantitative. The scale can be easily increased as necessary. The product, 1,4-dibutyryl-2-butene-1,4-diol is a colorless to slightly yellow, clear liquid.

Ozonolysis of the Protected Diol

1,4-Dibutyryl-2-butene-1,4-diol (1.365 mol) was dissolved in 4 L of dry CH_2Cl_2 in a dry, 5 L 3-neck flask equipped with a large drying tube and an open tube for the introduction of gas. The tube is optimally not a fritted, gas bubbling tube that will clog on exposure to the concentrated solution. The solution was stirred and cooled to −78° C. while inert gas was bubbled through the solution. The gas inlet was sealed once the solution had cooled sufficiently, and the flask and stirring apparatus were moved to the ozone generator. Oxygen was bubbled through the stirring solution for at least 20 minutes while maintaining the ice bath. A Cryocool is ideal to maintain the low temperature for this lengthy reaction. The ozone was then introduced at 8 to 8.5 psi. Upon completion, the ozone flow was stopped, and oxygen was bubbled through the solution for about a half an hour before 3 equivalents of Me_2S were added. The flask was removed from the cooling bath and transported to a hood where it was stirred for about 2 days to affect complete reduction. The solution was evaporated and put under vacuum for several hours.

This reaction typically yields approximately 95% of protected aldehyde (2-butyryloxyacetaldehyde), a colorless to yellow, clear liquid.

Cyclization of the Aldehyde With Mercaptoacetic Acid

The aldehyde (1.0 equivalent) was dissolved in toluene to provide a 0.80 to 0.85M solution in a flask equipped with a Dean Stark-type trap. Thioglycolic acid (1.1 equiv.) was added and the mixture was heated to reflux. Water was azeotropically removed via the trap. The reaction was completed in 3 hours and was allowed to cool to room temperature. The organic solution was washed twice with equal volumes of sat. $NaHCO_3$ water and once with water, dried over $MgSO_4$ and Norit, and vacuum filtered through celite before being evaporated in vacuo. The first $NaHCO_3$ wash was back extracted once with ether; the ether was washed once with water, dried over $MgSO_4$ and Norit®, vacuum filtered through celite®, and evaporated along with the other organic material from the toluene solution. The combined material was placed under vacuum overnight.

The reaction typically provides a 90% yield of 2-(butyryloxy)-methyl-5-oxo-1,3-oxathiolane.

Reduction of Lactone and Conversion to the Acetate

2-Butyryloxy-methyl-5-oxo-1,3-oxathiolane (1.00 equivalent) was dissolved in dry THF to give a 0.23M solution in a dry, 3-neck flask equipped with a mechanical stirrer and maintained under an inert atmosphere. The solution was stirred and cooled to 0° C. before 1.1 equivalent of 1.0M $Li(t\text{-}BuO)_3AlH$ in THF was added via canula. The reduction was complete in approximately three hours, as indicated by TLC using 2:1 ether/hexane solvent system and an anisaldehyde stain.

Approximately 10 equivalents of freshly distilled Ac_2O were then added and allowed to stir for 2 days to provide the acetylated product. The reaction was quenched by addition of saturated $NaHCO_3$, which was stirred overnight. The solution was then evaporated and stirred with more $NaHCO_3$ solution overnight. This was extracted with ether which was washed (carefully) twice with sat. $NaHCO_3$ and once with water, dried over $MgSO_4$ and Norit®, vacuum filtered through celite®, and evaporated. The product is a dark yellow, clear liquid. Gas chromatography (Init. T - 80°; Init. time=5 min.; Prog. rate - 10°/min; Final T=240° C.) typically indicates a purity of approximately 70%.

Silylation of 5-Fluorocytosine

5-Fluorocytosine (1.05 equivalents based on amount of acetylated lactol obtained in the previous step using GC indication of purity) was silylated by reflux in at least 10 equivalents of hexamethyldisilazane containing a catalytic amount of pure ammonium sulfate (0.05 to 0.10 eq.) for two hours after the solution turned clear. The flask was then sealed tightly and the solvent removed using a vacuum pump with an auxiliary trap. The product, a white solid, was left under vacuum over night until ready for use in the following coupling reaction.

Coupling of Silylated 5-Fluorocytosine With Acetylated Lactol

To silylated 5-fluorocytosine (33.86 gm. 0.124 mol) in dry dichloromethane (350 ml) was added $SnCl_4$ solution (135.6 ml, a 1 molar solution in CH_2Cl_2) under nitrogen atmosphere. The solution was stirred for 15 minutes at room temperature. This solution was cannulated to the solution of the lactol acetate (38 gm, 0.113 mol) in dichloromethane (400 ml) under nitrogen atmosphere over a period of 30 minutes.

The reaction solution was stirred for 2 hours, at which point the completion of reaction was indicated by TLC. The reaction solution was then diluted with dichloromethane (500 ml) and quenched with ammonium hydroxide solution. The ammonium hydroxide solution (100 ml) was added slowly maintaining the temperature of reaction below 30° C., resulting in the formation of a white precipitate.

The mixture was allowed to stir for another 30 minutes, and then passed through silica gel plug column (7 inch diameter 5 inch height). It was eluted sequentially with dichloromethane (2 L), ethyl acetate (2 L) and ethyl acetate:ethanol (9:1) (4 L). The ethyl acetate and ethyl acetate:ethanol eluents contained the desired product. These solutions were combined and evaporated at reduced pressure. The residual sticky solid was then washed with dry ether (200 ml) to give a white solid (25.35 gm; 71%), FTC-5'-butyrate.

FTC-5'-butyrate (8.74 gm; 0.026 mol) was dissolved in 250 ml methanol. Sodium methoxide (2.85 gm; 0.052 gm) was added at room temperature. The reaction was stirred for 1 hour, at which point the completion of reaction was confirmed by TLC. NH_4Cl solution (10 ml) was added to quench the reaction, and then the solvent was removed under reduced pressure. The residue was absorbed on silica gel (5 gm) and passed through a small column using ethyl acetate:ethanol as an eluent (9:1). The product-containing fractions were combined and evaporated to give a sticky solid which was washed with dry ether to give white solid FTC (6.00 gm, 88%). (^1H NMR: (DMSO-d^6) 8.18 (1H, d, H_6, J=8.4 Hz), 7.81 & 7.57 (2H, broad, NH_2), 6.12 (1H, dd, $H_{1'}$, J=5.7 & 4.2 Hz), 5.40 (1H, t, OH, J=5.7 Hz), 5.17 (1H, t, $1H_{4'}$, J=3–6 Hz), 3.74 (2H, m, $2H_{5'}$), 3.41 (1H, dd, $1H_{2'}$,

J=5.7 & 11.7 Hz), 3.11 (1H, dd, $1H_{2'}$, J=4.2 & 11.7 Hz); ^{13}C NMR: (DMSO-d^6) 157.85 (d, J=13.4 Hz), 153.28, 136.12 (d, J=241 HZ), 126.01 (d, J=32.6 Hz), 86.90, 86.84, 62.48, 37.07; mp 195–196° C.

III. Resolution of Nucleoside Enantiomers

A method is provided herein for the resolution of racemic mixtures of nucleoside enantiomers, including but not limited to the (+) and (−) enantiomers of FTC. The method can also be used to resolve racemic mixtures of carbohydrates or carbohydrate-like moieties, such as derivatives of 1,3-oxathiolane and 1,3-dioxolane. The method involves the use of an enzyme that preferentially catalyzes a reaction of one enantiomer in a racemic mixture. The reacted enantiomer is separated from the unreacted enantiomer on the basis of the new difference in physical structure. Given the disclosure herein, one of skill in the art will be able to choose an enzyme that is selective for the nucleoside enantiomer of choice (or selective for the undesired enantiomer, as a method of eliminating it), by selecting one of the enzymes discussed below or by systematic evaluation of other known enzymes. Given this disclosure, one of skill in the art will also know how to modify the substrate as necessary to attain the desired resolution. Through the use of either chiral NMR shift reagents, polarimetry, or chiral HPLC, the optical enrichment of the recovered ester can be determined.

The following examples further illustrate the use of enzymes to resolve racemic mixtures of enantiomers. Other known methods of resolution of racemic mixtures can be used in combination with the method of resolution disclosed herein. All of these modifications are considered within the scope of the invention.

Resolution Based on Hydrolysis of C5'-Nucleoside Esters

In one embodiment, the method includes reacting the C5'-hydroxyl group of a mixture of nucleoside racemates with an acyl compound to form C5'-esters in which the nucleoside is in the "carbinol" end of the ester. The racemic mixture of nucleoside C5'-esters is then treated with an enzyme that preferentially cleaves, or hydrolyses, one of the enantiomers and not the other, in a given time period.

An advantage of this method is that it can be used to resolve a wide variety of nucleosides, including pyrimidine and purine nucleosides that are optionally substituted in the carbohydrate moiety or base moiety. The method can also be used to resolve nucleoside derivatives that contain additional heteroatoms in the carbohydrate moiety, for example, FTC and BCH-189. The broad applicability of this method resides in part on the fact that although the carbinol portion of the ester plays a role in the ability of an enzyme to differentiate enantiomers, the major recognition site for these enzymes is in the carboxylic acid portion of the ester. Further, one may be able to successfully extrapolate the results of one enzyme/substrate study to another, seemingly-different system, provided that the carboxylic acid portions of the two substrates are the same or substantially similar.

Another advantage of this method is that it is regioselective. Enzymes that hydrolyse esters typically do not catalyze extraneous reactions in other portions of the molecule. For example, the enzyme lipase catalyses the hydrolysis of the ester of 2-hydroxymethyl-5-oxo-1,3-oxathiolane without hydrolysing the internal lactone. This contrasts markedly with "chemical" approaches to ester hydrolysis.

Still another advantage of this method is that the separation of the unhydrolysed enantiomer and the hydrolysed enantiomer from the reaction mixture is quite simple. The unhydrolysed enantiomer is more lipophilic than the hydrolysed enantiomer and can be efficiently recovered by simple extraction with one of a wide variety of nonpolar organic solvents or solvent mixtures, including hexane and hexane/ether. The less lipophilic, more polar hydrolysed enantiomer can then be obtained by extraction with a more polar organic solvent, for example, ethyl acetate, or by lyophilization, followed by extraction with ethanol or methanol. Alcohol should be avoided during the hydrolysis because it can denature enzymes under certain conditions.

Enzymes and Substrates

With the proper matching of enzyme and substrate, conditions can be established for the isolation of either nucleoside enantiomer. The desired enantiomer can be isolated by treatment of the racemic mixture with an enzyme that hydrolyses the desired enantiomer (followed by extraction of the polar hydrolysate with a polar solvent) or by treatment with an enzyme that hydrolyses the undesired enantiomer (followed by removal of the undesired enantiomer with a nonpolar solvent).

Enzymes that catalyze the hydrolysis of esters include esterases, for example pig liver esterase, lipases, including porcine pancreatic lipase and Amano PS-800 lipase, substillisin, and α-chymotrypsin.

FIG. **3** is a flow chart of the specificity of alkaline phosphatase and snake venom phosphodiesterase for the (+) and (−) enantiomers of FTC. As indicated, alkaline phosphatase hydrolyses the triphosphate of both of the enantiomers to FTC, and therefore is not effective as a separation means. Phosphodiesterase I preferentially hydrolyses the (+)-isomer of FTC to its monoester, which can then be exposed to 5'-nucleotidase to provide (+)-FTC.

The most effective acyl group to be used to esterify the C5'-position of the nucleoside can be determined without undue experimentation by evaluation of a number of homologs using the selected enzyme system. For example, when 1, 3-oxathiolane nucleosides are esterified with butyric acid, resolutions with both pig liver esterase and Amano PS-800 proceed with high enantioselectivity (94–100% enantiomeric excess) and opposite selectivity. Pig liver esterase preferentially hydrolyses the (+)-enantiomer of FTC, and Amano PS-800® preferentially hydrolyses the (−)-enantiomer of FTC. The percent enantiomeric excess reported in Table 1 is the amount of purified butyrate ester remaining in the enzyme treated mixture (i.e., the butyrate ester of (−)-FTC in the case of PLE and the butyrate ester of (+)-FTC in the case of Amano PS-800®).

Non-limiting examples of acyl groups that can be evaluated for use with a particular nucleoside enantiomeric mixture and particular enzyme include alkyl carboxylic acids and substituted alkyl carboxylic acids, including acetic acid, propionic acid, butyric acid, and pentanoic acid. With certain enzymes, it may be preferred to use an acyl compound that is significantly electron-withdrawing to facilitate hydrolysis by weakening the ester bond. Examples of electron-withdrawing acyl groups include α-haloesters such as 2-chloropropionic acid, 2-chlorobutyric acid, and 2-chloropentanoic acid. α-Haloesters are excellent substrates for lipases.

Resolution Conditions

The enzymatic hydrolyses are typically carried out with a catalytic amount of the enzyme in an aqueous buffer that has a pH that is close to the optimum pH for the enzyme in question. As the reaction proceeds, the pH drops as a result of liberated carboxylic acid. Aqueous base should be added to maintain the pH near the optimum value for the enzyme.

The progress of the reaction can be easily determined by monitoring the change in pH and the amount of base needed to maintain pH. The hydrophobic ester (the unhydrolysed enantiomer) and the more polar alcohol (the hydrolysed enantiomer) can be sequentially and selectively extracted from the solution by the judicious choice of organic solvents. Alternatively, the material to be resolved can be passed through a column that contains the enzyme immobilized on a solid support.

Enzymatic hydrolyses performed under heterogeneous conditions can suffer from poor reproducibility. Therefore, it is preferred that the hydrolysis be performed under homogeneous conditions. Alcohol solvents are not preferred because they can denature the enzymes. Homogeneity can be achieved through the use of non-ionic surfactants, such as Triton X-100. However, addition of these surfactants not only assists in dissolving the starting material, they also enhance the aqueous solubility of the product. Therefore, although the enzymatic reaction can proceed more effectively with the addition of a non-ionic surfactant than under heterogeneous conditions, the isolation of both the recovered starting material and the product can be made more difficult. The product can be isolated with appropriate chromatographic and chemical (e.g., selective salt formation) techniques. Diacylated nucleosides can be used but are often quite lipophilic and hard to dissolve in the medium used.

EXAMPLE 2

Enantioselective Lipase-Catalyzed Hydrolysis of FTC Esters

A number of 5'-O-acyl derivatives of FTC were prepared by selective O-acylation of the N-hydrochloride salt (see Table 1 and FIG. **4**) of (±)-FTC. The efficiency of the hydrolysis of the derivatives by lipases was investigated. As shown in Table 1, pig liver esterase (PLE) exhibits a high level of selectivity for the hydrolysis of the ester of the (+)-enantiomer of FTC, leaving predominately the butyrate of (−)-FTC in the HPLC-analyzed mixture. In contrast, PS-800 hydrolyses the ester of the (−)-enantiomer of FTC preferentially, leaving predominately the butyrate of the (+)-FTC in the HPLC-analyzed mixture. The rate of the hydrolysis was also found to be dependent on the nature of the acyl group; the acetyl derivative was significantly slower than the butyryl derivative. It has now been discovered that the rate of the hydrolysis of the propionic acid ester of FTC is even faster than that observed for the butyrate derivative. Percent recovery and percent of enantiomeric excess were both determined using HPLC. Although the enantioselectivity is excellent when employing PLE (typically 97% e.e. or higher), additional enrichment can be accomplished.by sequential enzymatic hydrolysis reactions in which the enantiomerically-enriched butyrate from a PLE-catalyzed hydrolysis is subjected to enzymatic hydrolysis by PS-800.

TABLE 1

Comparison of Effect of Ester on Enzyme Hydrolysis

Substrate	% Recovery	% E.E. (s.m.)
FTC Esters with PLE:		(−) −FTC
		(butyrate)
acetate	32.68	N.D.
propionate	39.87	N.D.
butyrate	48.00	98
butyrate	45.71	98.6
FTC Esters with PS800:		(+) −FTC
		butyrate
acetate	73.17	N.D.
propionate	52.67	N.D.
butyrate	58.34	N.D.
valerate	41.50	94

EXAMPLE 3

Procedure for the Preparation of (+)- and (−)-FTC via Enantioselective, Lipase-Catalyzed Hydrolysis of FTC Butyrate

The 5'-O-butyrate of (±)-FTC (0.47 mmol, 149 mg) was dissolved in 16 mL of a solution of 4:1 pH 8 buffer:CH_3CN. The clear solution was stirred and treated with 26 mg of pig liver esterase (PLE-A). The progress of the reaction was monitored by HPLC (FIG. **4**). After 20 hours (52% conversion), the reaction mixture was extracted with 2×80 mL of $CHCl_3$ and 80 mL of ethyl acetate. The organic layer extracts were combined, dried over anhydrous $MgSO_4$, filtered, and concentrated by rotary evaporation. The resulting residue was eluted on 2×1000 m pTLC plates using ethyl acetate as eluant (double elution) to give, after isolation, 53 mg (36% based on starting material) of FTC butyrate which was determined to have 98% enantiomeric excess (e.e.) by HPLC analysis. The enantiomerically-enriched butyrate was then treated with 1.6 mL of methanol followed by 0.38 mmol (20 mg) of sodium methoxide. The resulting mixture was stirred at room temperature, and the progress of the reaction was monitored by HPLC. The reaction was completed within 30 minutes. The solvent was removed by rotary evaporation to give a crude white solid (76 mg) that was eluted on a 1000 m PTLC using 5:1 ethyl acetate:ethanol. (−)-FTC was isolated as a white solid (33 mg; 82% yield). HPLC analysis of the FTC as its 5'-O-acetate derivative showed 97% e.e.; $[\alpha]$ (^{20},$_D$) −120.5° (c=0.88; abs. ethanol).

Emulsions in the work-up step can be avoided by adding $HCCl_3$ to the reaction mixture on completion (which also serves to denature the enzyme), stripping the solvents under vacuum, and then extracting with $HCCl_3$.

Similarly, 1.2 mmol (375 mg) of the 5'-O-butyrate of (±)-FTC was dissolved in 40 mL of 4:1 pH 8 buffer-CH_3CN. The clear solution was stirred and treated with 58 mg of pig liver esterase (PLE-A). The progress of the reaction was monitored by HPLC. After 90 minutes (38% conversion), the reaction mixture was added to 150 mL of $CHCl_3$. The layers were separated and the aqueous layer lyophilized to remove solvent. The white residue from the lyophilization was extracted with 3×10 mL of absolute ethanol. The extracts were filtered, combined, and concentrated in vacuo to yield 179 mg of crude oil. The crude material was eluted on a 45×30 mm column of silica gel using 3×75 mL of ethyl acetate followed by 5:1 ethyl acetate-ethanol. (+)-FTC was isolated as a white solid (109 mg; 37% based on starting butyrate). HPLC analysis of the (+)-FTC as its 5'-O-acetate derivative showed 97.4% e.e.; [a]O(^{20},$_D$) +113.4° (c=2.53; absolute ethanol)

A similar reaction was performed using 0.12 mmol (37 mg) of the 5'-O-butyrate of FTC and 7 mg of PS-800 in 4.0 mL of 4:1 pH 8 buffer:CH_3CN. The reaction was considerably slower than that with PLE-A and required 74 hours for 59% conversion. The recovered butyrate (11.4 mg; 31% of the initial amount) was found to exhibit 94% e.e. by HPLC.

Resolution of Nucleoside Enantiomers With Cytidine-Deoxycytidine Deaminase

In an alternative embodiment, cytidine-deoxycytidine deaminase is used to resolve racemic mixtures of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane and its derivatives, including 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1, 3-oxathiolane. The enzyme catalyses the deamination of the cytosine moiety to a uracil. It has been discovered that one of the enantiomers of 1,3-oxathiolane nucleosides is a preferred substrate for cytidine-deoxycytidine deaminase. The enantiomer that is not converted to a uracil derivative (and therefore is still basic) is extracted from the solution with an acidic solution. Care should be taken to avoid strong acidic solutions (pH below 3.0), that may cleave the oxathiolane ring.

Cytidine-deoxycytidine deaminase can be isolated from rat liver or human liver, or expressed from recombinant sequences in a procaryotic system such as in *E. coli.*

The method of resolution of cytidine nucleoside enantiomers using cytidine-deoxycytidine deaminase can be used as the sole method of resolution or can be used in combination with other methods of resolution, including resolution by enzymatic hydrolysis of 5'-O-nucleoside esters as described above.

Combination of Enzymatic Resolution With Classical Resolution Methods

The process described above for resolving racemic mixtures of nucleoside enantiomers can be combined with other classical methods of enantiomeric resolution to increase the optical purity of the final product.

Classical methods of resolution include a variety of physical and chemical techniques. Often the simplest and most efficient technique is recrystallization, based on the principle that racemates are often more soluble than the corresponding individual enantiomers. Recrystallization can be performed at any stage, including on the acylated compounds or the final enantiomeric product. If successful, this simple approach represents a method of choice.

When recrystallization fails to provide material of acceptable optical purity, other methods can be evaluated. If the nucleoside is basic (for example, a cytidine) one can use chiral acids that form diastereomeric mixtures that may possess significantly different solubility properties. Nonlimiting examples of chiral acids include malic acid, mandelic acid, dibenzoyl tartaric acid, 3-bromocamphor-8-sulfonic acid, 10-camphorsulfonic acid, and di-p-toluoyltartaric acid. Similarly, acylation of the free hydroxyl group with a chiral acid derivative also results in the formation of diastereomeric mixtures whose physical properties may differ sufficiently to permit separation.

Small amounts of enantiomerically enriched nucleosides can be obtained or purified by passing the racemic mixture through an HPLC column that has been designed for chiral separations, including cyclodextrin bonded columns marketed by Rainin Corporation.

EXAMPLE 4

Separation of Racemic Mixtures of Nucleosides by HPLC

The resolutions of the C4'-enantiomers of (±)-FTC were performed using a chiral cyclodextrin bonded (cyclobond AC-I) column obtained from Rainin Corporation (Woburn, Mass.). The conditions were as follows: Isocratic 0.5% methanol in water; flow rate 1 ml/min., UV detection at 262 nm. HPLC grade methanol was obtained from J. T. Baker (Phillipsburg, N.J.). The racemic mixtures were injected and fractions were collected. Fractions containing each of the enantiomers were pooled, frozen, and then lyophilized. The compounds were characterized by UV spectroscopy and by their retention times on HPLC. In general, the (−)-enantiomers have lower retention times than the (+)-enantiomers (see *J. Liouid Chromatography* 7:353–376, 1984). The concentrations of the compounds were determined by UV spectroscopy, using a stock solution of known concentration (15 μM) prepared in water for biological evaluation. The retention times for the separated enantiomers are provided in Table 2.

TABLE 2

Retention Times of Enantiomers of FTC

Compound	R_f (min)
(−) −FTC	8.3
(+) −FTC	8.7

EXAMPLE 5

Alternative Methods for Separating FTC Enantiomers Using a Chiral Column

Using a Cyclobond I-Ac column (5 μm, 25 cm×4.6 mm, Rainin Corporation, Woburn, Mass., catalog no. AST-41049), with a flow rate of 0.6 ml/min of 0.5% isocratic methanol (Fisher Scientific, Inc. HPLC grade, cat no. A-452-4 in water), and UV detection at 262 nm, the FTC enantiomers exhibited retention times of 12.68 minutes ((−)-FTC) and 13.20 minutes ((+)-FTC).

Using a Chiralpak AS column (10 μm, 25 cm×4.6 mm, J. T. Baker Inc., Phillisburg, N.J., catalog no. 7406-00, serial no. 09-29-10320) with a flow rate of 0.8 ml/min of isopropyl alcohol (HPLC grade, Fisher Scientific, Inc., cat no. A-451-4) and UV detection of 262 nm, the FTC enantiomers exhibited retention times of 5.9 minutes ((−)-FTC), and 9.8 minutes ((+)-FTC)

IV. Ability of 2-Hydroxymethyl-5-(5-fluorocytosin-1-yl)-1, 3-oxathiolane ("FTC") to Inhibit the Replication of HIV

It is often desirable to screen a number of racemic mixtures of nucleosides as a preliminary step to determine which warrant further resolution into enantiomerically enriched components and further evaluation of antiviral activity. The ability of nucleosides to inhibit HIV can be measured by various experimental techniques. The technique used herein, and described in detail below, measures the inhibition of viral replication in phytohemagglutinin (PHA) stimulated human peripheral blood mononuclear (PBM) cells infected with HIV-1 (strain LAV). The amount of virus produced is determined by measuring the virus-coded reverse transcriptase enzyme. The amount of enzyme produced is proportional to the amount of virus produced. Table 3 provides the EC_{50} values (concentration of nucleo

side that inhibits the replication of the virus by 50% in PBM cells, estimated 10% error factor) and IC_{50} values (concentration of nucleoside that inhibits 50% of the growth of mitogen-stimulated uninfected human PBM cells) of a number of (±)-1,3-oxathiolane and nucleosides.

EXAMPLE 6

Anti-HIV Activity of (±)-1,3-Oxathiolans Nucleosides

A. Three-day-old phytohemagglutinin-stimulated PBM cells (10^6 cells/ml) from hepatitis B and HIV-1 seronegative healthy donors were infected with HIV-1 (strain LAV) at a concentration of about 100 times the 50% tissue culture infectious dose (TICD 50) per ml and cultured in the presence and absence of various concentrations of antiviral compounds.

B. Approximately one hour after infection, the medium, with the compound to be tested (2 times the final concentration in medium) or without compound, was added to the flasks (5 ml; final volume 10 ml). AZT was used as a positive control.

C. The cells were exposed to the virus (about 2×10^5 dpm/ml, as determined by reverse transcriptase assay) and then placed in a CO_2 incubator. HIV-1 (strain LAV) was obtained from the Center for Disease Control, Atlanta, Ga. The methods used for culturing the PBM cells, harvesting the virus and determining the reverse transcriptase activity were those described by McDougal et al. (*J. Immun. Meth.* 76, 171–183, 1985) and Spira et al. (*J. Clin. Meth.* 25, 97–99, 1987), except that fungizone was not included in the medium (see Schinazi, et al., *Antimicrob. Agents Chemother.* 32, 1784–1787 (1988); Id., 34:1061–1067 (1990)).

D. On day 6, the cells and supernatant were transferred to a 15 ml tube and centrifuged at about 900 g for 10 minutes. Five ml of supernatant were removed and the virus was concentrated by centrifugation at 40,000 rpm for 30 minutes (Beckman 70.1 Ti rotor). The solubilized virus pellet was processed for determination of the levels of reverse transcriptase. Results are expressed in dpm/ml of sampled supernatant. Virus from smaller volumes of supernatant (1 ml) can also be concentrated by centrifugation prior to solubilization and determination of reverse transcriptase levels.

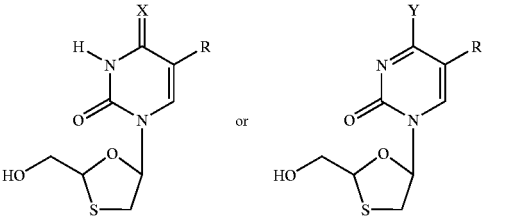
The median effective (EC_{50}) concentration was determined by the median effect method (*Antimicrob. Agents Chemother.* 30, 491–498 (1986). Briefly, the percent inhibition of virus, as determined from measurements of reverse transcriptase, is plotted versus the micromolar concentration of compound. The EC_{50} is the concentration of compound at which there is a 50% inhibition of viral growth.

E. Mitogen stimulated uninfected human PBM cells (3.8×10^5 cells/ml) were cultured in the presence and absence of drug under similar conditions as those used for the antiviral assay described above. The cells were counted after 6 days using a hemacytometer and the trypan blue exclusion method, as described by Schinazi et al., *Antimicrobial Agents and Chemotherapy*, 22(3), 499 (1982). The IC_{50} is the concentration of compound which inhibits 50% of normal cell growth.

TABLE 3

EC_{50} and IC_{50} of Various Analogues of 1,3-Oxathiolane Nucleosides in Human PBM Cells

			Antiviral Cytotoxicity	
Code	X or Y	R	EC_{50}, μM	IC_{50}, μM
DLS-009	X = O	H	>100	>100
DLS-010	X = O	Me	64.4	>100
DLS-027	X = O	F	>100	>100
DLS-028	X = O	Cl	60.8	>100
DLS-044	X = O	Br	>100	>100
DLS-029	X = O	I	>100	>100
DLS-020	Y = NH_2	H	0.02	>100
DLS-011	Y = NH_2	Me	>10	>100
DLS-022	Y = NH_2	F	0.01	>100
DLS-023	Y = NH_2	Cl	38.7	>100
DLS-021	Y = NH_2	Br	77.4	>100
DLS-026	Y = NH_2	I	0.72	>100
DLS-058(−)	Y = NH_2	F	0.008	>100
DLS-059(+)	Y = NH_2	F	0.84	>100
DLS-053	Y = NH_2	CF_3	60.7	>100



As indicated in Table 3, in general, the substituted cytosine 1,3-oxathiolane nucleosides are more active than the corresponding uracil nucleosides. The error in EC_{50} and IC_{50} measurements are estimated at ±10%.

One of the compounds, (±)-FTC, (referred to as "DLS-022", compound 8) not only exhibits exceptional activity (approximately 10 nM in PBM cells), but also quite low toxicity (>100 μM in PBM, Vero and CEM cells). Further, the (−)-enantiomer of FTC (DLS-058), exhibits significantly greater activity than the racemic mixture.

The IC_{50} of (±)-FTC was over 100 μM, indicating that the compound was not toxic in uninfected PBM cells evaluated up to 100 μM.

EXAMPLE 7

Antiviral Activity of the Enantiomers of FTC Resolved by UPLC

The enantiomers of FTC were isolated by the method of Example 4, and the antiviral activity evaluated by the method of Example 6. The results are provided in Table 4, and illustrated in FIG. **5**.

TABLE 4

Antiviral Activity of the (+) and (−) Enantiomers of FTC

Treatment	Concn., μM	DPM/ml	% Inhibition (Corrected)	EC_{50}: μM
FTC (±)	0.0001	73,755	26.6	0.018
	0.005	83,005	16.3	
	0.01	60,465	41.3	
	0.05	34,120	70.4	
	0.1	14,160	92.4	
	0.5	18,095	88.1	
	1	7,555	99.7	
	5	7,940	99.3	
	10	5,810	101.7	
FTC (−)	0.001	76,275	23.8	0.02

TABLE 4-continued

Antiviral Activity of the (+) and (−) Enantiomers of FTC

Treatment Concn., μM	DPM/ml	% Inhibition (Corrected)	EC_{50}: μM
0.005	58,590	43.3	
0.01	75,350	24.8	
0.05	28,890	76.2	
0.1	13,175	93.5	
0.5	9,485	97.6	
FTC (+) 0.001	94,340	3.8	0.28
0.005	107,430	−10.6	
0.01	99,465	−1.8	
0.05	87,120	11.8	
0.1	86,340	12.7	
0.5	33,225	71.4	

As indicated in Table 4, in this experiment the (−)-enantiomer of FTC appears to be approximately one order of magnitude more potent than the (+)-FTC enantiomer, and has approximately the same anti-HIV activity as the racemic mixture. Neither the enantiomers nor the racemic mixture is toxic up to 100 μM as measured by the Trypan Blue exclusion method in human PBM cells.

EXAMPLE 8

Antiviral Activity of FTC Enantiomers Resolved by Method of Example 3

The enantiomers of (±)-FTC were also resolved by the method of Example 3, and the antiviral activity evaluated by the method of Example 6. The results are illustrated in FIG. **6**. As indicated in FIG. **6**, the EC_{50} of the racemic mixture of FTC was 0.017 μM, the EC_{50} of (−)-FTC at 0.0077 4μM, and the EC_{50} of (+)-FTC at 0.84 μM.

EXAMPLE 9

Uptake of (±)-FTC Into Human PBX Cells

Studies were undertaken using radiolabeled FTC to follow the intracellular profiles of the parent drug and metabolites detected within the cell. All studies were conducted in duplicate. Human peripheral blood mononuclear cells (PBM cells) were suspended in RPMI 1640 medium containing 10% fetal calf serum and antibiotics (2×10^6 cells/ml), 10 ml per timepoint) and incubated with addition of 10 μM FTC (specific activity about 700 dpm/pmol). Cells were exposed to the drug for 2, 6, 12, and 24 hours. At these timepoints, the medium was removed and the cells were washed two times with cold Hank's balanced salt solution. Extraction was performed with addition of 0.2 ml of 60% cold methanol/water and stored overnight at −70° C. The following morning, the suspensions were centrifuged and extractions were repeated two times for 0.5 hours at −700° C. The total supernatants (0.6 ml) were lyophilized to dryness. The residues were resuspended in 250 μl of water and aliquots of between 50 and 100 μl were analyzed by HPLC. Quantitation of intracellular parent drug and metabolic derivatives were conducted by HPLC. Because of the potential acid lability of some compounds, a buffer system close to physiological pH was used for the separation of the metabolites.

FIG. **7** is a graph of the presence (uptake) of tritiated (±)-FTC in human PBM cells (average of two determinations) in time (hours) versus pmol/10^6 cells. The uptake studies indicate that radiolabeled FTC is readily taken up in human lymphocytes, that produce very large amounts of the 5'-triphosphate derivative of FTC.

EXAMPLE 10

Antiretroviral Activity of PTC in Various Cell Lines

The antiretroviral activity of FTC was measured in a number of cell lines using procedures similar, but not identical, to that set out in Example 6. Cell lines were obtained from either human donors, AIDS Research and Reference Reagent Program, NIH, Rockville, Md., ATCC, or the Red Cross. The CEM thymidine kinase deficient cells were prepared by sequential passage of CEM cells in the presence of 5-bromo-2'-deoxyuridine. The results are provided in Table 5.

TABLE 5

Antiretroviral Activity of FTC In Different Cell Systems

Cell system (Virus strain)	EC_{50} (μM) (+) −FTC
HIV-1	
PBMC (LAV-1)	0.027
MT2 ($HTLV_{IIIB}$)	0.89
CEM (LAV-1)	0.08
CEM-$TK^{(-)}$ (LAV-1)	0.026
CEM ($HTLV_{IIIB}$) NIH	0.09
HIV-2	
PBMC (ROD2)	0.0038 (±) −FTC
	0.0007 (−) −FTC
	0.026 (+) −FTC
SIV	
AA-2 (SIV251)	4.6
C-8166 (SIV251)	<8.0
FIV	
CrFK (61E)	≦1

EXAMPLE 11

Egress of (±)-FTC, from Human PBM Cells

Studies were performed using radiolabeled FTC to follow the intracellular profiles of the parent drug and metabolites detected within the cell after incubation in media with drug for 24 hours, and then removal of drug. This study measures the time needed for intracellular levels of triphosphates to decline. Studies were conducted in duplicate. Uninfected cells (2×10^6 ml) were suspended in the appropriate medium supplemented with serum (10 ml per timepoint) and incubated at 37° C. in a 5% CO_2 incubator. The radiolabeled FTC concentration was 10 μM. After pulsing the cells with the labeled compound for 24 hours, the cells were thoroughly washed and then replenished with fresh medium without the antiviral drugs (0 hr). At 0, 2, 4, 6, 12, 24, and 48 hours (second incubation time), the cells were removed, and immediately extracted with 60% cold methanol/water. The extract was obtained by centrifugation and removal of the cell pellet. The extracts were lyophilized and then stored at −70° C. Prior to analysis, the material was resuspended in 250 microliters of HPLC buffer and immediately analyzed. Quantitation of intracellular parent drug and metabolic derivatives was conducted by HPLC, using either a Micromeritics or Hewlett-Packard model 1090 PHLC system with an anion exchange Partisil 10 SAX column (Whatman, Inc.), at a flow rate of 1 ml/min, 1 kpsi pressure, with UV detection at 262 nm. The mobile phase consisted of

deionized water (A), 2 mM NaH_2PO_4/16 mM NaOAc (pH=6.6) (B), 15 mM NaH_2PO_4/120.2 mM NaOAc (pH=6.6) (C), and 100 mM NaH_2PO_4/800 mM NaOAc (pH=6.6) (D).

Separation method: isocratic for 5 minutes with A, followed by a 15 minute linear gradient to 100% B, followed by a 20 minute linear gradient to 100% C, followed by 10 minute linear gradient to 100% D, followed by 30 minutes isocratic with 100% D.

Retention times (minutes) in Human Cells:

Compound	Unchanged	Mono-phosphate	Diphosphate	Triphosphate
(±) –FTC	5.0	39.0	55.0	68.0

FIG. **8** is a graph of the egress of radiolabeled (±)-FTC from human PBM cells, measured in hours after drug removal versus concentration (pmol/10^6 cells). As indicated in the FIG., FTC-triphosphate has an intracellular half-life of approximately 12 hours and can be easily detected intracellularly at concentrations of 1–5 μM 48 hours after the removal of the extracellular drug, which is well above the EC_{50} for the compound. Further, the affinity (K^I) for (±)-FTC triphosphate using HIV RT is 0.2 μM, which is below the 48 hour concentration level.

EXAMPLE 12

Anti-HIV Activity of Pharmaceutically Acceptable Derivatives of (±)-PTC

a. A number of pharmaceutically acceptable derivatives of (±)-FTC prepared by derivatizing the 5'and N^4 positions were evaluated for anti-HIV activity in PBM cells using a procedure similar to that described in Example 6. The results are as follows. The 5'-O-butyrate ester of (±)-FTC exhibited an EC_{50} of 0.0017. The N^4-acetyl derivative of (±)-FTC exhibited an EC_{50} of 0.0028. The 5'-O-butyrate, N^4-ester of (±)-FTC exhibited an EC_{50} =0.0058.

b. The anti-HIV activity of the 5'-O-butyrate ester of (±)-FTC in the MT4 system (EC_{50}) was 0.04 μM. In the same assay, the unacylated (±)-FTC exhibited an IC50 of 0.52 μM. The IC50 for AZT in this system was 0.09 μM.

V. Ability of FTC to Inhibit the Replication of HBV

EXAMPLE 13

Evaluation of Activity of (+) and (−)-Enantiomers of FTC in 2.2.15 Cell Cultures

The ability of the enantiomers of FTC to inhibit the growth of virus in 2.2.15 cell cultures (HepG2 cells transformed with hepatitis virion) is described in detail below.

A summary and description of the assay for antiviral effects in this culture system and the analysis of HBV DNA has been described (Korba and Milman, 1991, *Antiviral Res.*, 15:217). The antiviral evaluations were performed on two separate passages of cells. All wells, in all plates, were seeded at the same density and at the same time.

Assay Parameters

Due to the inherent variations in the levels of both intracellular and extracellular HBV DNA, only depressions greater than 3.5-fold (for HBV virion DNA) or 3.0-fold (for HBV DNA replication intermediates) from the average levels for these HBV DNA forms in untreated cells are considered to be statistically significant [P<0.05]. The levels of integrated HBV DNA in each cellular DNA preparation (which remain constant on a per cell basis in these experiments) were used to calculate the levels of intracellular HBV DNA forms, thereby ensuring that equal amounts of cellular DNA were compared between separate samples.

Typical values for extracellular HBV virion DNA in untreated cells ranged from 50 to 150 pg/ml culture medium (average of approximately 76 pg/ml). Intracellular HBV DNA replication intermediates in untreated cells ranged from 50 to 100 pg/μg cell DNA (average approximately 74 pg/μg cell DNA). In general, depressions in the levels of intracellular HBV DNA due to treatment with antiviral compounds are less pronounced, and occur more slowly, than depressions in the levels of HBV virion DNA (Korba and Milman, 1991, *Antiviral Res.*, 15:217).

The manner in which the hybridization analyses were performed for these experiments resulted in an equivalence of approximately 1.0 pg of intracellular HBV DNA to 2–3 genomic copies per cell and 1.0 pg/ml of extracellular HBV DNA to 3×10^5 viral particles/ml.

Toxicity Analysis

Toxicity analyses were performed to assess whether any observed antiviral effects were due to a general effect on cell viability. The method used herein was the measurement of the uptake of neutral red dye, a standard and widely used assay for cell viability in a variety of virus-host systems, including HSV and HIV. Toxicity analyses were performed in 96-well flat bottomed tissue culture plates. Cells for the toxicity analyses were cultured and treated with test compounds with the same schedule as described for the antiviral evaluations below. Each compound was tested at 4 concentrations, each in triplicate cultures (wells "A", "B", and "C"). Uptake of neutral red dye was used to determine the relative level of toxicity. The absorbance of internalized dye at 510 nm (A_{sin}) was used for the quantitative analysis. Values are presented as a percentage of the average A_{sin} values in 9 separate cultures of untreated cells maintained on the same 96-well plate as the test compounds. Dye uptake in the 9 control cultures on plate 5 ranged from 91.6% to 110.4%, and on plate 6 from 96.6% to 109%. The results are provided in Table 6.

TABLE 6

Toxicity Analysis of Test Compounds in 2.2.15 Cells

PLATE	COMPOUND	CONC. (μM)	DYE UPTAKE (% OF CONTROL) WELL A	WELL B	WELL C
5	DMSO	10.0*	0.7	1.6	0.9
		3.3	55.9	68.7	61.7
		1.0	91.2	96.4	106.8
		0.3	98.7	102.9	93.5
6	(−) –FTC	300	53.0	51.1	51.5
		100	64.1	66.6	77.6
		30	98.7	94.3	96.4
		10	94.3	94.9	92.2
6	(+) –FTC	300	43.4	56.7	58.5
		100	77.7	66.3	72.1
		30	81.1	88.3	88.1
		10	90.9	99.4	90.5

*For DMSO, concentrations are presented as percent of original stock solution.

Toxicity Evaluation

As indicated in Table 6, no significant toxicity (greater than 50% depression of the dye uptake levels observed in untreated cells) was observed for the test compounds at the concentrations used for the antiviral evaluations. Both test compounds, (−)-FTC and (+)-FTC, appeared to be toxic at the highest concentration used for the toxicity tests (330 μM).

Antiviral Evaluations

Controls

Within normal variations, levels of HBV virion DNA and intracellular HBV replication intermediates [HBV RI] remained constant in the untreated cells over the challenge period. DMSO, at a concentration of 1%, did not affect the levels of HBV replication in 2.2.15 cell cultures.

Test Compounds

As indicated in Table 7, both (−)-FTC and (+)-FTC significantly inhibited the replication of HBV at the tested levels. As indicated in Table 8, (−)-FTC still significantly inhibits the synthesis of HBV virion DNA and intracellular HBV DNA at concentrations of 4, 1, and 0.25 μM.

TABLE 7

Effect of Test Compounds on HBV Production In 2.2.15 Cell Cultures

		HBV Virion DNA* (pg/ml Culture Medium)			Intracellular HBV DNA (pg/ug Cell DNA)	
WELL	TREATMENT	DAY 0	DAY 4	DAY 9	MONO.	RI
7A	Untreated Cells	59	75	94	2.7	93
7B	Untreated Cells	47	64	88	2.5	93
8A	Untreated Cells	65	100	71	2.2	97
8B	Untreated Cells	77	65	110	2.4	62
7K	DMSO @ 1.00%	100	50	48	1.9	95
7L	DMSO @ 1.00%	48	96	54	2.8	98
8K	DMSO @ 1.00%	93	63	68	2.2	86
8L	DMSO @ 1.00%	66	57	59	1.6	97
9U	(−) −FTC @ 10 μM	120	36	1	1.1	14
9V	(−) −FTC 10 μM	89	48	1	1.5	19
10U	(−) −FTC 10 μM	58	41	0.1	1.9	13
10V	(−) −FTC 10 μM	110	32	0.1	1.2	16
9W	(+) −FTC @ 10 μM	88	42	0.1	0.8	14
9X	(+) −FTC 10 μM	58	57	0.2	0.4	19
10W	(+) −FTC 10 μM	69	55	0.1	0.7	17
10X	(+) −FTC 10 μM	45	39	0.1	0.4	15

*Sensitivity cutoff for HBV virion DNA was 0.1 pg/ml.

@ Intracellular HBV DNA was analyzed 24 hours following the 9th day of treatment. The levels of integrated HBV DNA in each cell DNA preparation were used to calculate the levels of episomal 3.2 Kb HBV genomes (MONO.) and HBV DNA replication intermediates (RI).

TABLE 8

Effect of Test Compounds on KBV Production in 2.2.15 Cell Cultures

		HBV VIRION DNA* (pg/ml CULTURE MEDIUM)			INTRACELLULAR HBV DNA* (pg/μg CELL DNA)	
WELL	TREATMENT	DAY 0	DAY 4	DAY 9	MONO.	RI
31A	untreated cells	64	54	65	2.8	65
31B	"	51	54	77	2.0	53
32A	"	100	76	56	3.5	81
32B	"	53	97	83	3.1	68
35A	(−) −FTC @ 4 μM	74	27	>0.1	1.4	1
35B	"	87	28	>0.1	0.5	1
36A	"	120	20	1	0.9	1
36B	"	59	16	0.2	0.2	2
35C	(−) −FTC @ 1 μM	70	13	>0.1	1.7	2
35D	"	62	15	>0.1	1.2	3
36C	"	60	22	1	1.4	2
36D	"	89	28	0.3	1.5	4
35E	(−) −FTC @ 0.25 μM	84	15	>0.1	1.5	4
35F	"	89	16	4	2.2	4
36E	"	66	13	1	1.8	8
36F	"	49	19	0.1	0.3	9

*Sensitivity cutoff for HBV virion DNA was 0.1 pg/ml.

+ Analysis of intracellular HBV DNA was 24 hours following the 9th day of treatment. The levels of integrated HBV DNA in each cell DNA preparation were used to calculate the levels of episomal 3.2 kb HBV genomes (MONO.) and HBV DNA replication intermediates (RI).

EXAMPLE 14

Uptake of (±)-FTC into Human Liver Cells; HVB Activity of FTC

The procedure of Example 9 was repeated with human liver cells (HepG2 cells, available from the ATCC) to determine the uptake and metabolism of FTC in these cells. As shown in FIG. **9**, (±)-FTC is taken up by HepG2 cells in large amounts. These human liver cells metabolize a large percentage of the (±)-FTC to (±)-FTC triphosphate.

This data, in conjunction with other data provided herein, indicate that (±)-FTC, as well as its (−) and (+) enantiomers, are phosphorylated in liver cells. These cells can be transformed with hepatitis B virus.

EXAMPLE 15

Egress of FTC in Human HepG2 Cells

FIG. **10** illustrates the egress of [^{3}H]-(±)-FTC and its phosphorylated derivatives in human HepG2 in pmol/10^6 cells over time cells after pulsing cells with 10 μM [^{3}H]-(±)-FTC (700 DPM/pmole) for 24 hours, and evaluating the concentration of compound 24 hours after removal.

FIG. **11** illustrates the decrease in the combined concentration of [^{3}H]-(±)-FTC and its phosphorylated derivatives from human HepG2 cells after incubation with 10 μM [^{3}H]-(±)-FTC (700 DPM/pmole) for 24 hours, in pmol/10^6 cells over time.

As illustrated, even at 48 hours, over 1 μM of active compound (which is significantly higher than the EC_{50} for the compound) is still present in the cells.

V. Toxicity in Granulocyte-Macrophage Precursor Cells

EXAMPLE 16

Effect of FTC on Colony Formation of Granulocyte-Macrophage Precursor Cells

FIG. **12** is a graph of the effect of the (−) and (+) enantiomers of FTC on colony formation of granulocytes-macrophage precursor cells, as measured in percent survival versus concentration in μM ((−)-FTC, open circle; (+)-FTC, darkened circle; AZT, darkened square. As indicated, the (−)-enantiomer of FTC appears to be less toxic i.e., have a higher IC_{50}, than either the (+)-enantiomer or AZT in this cell line.

VI. Pharmacokinetics of FTC

EXAMPLE 17

Metabolism of FTC on Administration to Rats

(±)-FTC was administered intravenously at dosages of 10, 50 and 100 mg/kg to rats, and the area under the plasma drug concentration versus time (AUC), total clearance (CL_T), steady-state volume of distribution (V_{SS}), mean residence time (MRT) and half-life ($t_{1/2}$), evaluated. The results are provided in Table 9.

TABLE 9

Pharmacokinetic Parameters of FTC After Intravenous Administration of 10, 50, 100 mg/kg to Rats*

Dose mg/kg	AUC mg h/L	CL_T L/h/kg	V_{SS} L/kg	MRT h	$t_{1/2}$ h
10	9.65	0.988	0.758	0.768	0.757
50	57.11	0.874	0.699	0.800	0.815
100	120.72	0.830	0.663	0.798	0.969

*AUC = area under the plasma drug concentration versus time curve;
CL = total clearance;
V_{SS} = steady-state volume of distribution;
MRT = mean residence time;
and $t_{1/2}$ = half-life.

EXAMPLE 18

Pharmacokinetic Parameters for FTC After Intravenous and Oral Administration of FTC

Model-independent pharmacokinetic parameters were derived for (±)-FTC by administration (intravenous (I.V.) and oral (P.O.)) of 33.3 mg/kg to Rhesus Monkeys. The results are provided in Table 10. Importantly, the mean bioavailability of the compound in monkeys was 73% (±6).

TABLE 10

Model-Independent Pharmacokinetic Parameters Derived for FTC After Intravenous (I.V.) or Oral (P.O.) Administration of 33.3 mg/kg to Rhesus Monkeys*

Monkey	AUC mg h/L	CL_T L/h/kg	V_{SS} L/kg	MRT h	$t_{1/2}$ h	Ka_1 h^-	F %
I.V.							
RUh	19.14	1.74	2.71	1.56	1.28		
RMi	26.31	1.26	1.97	1.56	1.22		
RJd	22.51	1.48	2.00	1.36	1.47		
Mean ±	22.65	1.49	2.23	1.49	1.32		
S.D.	3.59	0.24	0.42	0.12	0.13		
P.O.							
RUh	13.21			2.07	1.58	0.48	71
RMi	21.11			2.32	1.08	0.43	80
RJd	15.29			3.23	1.47	0.31	68
Mean ±	16.54			2.54	1.38	0.41	73.00 (±6)
S.D.	4.09			0.61	0.26	0.09	6.24

*AUC = area under the plasma drug concentration versus time curve;
CL = total clearance;
V_{SS} = steady-state volume of distribution;
MRT = mean residence time;
and $t_{1/2}$ = half-life;
F = bioavailability;
and K_a = first order absorption rate constant.

TABLE 11

CSF/Serum Ratio of FTC and Its Deaminated Metabolite 1 Hour After Treatment

Monkey	Route	FTC	Metabolite (FTU)
RUh	I.V.	0.076	0.024
RMi	I.V.	0.062	0.032
RJd	I.V.	0.162	0.052
Mean ±		0.100	0.036
S.D.		0.054	0.014
RUh	P.O.	0.048	0.026
RMi	P.O.	0.039	0.037
RJd	P.O.	0.117	0.055

TABLE 11-continued

CSF/Serum Ratio of FTC and Its Deaminated Metabolite 1 Hour After Treatment

Monkey	Route	FTC	Metabolite (FTU)
Mean ± S.D.		0.068 0.043	0.039 0.015

EXAMPLE 19

CSF/Serum Ratio of FTC and its Metabolites in Rhesus Monkeys

The ability of (±)-FTC to cross the blood-brain barrier was evaluated by administering 33.3 mg/kg of the active compound to rhesus monkeys, and measuring the amount of (±)-FTC in the cerebral spinal fluid (CSF) and blood serum one hour after administration. The results are provided in Table 11. The data indicates that a significant amount of active compound passes through the blood-brain barrier in this mammal.

III. Preparation of Pharmaceutical Compositions

Humans suffering from diseases caused by HIV or HBV infection can be treated by administering to the patient an effective amount of (±)-FTC, or its (−) or (+) enantiomer or a pharmaceutically acceptable derivative or salt thereof in the presence of a pharmaceutically acceptable carrier or diluent. The active materials can be administered by any appropriate route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, or topically, in liquid or solid form.

The active compound is included in the pharmaceutically acceptable carrier or diluent in an amount sufficient to deliver to a patient a therapeutically effective amount of compound to inhibit viral replication in vivo, especially HIV and HBV replication, without causing serious toxic effects in the patient treated. By "inhibitory amount" is meant an amount of active ingredient sufficient to exert an inhibitory effect as measured by, for example, an assay such as the ones described herein.

A preferred dose of (−), (+), or (±)-FTC for all of the above-mentioned conditions will be in the range from about 1 to 50 mg/kg, preferably 1 to 20 mg/kg, of body weight per day, more generally 0.1 to about 100 mg per kilogram body weight of the recipient per day. The effective dosage range of the pharmaceutically acceptable derivatives can be calculated based on the weight of the parent nucleoside to be delivered. If the derivative exhibits activity in itself, the effective dosage can be estimated as above using the weight of the derivative, or by other means known to those skilled in the art.

The compound is conveniently administered in unit any suitable dosage form, including but not limited to one containing 7 to 3000 mg, preferably 70 to 1400 mg of active ingredient per unit dosage form. A oral dosage of 50–1000 mg is usually convenient.

Ideally the active ingredient should be administered to achieve peak plasma concentrations of the active compound of from about 0.2 to 70 μM, preferably about 1.0 to 10 μM. This may be achieved, for example, by the intravenous injection of a 0.1 to 5% solution of the active ingredient, optionally in saline, or administered as a bolus of the active ingredient.

The concentration of active compound in the drug composition will depend on absorption, inactivation, and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at varying intervals of time.

A preferred mode of administration of the active compound is oral. Oral compositions will generally include an inert diluent or an edible carrier. They may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition.

The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar, shellac, or other enteric agents.

(±)-FTC, or its (−) or (+)-enantiomer or pharmaceutically acceptable salts thereof can be administered as a component of an elixir, suspension, syrup, wafer, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

(±)-FTC, or its (−) or (+)-enantiomers, or pharmaceutically acceptable derivatives or salts thereof can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action, such as antibiotics, antifungals, antiinflammatories, or other antivirals, including other nucleoside anti-HIV compounds.

Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

If administered intravenously, preferred carriers are physiological saline or phosphate buffered saline (PBS).

In a preferred embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc.

Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) are also preferred as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811 (which is incorporated herein by reference in its entirety). For example, liposome formulations may be prepared by dissolving appropriate lipid(s) (such as stearoyl phosphatidyl ethanolamine, stearoyl phosphatidyl choline, arachadoyl phosphatidyl choline, and cholesterol) in an inorganic solvent that is then evaporated, leaving behind a thin film of dried lipid on the surface of the container. An aqueous solution of the active compound or its monophosphate, diphosphate, and/or triphosphate derivatives are then introduced into the container. The container is then swirled by hand to free lipid material from the sides of the container and to disperse lipid aggregates, thereby forming the liposomal suspension.

IV. Preparation of Phosphate Derivatives of FTC

Mono, di, and triphosphate derivative of FTC can be prepared as described below.

The monophosphate can be prepared according to the procedure of Imai et al., *J. Org. Chem.*, 34(6), 1547–1550 (June 1969). For example, about 100 mg of FTC and about 280 μl of phosphoryl chloride are reacted with stirring in about 8 ml of dry ethyl acetate at about 0° C. for about four hours. The reaction is quenched with ice. The aqueous phase is purified on an activated charcoal column, eluting with 5% ammonium hydroxide in a 1:1 mixture of ethanol and water. Evaporation of the eluant gives ammonium FTC-5'-monophosphate.

The diphosphate can be prepared according to the procedure of Davisson et al., *J. Org. Chem.*, 52(9), 1794–1801 (1987). FTC diphosphate can be prepared from the corresponding tosylate, that can be prepared, for example, by reacting the nucleoside with tosyl chloride in pyridine at room temperature for about 24 hours, working up the product in the usual manner (e.g., by washing, drying, and crystallizing it).

The triphosphate can be prepared according to the procedure of Hoard et al., *J. Am. Chem. Soc.*, 87(8), 1785–1788 (1965). For FTC is activated (by making a imidazolide, according to methods known to those skilled in the art) and treating with tributyl ammonium pyrophosphate in DMF. The reaction gives primarily the triphosphate of the nucleoside, with some unreacted monophosphate and some diphosphate. Purification by anion exchange chromatography of a DEAE column is followed by isolation of the triphosphate, e.g., as the tetrasodium salt.

This invention has been described with reference to its preferred embodiments. Variations and modifications of the invention, will be obvious to those skilled in the art from the foregoing detailed description of the invention. It is intended that all of these variations and modifications be included within the scope of the appended claims.

We claim:

1. A method for treating HIV infection in humans comprising administering an effective amount of (−)-β-L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane, or its physiologically acceptable salt, optionally in a pharmaceutically acceptable carrier.

2. The method of claim **1**, wherein the carrier is suitable for oral delivery.

3. The method of claim **1**, wherein the carrier comprises a capsule.

4. The method of claim **1**, wherein the carrier is in the form of a tablet.

5. The method of claim **1**, wherein the administration is parenteral.

6. The method of claim **1**, wherein β-L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane is administered in a form that is at least 95% free of its corresponding β-D-enantiomer.

7. The method of claim **1**, wherein β-L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane is administered in a form that is at least 95% free of its corresponding β-D-enantiomer.

8. The method of claim **1**, wherein β-L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane is administered as an isolated enantiomer.

9. A method for treating HIV infection in humans comprising administering an effective amount of (+)-β-D-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane, or its physiologically acceptable salt, optionally in a pharmaceutically acceptable carrier.

10. The method of claim **9**, wherein the carrier is suitable for oral delivery.

11. The method of claim **9**, wherein the carrier comprises a capsule.

12. The method of claim **9**, wherein the carrier is in the form of a tablet.

13. The method of claim **9**, wherein the administration is parenteral.

14. The method of claim **9**, wherein β-D-2-hydroxymethyl-5-(5-fluorocytosin- 1-yl)-1,3-oxathiolane is administered in a form that is at least 95% free of its corresponding β-L enantiomer.

15. The method of claim **9**, wherein β-D-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane is administered in a form that is at least 95% free of its corresponding β-L-enantiomer.

16. The method of claim **9**, wherein β-D-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane is administered as an isolated enantiomer.

17. A method for treating HIV infection in humans comprising administering an effective amount of the monphosphate, diphosphate or triphosphate of β-L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane, or its physiologically acceptable salt, optionally in a pharmaceutically acceptable carrier.

18. The method of claim **17**, wherein the phosphate of β-L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane is administered in a form that is at least 95% free of its corresponding β-D-enantiomer.

19. The method of claim **17**, wherein the phosphate of β-L-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane is administered as an isolated enantiomer.

20. A method for treating HIV infection in humans comprising administering an effective amount of the monophosphate, diphosphate, or triphosphate of β-D-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane, or its physiologically acceptable salt, optionally in a pharmaceutically acceptable carrier.

21. The method of claim **20**, wherein the phosphate of β-D-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane is administered in a form that is at least 95% free of its corresponding β-L-enantiomer.

22. The method of claim **20**, wherein the phosphate of β-D-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane is administered as an isolated enantiomer.

* * * * *

EXHIBIT B

US006703396B1

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

(10) **Patent No.: US 6,703,396 B1**
(45) **Date of Patent: Mar. 9, 2004**

(54) **METHOD OF RESOLUTION AND ANTIVIRAL ACTIVITY OF 1,3-OXATHIOLANE NUCLESOSIDE ENANTIOMERS**

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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 641 days.

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(63) Continuation of application No. 08/092,248, filed on Jul. 15, 1993, now abandoned, which is a continuation of application No. 07/736,089, filed on Jul. 26, 1991, now abandoned, which is a continuation-in-part of application No. 07/659,760, filed on Feb. 22, 1991, now Pat. No. 5,210,085, which is a continuation-in-part of application No. 07/473,318, filed on Feb. 1, 1990, now Pat. No. 5,204,466.

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(58) **Field of Search** 544/243, 317; 514/274, 86

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,000,137	A		12/1976	Dvonoch et al. 544/313
4,336,381	A		6/1982	Nagata et al. 544/313
4,861,759	A		8/1989	Mitsuya et al. 514/46
4,879,277	A		11/1989	Mitsuya et al. 514/49
4,900,828	A		2/1990	Belica et al. 544/317
4,916,122	A		4/1990	Chu et al. 514/50
4,963,533	A		10/1990	de Clerq et al. 514/49
5,041,449	A		8/1991	Belleau et al. 514/274
5,047,407	A		9/1991	Belleau et al. 544/317
5,059,690	A		10/1991	Zahler et al. 544/276
5,071,983	A		12/1991	Koszalka et al. 544/317
5,179,104	A		1/1993	Chu et al. 544/310
5,185,437	A		2/1993	Koszalka et al. 544/317
5,204,466	A	*	4/1993	Liotta et al. 544/317
5,210,085	A	*	5/1993	Liotta et al. 544/317
5,234,913	A		8/1993	Furman, Jr. et al. 514/49
5,248,776	A		9/1993	Chu et al. 544/310
5,270,315	A		12/1993	Belleau et al. 544/265
5,538,975	A		7/1996	Dionne
5,618,820	A		4/1997	Dionne
5,814,639	A		9/1998	Liotta et al.

FOREIGN PATENT DOCUMENTS

EP	0 217 580	4/1987
EP	0337713	4/1989
EP	0 361 831	4/1990
EP	0375329	6/1990
EP	0433898	6/1991
EP	0515157	5/1992
EP	0494119	7/1992
EP	494119	7/1992
EP	0515144	11/1992
EP	0515156	11/1992
EP	0526253	2/1993
WO	WO 88/07532	10/1988
WO	WO 90/12023	10/1990
WO	WO 91/11186	8/1991
WO	WO 91/17159	11/1991
WO	WO 92/08717	5/1992
WO	WO 92/10496	6/1992
WO	WO 92/10497	6/1992
WO	WO 92/14743	9/1992
WO	WO 92/15308	9/1992
WO	WO 92/18517	10/1992
WO	WO 94/04154	3/1994

OTHER PUBLICATIONS

Daniels, T.C. et al. *Textbook of Organic Medicinal and Pharmaceutical Chemistry,* ed. by Wilson, C.O. et al. (Lippincott Co., Philadelphia), pp. 31–36 (1977).*

Carter, S.O. et al. *Antimicrob. Agents Chemotherapy,* vol. 34, pp. 1297–1300 (1990).*

March, J. *Advanced Organic Chemistry* (Wiley and Sons, New York), pp. 82–107 (1985).*

Belleau, B., et al., "Design and Activity of a Novel Class of Nucleoside Analogs Effective Against HIV–1," *International Conference on AIDS,* Montreal, Quebec, Canada Jun. 4–9, 1989.

Krenitsky, T.A., et al., "3'–Amino–2',3'–Dideoxyribonucleosides of Some Pyrimidines: Synthesis and Biological Activities," *J. Med. Chem,* Vo. 26 (1993).

Sterzycki, R.Z., et al., "Synthesis and anti–HIV activity of several 2'–fluoro–containing pyrimidine nucleosides," J. Med. Chem. 33:2150–2157 (1990).

Storer, B., et al., "The Resolution and Absolute Stereochemistry of the Enantiomeris of cis–1–[2–(Hydromethyl)–1, 3–Oxathiolan–5–yl)cytosine (BCH189): Equipotent Anti–HIV Agents," *Nucleosides & Nucleotides,* 12(2) 225–236 (1993).

(List continued on next page.)

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(57) **ABSTRACT**

A process for the resolution of a racemic mixture of nucleoside enantiomers that includes the step of exposing the racemic mixture to an enzyme that preferentially catalyzes a reaction in one of the enantiomers. The nucleoside enantiomer (−)-2-hydroxymethyl-5-(5-flurocytosin-1-yl)-1,3-oxathiolane is an effective antiviral agent against HIV, HBV, and other viruses replicating in a similar manner.

28 Claims, 5 Drawing Sheets

OTHER PUBLICATIONS

Van Roey, P., "Solid State Conformation of Anti–Human Immunosudeficiency Virus Type–1 Agents: Crystal Structures of Three 3'–Azido–3'–deoxythymidine Analogues," *J. Am. Chem. Soc.* (1988) 110, pp. 2277–2782.

Wilson, L.J., et al., "The Synthesis and Anti–HIV Activity of Pyrimidine Dioxolanyl Nucleosides," *Bioorganic & Medicinal Chemistry Letters,* 13(2), pp. 169–174 (1993).

Balzarini, J., et al., "Potent and Selective Anti–HTI V–III/I AV Activity of 2',3'–Dideoxycytidinene, the 2'–3'–Unsaturated Derivative of 2',3'–Dideoxycytidine," *Biochemical and Biophysical Reserach Communications,* vol. 140, No. 2, pp. 735–742 (1986).

Carter et al., "Activties of (–)–Carbovir and 3'–Azido–3'–Deoxythymidine Against Human Immunodeficiency Virus In Vitro," *Antimicrobial Agents and Chemotherapy,* vol. 34, No. 6, pp. 1297–1300 (1990).

Chang, Chien–Neng, et al., "Deoxycytidine Deaminase–resistant Stereoisomer Is the Active Form of (±)–2', 3'–Dideoxy–3'–thiacytidine in the Inhibition of Hepatitis B Virus Replication," *The Journal of Biological Chemistry,* vol. 357, No. 20, pp. 13938–13942 (1992).

Chu et al., "Structure–Activity Relationships of Pyrimidine Nucleosides as Antiviral Agents for Human Immunodeficiency Virus Type 1 in Peripheral Blood Mononuclear Cells," *J. Med. Chem.* vol. 32, p. 612 (1989).

Chu, et al., "Comparative Activity of 2',3'–Saturated and Unsaturated Pyrimidine and Purine Nucleosides Against Human Immunodeficiency Virus Type 1 in Peripheral Blood Mononuclear Cells," *Biochem. Pharm.,* vol. 37, No. 19, pp. 3543–3548 (1988).

Chu, C.K., et al., "An Efficient Total Synthesis of 3'–Azido–3'–Deoxythiymidine (AZT) and 3'–Azido–2', 3'–Dideoxyuridine (AZDDU, CS–87) from D–Mannitol," *Tetrahedron Lett.* 1988, 5349.

Cretton, E., et al., "Catabolism of 3'–Azido–3'–Deoxythymidine in Hepatocytes and Liver Microsomes, with Evidence of Formation of 3'–Amino–3'–Deoxythymidine, a Highly Toxic Catabolite for Human Boane Marrow Cells," *Molecular Pharmacology,* vol. 39, pp. 258–266 (1991).

Cretton, E., et al., "Pharmokinetics of 3'–Azido–3'–Dexoythymidine and its Catabolites and Interactions with Probenecid in Rhesus Monkeys," *Antimicrobial Agents and Chemotheraphy,* pp. 801–807 (1991).

Furman, et al., "The Anti–Hepatitis B Virus Activities, Cytotoxicities, and Anabolic Profiles of the (–) and (+) Enantiomers of cis–5–Fluoro–1–[2–(Hydromethyl)–1, 3–Oxthiolan–5–yl]Cytosine," *Antimicrobial Agents and Chemotherapy,* vol. 36, No. 12, pp. 2686–2692 (1992).

Jeong, L., et al., "Asymmetric Synthesis and Biological Evaluation of β–L–(2R,5S)– and α–L–(2R–5R)–1,3–Oxathiolane–Pyrimidine and –Purine Nucleosides and Potential Anti–HIV Agents," *J. Med. Chem.,* vol. 36 (1993).

Lin, et al., "Potent and Selective In Vitro Activity of 3'–Deoxythmindin–2–Ene–(3'–Deoxy–2+,3'–Didehydrothymidine) Against Human Immunodeficiency Virus," *Biochem. Pharm.,* vol. 36, No. 17, p. 2716 (1987).

Mitsuya, H., et al., "Rapid in Vitro Systems for Assessing Activity of Agents Against HTLV–III/LAV," *AIDS: Modern Concepts and Therapeutic Challenges,* S. Broder, Ed. (Marcel–Dekker, New York, 1987), pp. 303.

Mitsuya, J., et al., 3'–Azido–3'–Deoxythymidine (BW A 509U): An Antiviral Agent that Inhibits the Infectivity and Cytopathic Effect of Human T–Lymphotropic Virus Type III/Lymphadenopathy–Associated Virus In Vitro, *Proc. Natl. Acad. Sci., USA,* vol. 82, pp. 7097–7100 (1985).

Mitsuya, H., et al., "Molecular Targets for AIDS Therapy," *Science,* vol. 249, pp. 1533–1544. (1990).

Norbeck, D., et al., "A New 2',3'–Dideoxynucleoside Prototype with In Vitro Activity Against HIV," *Tetrahedron Lett.* 1989, 6263.

Okabe, M., et al., "Synthesis of the Dideoxynucleosides ddC and CNT from Glutamic Acid, Ribonolactone, and Pyrimidine Bases," *J. Org. Chem.* 1989.

Richman, D. D., et al., "The Toxicity of Azidothymidine (AZT) in the Treatment of Patients with AIDS and AIDS–Related Complex," *N. Eng. J. Med.* 1987, 317:192.

Satsumabayashi, S. et al., "The Synthesis of 1,3–Oxathiolan–5–one Derivatives," *Bull, Chem. Soc. Japan,* 1972, 45,913.

Schinazi, R.F., et al., "Activities of the Four Optical Isomers of 2'–3'–Dideoxy–3'–Thiacytidine (BCH–189) against Human Immunodeficiency Virus Type 1 in Human Lymphocytes," *Antimicrobial Agents and Chemotherapy* 36(3) 672–676 (1992).

Schinazi, R.F., et al., "Insights into HIV Chemotherapy," *AIDS Research and Human Retroviruses* 8(6) (1992) 963–990.

Schinazi, R.F., et al., "Pharmacokinetics and Metabolism of Racemic 2'–3'–Dideoxy–5–Fluoro–3'–Thiacytidine in Rhesus Monkeys," *Antimicrobial Agents and Chemotherapy* 36(11) 2432–2438. (1992).

Schinazi, R.F., et al., "Selective Inhibition of Human Immunodeficiency Viruses by Racemates and Enantiomers of cis–5–Fluoro–1–[2–(Hydroxymethyl)–1, 3–Oxathiolan–5–yl]Cytosine," *Antimicrobial Agents and Chemotherapy* 36(11) 2423–2431. (1992).

Schinazi, R.F., et al., "Substrate Specificity of *Escherichia coli* Thymidine Phosphorylase for Pyrimidine Nucleoside with an Anti–Human Immunodeficiency Virus Activity," *Biochemical Pharmacology* 44(2) (1992) 199–204.

Vorbrüggen et al, "Nucleoside Synthesis with Trimethylsilyl Triflate and Perchlorate as Catalysts," *Chem. Ber.* 1981, 114:1234–1255.

Wilson, L.J., et al., "A General Method for Controlling Glycosylation Stereochemistry in the Synthesis of 2'–Deoxyribose Nucleosides," *Tetrahedron Lett.* 1990, 1815.

Zhu, Zhou, et al., "Cellular Metabolism of 3'–Azido–2', 3'–Dideoxyuridine with Formation 5'–O–Diphophoshexase Derivative by Previously Unrecognized Metabolic Pathways of 2'–Deoxyuridine Analogs," *Molecular Pharmacology,* vol. 38, pp. 929–938 (1990).

Soudeyns, et al., "Anti–Human Immunodeficiency Virus Type 1 Activity and In Vitro Toxicity of 2'–Deoxy–3'–Thiacytidine (BCH–189), a Novel Heterocyclic Nucleoside Analog," *Antimicrobial Agents And Chemotherapy,* pp. 1386–1390 (1991).

Shin–Lian, et al., *Natl. Acad. Sci.* 88, 8495 (1991).

ASAI, J. Chem. Pharm. Bull, 1967, 1863–1870, 15(12).

Armstrong, Chiral Stationary Phase 1984.

Armstrong, Chromatographic Chiral Separation, 1986, vol. 40.

Armstrong, Analytical Chemistry, 1987, 84–91, 59(2).

Allenmark, Chromographic Enantioseparation: Method and Applications, 1988.

Balzarini, J. Med. Chem, 1985, 1385–1386, 28.
Balzarini, J. Med. Chem, 1989, 1861–1865, 32.
R. Baum and R. Dagoni, Chemical Engineering News, Jun. 26, 1989, p. 11.
Beach, J. Org. Chem., 1992, 2217–2219, 57.
Belleau, Aids Abstract, 1989, 515.
Belleau, Aids Abstract, 1989, 552.
Boehm, Anal. Chem., 1988, 522–528, 60.
Bzowska, Biochemical Pharmacology, 1991, 1709–1803, 41(12).
Carruthers, Some Modern Methods Of Organic Synthesis, 1986.
Coates, Antimibrobial Agents and Chemtherapy, Jan. 1992, 202–205.
Collet, Enantiomers, Racemates, and Resolutions, 1981.
Dappen, Journal of Chromatography, 1986, 1–20, 373.
Davies, Biotransformations in Preparative Organic Chemistry, 1989.
Eliel, Ernest, *Stereochemistry of Carbon Compounds,* John Wiley & Sons, 1962, p. 328.
Greene, *Protective Groups In Organic Synthesis,* John Wiley & Sons, 1981, pps. 10–72.
Forsman, J. of Chromatography, 1984, 217–221, 303.
Han, J. of Chromatography, 1988, 376–381, 441.
Hesse, Chromatographia, 1973, 277–280, 6(6).
Hinze, Anal. Chem. 1985, 237–232, 57.
Hooper, Clinical Pharmacology and Therapeutics, 1990, 633–640, 48(6).
Holy, Chem. Commun., 1971, 3282–3299, 36.
Humber, Tetrahedron Letters, 1992, 4625–4628, 33(32).
Ikeda, Chemistry Letters, 1989, 1089–1090.
Johnson, J. of Chromatography, 1984, 449–452, 291.
D24 Jones, Illustrative Examples of Enzymes in Organic Synthesis, 1986.
D25 Jones, Enzymes in Organic Synthesis, Tetrahedron, 42:13 1986.
D13 Kim, J. Med. Chem. 30:1987.
Kim, J. Med. Chem., 1987, 862–866, 30.
Lin, J. Med. Chem., 1988, 336–340, 31.
Lipkpwitz, Anal. Chem., 1986, 1611–1617, 58.
Lim, Drug Metabolism and Disposition, 1989, 212–217, 17(2).
Lindner, J. of Chromatography, 1980, 308–310, 193.
D14 Lin, J. Med. Chem. 32:1989.
D19 Mackie and Smith, Guidebook to Organic Synthesis, 1982.
76 Lin Synthesis and Antivity 1976.
18 Nasr Structure–Activity Nasr, Annual NY Academy of Science 616:1990, Based on AIDS lecture.
75 Nogradi Stereoselective Synthesis, Weinheim; New York: VCH, 1986, 43–47.
71 Okamoto, J. of Chromotography, 1987, 389:95–102.
D26 Ohno, Organic Reactions, 37:1989.
57. O'Shea, Chirality, 1990, 257–262, 2.
17 Prince A Comparative Study Prince, B.S.E.B.M. 110:1969.
Stoner, The Resolution and Absolute . . . , Nucleosides & Nucleotides, 1993, 225–236, 12.
Scheit, Nucleotide Analogues . . . , 1980, 153–160.
Shimizu, Synthesis of Anomeric . . . , Chem. Pharm. Bull., 1967, 2011–2014, 15.
Seebach, Organic Synthesis . . . , Angew, Chem. Int. Ed. Engl., 1990, 1320–1367, 29.
Shealy, Carbocyclic Analogues . . . , J.Med. Chem., 1983, 156–161, 26.
Wainer, Drug Analysis . . . , Stevenson Chiral Separations, 1988, 11–21.
Sih, Topics in Stereochemistry, 1989.
Townsend (ed), Chemistry of Nucleosides and Nucleotides 1988.
Vince, Resolution . . . , Biochem. Biophys. Res. Comm., 1990, 912–916, 168.
Wainer, Proposal for . . . , trends in analytical chem., 1987, 125–134, 6.
Wainer, A Practical . . . , 1988, 1–38.
Wainer, Drug Stereochemistry, 1988.
Wilen, Tables of Resolving Agents and Optical Resolutions, 1972.
Wilen Resolving Agents.
Walker, "Nucleosides" in Comprehensive Chemistry: The Synthesis and Reactions of Organic Compounds 1979.
Whitesides, Chem. Int.Ed.Engl., 24:8 1985.
Haslam, Comprehensive Organic Chemistry . . . , 1979, 95–100, Pergamon Press, New York, USA.
Ueda, Synthesis . . . , Chemistry of Nucleosides and Nucleotides, 1–112, Plenum Press, New York.
Pirkle, Chiral Stationary . . . , Advances in Chromo. vol 27, 1987, 73–127, Marcel Deker, NY, USA.
Brown, State of the art . . . , Advances in Chromatography, 1980, 101–139, 1980.
1982–1986 gen subject list, Chemical Abstracts, 5654CS.
1987–1991, gen subject list, Chemical Abstracts, 6982GS, 7065GS–1067GS.
March, Advanced Organic Chemistry . . . , 1977, 108–111, McGraw–Hill, New York, USA.
Macherey–Nagel Duren, Catalog for The Separation of chiral molecule . . . .
Daicel Chemical Industries, Application Guide for chiral column . . . , 1989.
Alltech Associates, Inc., The Separation of Optical Isomers, Bulletin #87.
Daicel Chemical Industries, Crownpak, Chiralpak . . . , Catralogue.
Published abstract together with a transcript of an oral presentation given by Bernard Belleau at the 5th International Conference on AIDS, Jun. 6, 1989, plus copies of the poster used in the presentation.
Letter of Feb. 6, 1998 from the European patent representatives acting for the patentee (Emory University) to the EPO regarding the opposed patent (European Patent Application No. 91904454.5).
Declaration of Dennis C. Liotta in respect of the Opposition by Emory University against European Patent No. 0382526 B.
Declaration of Dr. Irving Wainer in relation to the Opposition by Emory University against European Patent No. 0382526 B.
Declaration of Ian M. Mutton in relation to the Opposition by Emory University against European Patent No. 0382526 B.
Declaration of Michael John Dawson in relation to the Opposition by Emory University against European Patent No. 0382526 B.
Borthwick, et al., "Synthesis and Enzymatic Resolution of Carbocyclic 2'–Ara–Fluoro–Guanosine: A Potent New Anti– Herpetic Agent," *J. Chem. Soc. Commun.,* vol. 10. pp. 656–658 (1988).

Herdewijn, et al., "Resolution of Aristeromycin Enantiomers," *J. Med. Chem.,* 1985, vol. 28, 1385–1386.

Krenitsky, et al., "An Enzymic Synthesis of Purine D–arabinonucleosdes," *Carbohydrate Research,* Vol 97, pp. 139–146 (1981).

Mahmoudian, et al., "Enzymatic Production of Optically Pure (2'R–cis)–2'–deoxy–3'–thiacytidine (3TC, Lamivudine): A Potent Anti–HIV Agent," *Enzyme Microb. Technol.,* Sep. 1993, vol. 15, 749–755, published by the Glaxo Group Research Ltd.

Pirkle and Pochansky, "Chiral Stationary Phases for the Direct LC Separation of Enantiomers," *Advances in Chromatography,* Giddings, J.C., Grushka, E., Brown, P.R., eds.: Marcel Dekker: New York, 1987; vol. 27, Chap. 3, pp. 73–127.

Secrist, et al., "Resolution of Racemic Carbocyclic Analogues of Purine Nucleosides Through the Action of Adenosine Deaminase Antiviral Activity of the Carbocyclic 2'–Deoxyguanosine Enantimers," *J. Med. Chem.,* vol. 30, pp. 746–749 (1987).

* cited by examiner

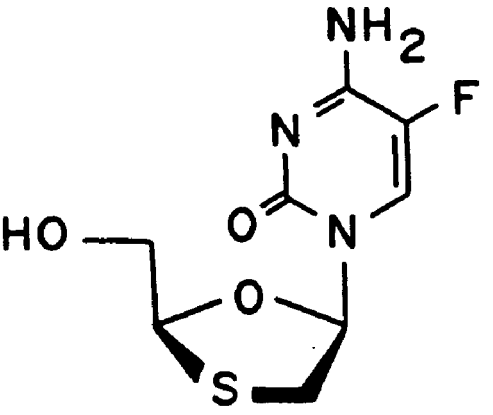


FIG. 1

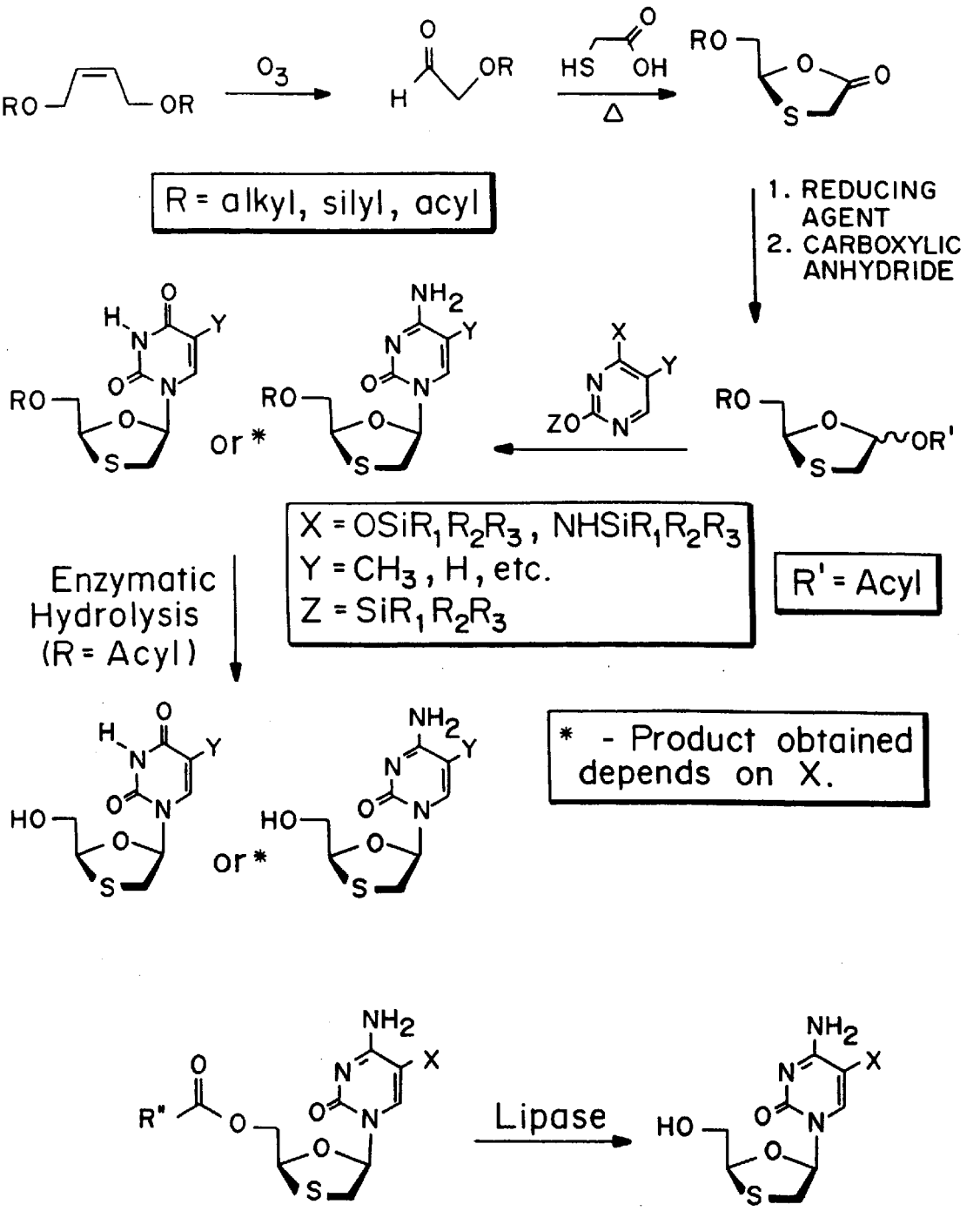

RO
OR
O_3
H
HS
OH
Δ
S
R = alkyl, silyl, acyl
1. REDUCING AGENT
2. CARBOXYLIC ANHYDRIDE
or *
ZO
OR'
X = $OSiR_1R_2R_3$, $NHSiR_1R_2R_3$
Y = CH_3, H, etc.
Z = $SiR_1R_2R_3$
R' = Acyl
Enzymatic Hydrolysis (R = Acyl)
HO
* - Product obtained depends on X.
R"
Lipase
R" = alkyl, substituted alkyl; X=H, F, alkyl, halogen, etc.
Lipase = pig liver esterase, porcine pancreatic lipase. Amano PS-800, subtilisin, etc.

FIG. 2

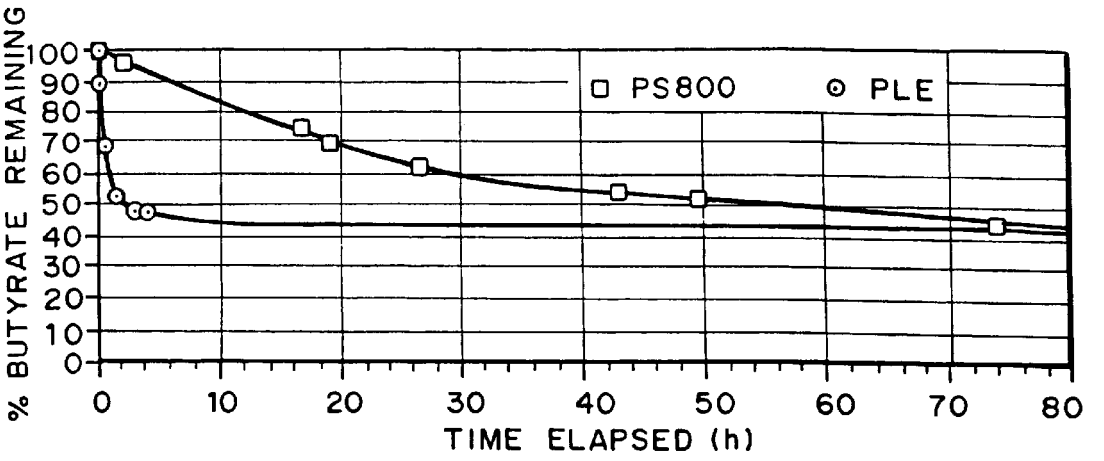

% BUTYRATE REMAINING
100
90
80
70
60
50
40
30
20
10
0
PS800
PLE
0
10
20
30
40
50
60
70
80
TIME ELAPSED (h)

FIG. 3

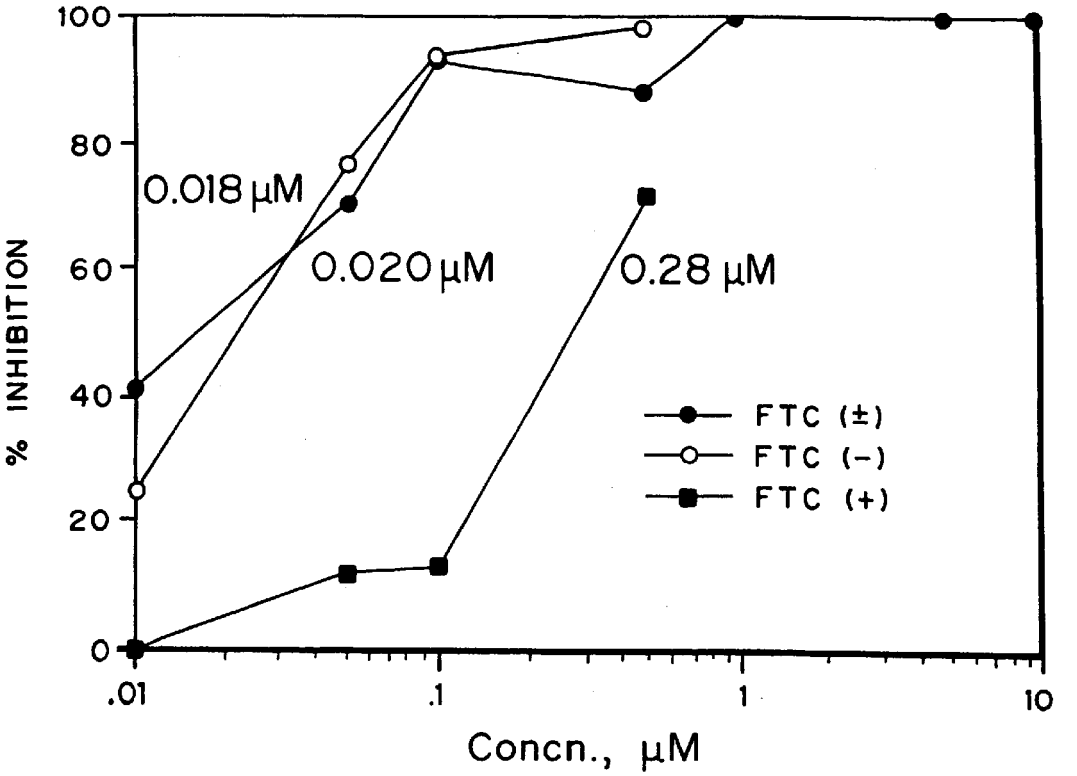

% INHIBITION
100
80
60
40
20
0
0.018 μM
0.020 μM
0.28 μM
FTC (±)
FTC (−)
FTC (+)
.01
.1
1
10
Concn., μM

FIG. 4

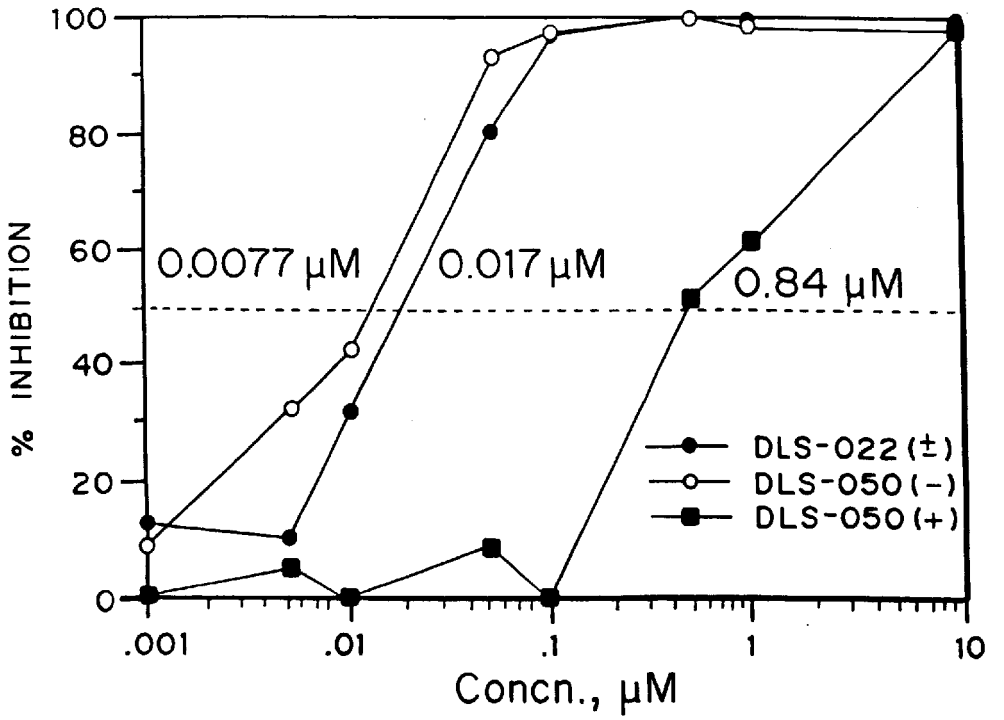

100
80
60
40
20
0
% INHIBITION
0.0077 µM
0.017 µM
0.84 µM
DLS-022 (±)
DLS-050 (-)
DLS-050 (+)
.001
.01
.1
1
10
Concn., µM

FIG. 5

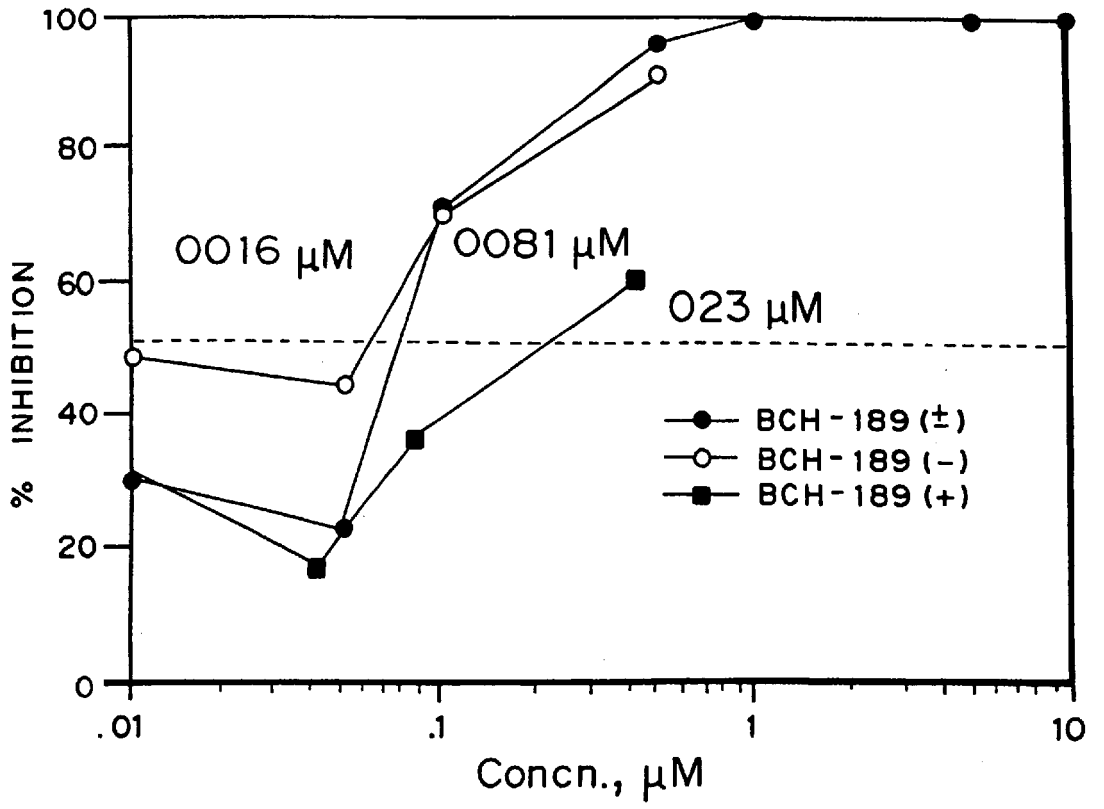

100
80
60
40
20
0
% INHIBITION
0016 µM
0081 µM
023 µM
BCH-189 (±)
BCH-189 (-)
BCH-189 (+)
.01
.1
1
10
Concn., µM

FIG. 6

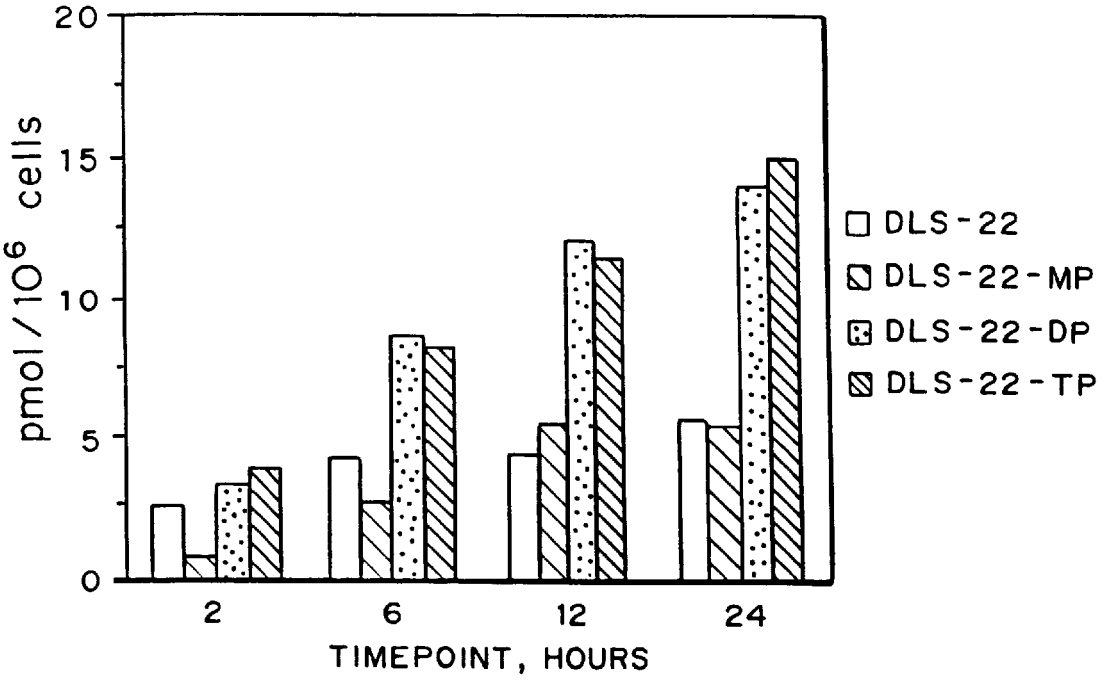

20
15
10
5
0
pmol / 10^6 cells
2
6
12
24
TIMEPOINT, HOURS
DLS-22
DLS-22-MP
DLS-22-DP
DLS-22-TP

FIG. 7

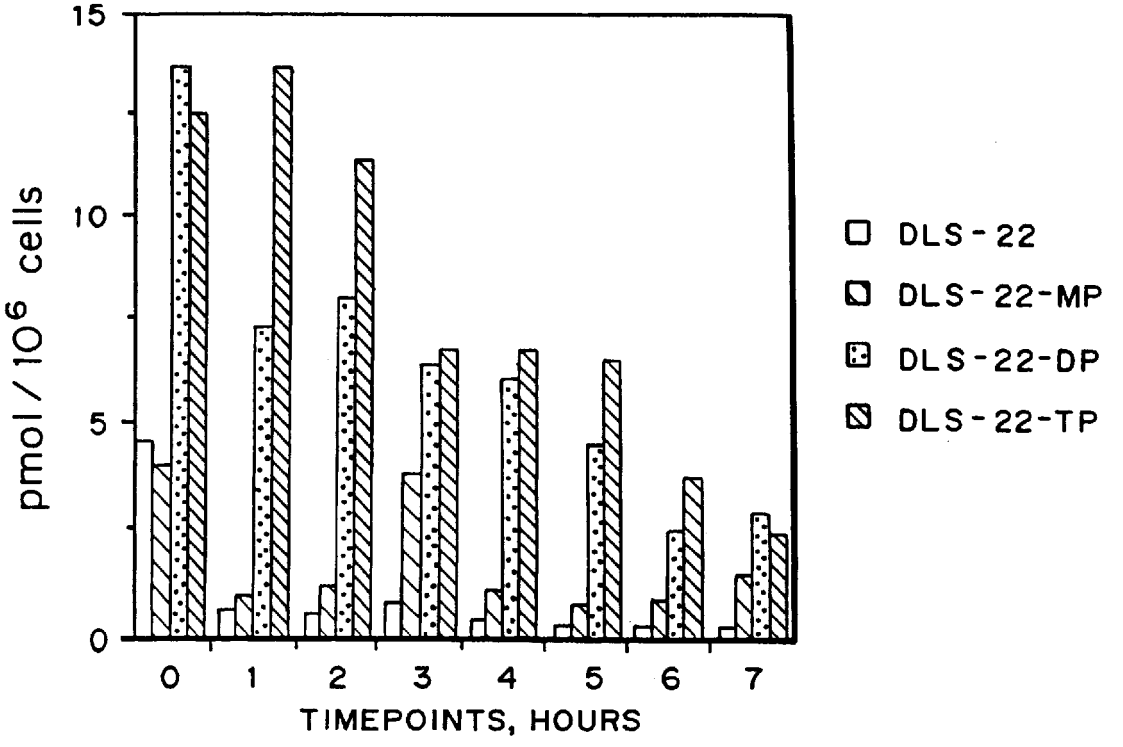

15
10
5
0
pmol / 10^6 cells
0
1
2
3
4
5
6
7
TIMEPOINTS, HOURS
DLS-22
DLS-22-MP
DLS-22-DP
DLS-22-TP

FIG. 8

METHOD OF RESOLUTION AND ANTIVIRAL ACTIVITY OF 1,3-OXATHIOLANE NUCLESOSIDE ENANTIOMERS

This application is a continuation of application U.S. Ser. No. 08/092,248, filed on Jul. 15, 1993, now abandoned, which is a continuation of U.S. Ser. No. 07/736,089, filed on Jul. 26, 1991, now abandoned, which is a continuation-in-part of U.S. Ser. No. 07/659,760, filed on Feb. 22, 1991, now U.S. Pat. No. 5,210,085, which is a continuation-in-part of U.S. Ser. No. 07/473,318, filed on Feb. 1, 1990, now U.S. Pat. No. 5,204,466.

U.S. Government has rights in this invention arising out of the partial funding of work leading to this invention through the National Institutes of Health Grant Nos. NIH 5-21935 and NIH AI-26055, as well as a Veteran's Administration Merit Review Award.

BACKGROUND OF THE INVENTION

This invention is in the area of biologically active nucleosides, and specifically includes a method for the resolution of nucleoside enantiomers, including 1,3-oxathiolane nucleosides, and antiviral compositions that include the enantiomerically enriched 1,3-oxathiolane nucleosides, (−) and (+)-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane ("FTC").

This application is a continuation-in-part application of U.S. Ser. No. 07/659,760, entitled "Method for the Synthesis, Compositions and Use of 2'-Deoxy-5-Fluoro-3'-Thiacytidine and Related Compounds", filed on Feb. 22, 1991, by Dennis C. Liotta, Raymond Schinazi, and Woo-Baeg Choi, that is a continuation-in-part application of U.S. Ser. No. 07/473,318, entitled "Method and Compositions for the Synthesis of BCH-189 and Related Compounds," filed on Feb. 1, 1990, by Dennis C. Liotta and Woo-Baeg Choi.

In 1981, acquired immune deficiency syndrome (AIDS) was identified as a disease that severely compromises the human immune system, and that almost without exception leads to death. In 1983, the etiological cause of AIDS was determined to be the human immunodeficiency virus (HIV). In December, 1990, the World Health Organization estimated that between 8 and 10 million people worldwide were infected with HIV, and of that number, between 1,000,000 and 1,400,000 were in the U.S.

In 1985, it was reported that the synthetic nucleoside 3'-azido-3'-deoxythymidine (AZT) inhibits the replication of human immunodeficiency virus type 1. Since then, a number of other synthetic nucleosides, including 2',3'-dideoxyinosine (DDI), 2',3'-dideoxycytidine (DDC), 3'-fluoro-3'-deoxythymidine (FLT), 2',3'-dideoxy-2',3'-didehydrothymidine (D4T), and 3'-azido-2',3'-dideoxyuridine (AZDU), have been proven to be effective against HIV. A number of other 2',3'-dideoxynucleosides have been demonstrated to inhibit the growth of a variety of other viruses in vitro. It appears that, after cellular phosphorylation to the 5'-triphosphate by cellular kinases, these synthetic nucleosides are incorporated into a growing strand of viral DNA, causing chain termination due to the absence of the 3'-hydroxyl group.

In its triphosphate form, 3'-azido-3'-deoxythymidine is a potent inhibitor of HIV reverse transcriptase and has been approved by the FDA for the treatment of AIDS. However, the benefits of AZT must be weighed against the severe adverse reactions of bone marrow suppression, nausea, myalgia, insomnia, severe headaches, anemia, peripheral neuropathy, and seizures. These adverse side effects often occur immediately after treatment begins, whereas a minimum of six weeks of therapy is necessary to realize AZT's benefits. DDI, which has recently been approved by an FDA Committee for the treatment of AIDS, is also associated with a number of side effects, including sporadic pancreatis and peripheral neuropathy.

Both DDC and D4T are potent inhibitors of HIV replication with activities comparable (D4T) or superior (DDC) to AZT. However, both DDC and D4T are not efficiently converted to the corresponding 5'-triphosphates in vivo. Both compounds are also toxic and can cause peripheral neuropathies in humans.

The success of various 2',3'-dideoxynucleosides in inhibiting the replication of HIV in vivo or in vitro has led a number of researchers to design and test nucleosides that substitute a heteroatom for the carbon atom at the 3'-position of the nucleoside. Norbeck, et al., disclose that (±)-1-[(2β, 4β)-2-(hydroxymethyl)-4-dioxolanyl]thymine (referred to below as (±)-dioxolane-T) exhibits a modest activity against HIV (EC_{50} of 20 μm in ATH8 cells), and is not toxic to uninfected control cells at a concentration of 200 μm. *Tetrahedron Letters* 30 (46), 6246, (1989).

European Patent Application Publication No. 0 382 526 filed by IAF Biochem International, Inc. discloses a number of substituted 1,3-oxathiolanes with antiviral activity, and specifically reports that the racemic mixture (about the C4'-position) of the C1'-β isomer of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane (referred to below as (±)-BCH-189) has approximately the same activity against HIV as AZT, and no cellular toxicity at therapeutic levels. (±)-BCH-189 has also been found to inhibit the replication of AZT-resistant, HIV isolates from patients who have been treated with AZT for longer than 36 weeks.

To market a nucleoside for pharmaceutical purposes, it must not only be efficacious with low toxicity, it must also be cost effective to manufacture. An extensive amount of research and development has been directed toward new, low cost processes for large scale nucleoside production. 2',3'-Dideoxynucleosides are currently prepared by either of two routes: derivatization of an intact nucleoside or condensation of a derivatized sugar moiety with a heterocyclic base. Although there are numerous disadvantages associated with obtaining new nucleoside analogues by modifying intact nucleosides, a major advantage of this approach is that the appropriate absolute stereochemistry has already been set by nature. However, this approach cannot be used in the production of nucleosides that contain either nonnaturally occurring bases or nonnaturally occurring carbohydrate moieties (and which therefore are not prepared from intact nucleosides), such as 1,3-oxathiolane nucleosides and 1,3-dioxolane nucleosides.

When condensing a carbohydrate or carbohydrate-like moiety with a heterocyclic base to form a synthetic nucleoside, a nucleoside is produced that has two chiral centers (at the C1' and C4'-positions), and thus exists as a diastereomeric pair. Each diastereomer exists as a set of enantiomers. Therefore, the product is a mixture of four enantiomers.

It is often found that nucleosides with nonnaturally-occurring stereochemistry in either the C1' or the C4'-positions are less active than the same nucleoside with the stereochemistry as set by nature. For example, Carter, et al., have reported that the concentration of the (−)-enantiomer of carbovir (2',3'-didehydro-2',3'-dideoxyguanosine) required to reduce the reverse transcriptase activity by 50% (EC_{50})

is 0.8 μM, whereas the EC_{50} for the (+)-enantiomer of carbovir is greater than 60 μM. *Antimicrobial Agents and Chemotherapy,* 34:6, 1297–1300 (June 1990).

U.S. Ser. No. 07/659,760 discloses that 1,3-oxathiolane and 1,3-dioxolane nucleosides can be prepared with high diastereoselectivity (high percentage of nucleoside with a β configuration of the bond from the C1'-carbon to the heterocyclic base) by careful selection of the Lewis acid used in the condensation process. It was discovered that condensation of a 1,3-oxathiolane nucleoside with a base occurs with almost complete β-stereospecificity when stannic chloride is used as the condensation catalyst, and condensation of 1,3-dioxolane with a base occurs with almost complete β-stereospecificity when various chlorotitanium catalysts are employed. Other Lewis acids provide low (or no) C1'-β selectivity or simply fail to catalyze the reactions.

There remains a strong need to provide a cost effective, commercially viable method to obtain β-stereospecificity of synthetic nucleosides prepared by condensing a carbohydrate-like moiety with a base. This is important because it is likely that many synthetic nucleoside inhibitors of viral replication now emerging from academic and commercial laboratories will require resolution. An economical and facile method for resolving racemic mixtures of nucleosides would greatly facilitate antiviral research and ultimately, commercial manufacture. Further, resolution of racemic mixtures of nucleosides may provide a route to increase the activity of synthetic nucleosides by eliminating or minimizing the undesired enantiomer.

Therefore, it is an object of the present invention to provide a method for the resolution of racemic mixtures of nucleosides.

It is another object of the present invention to provide enantiomerically enriched 1,3-oxathiolane nucleosides.

It is still another object of the present invention to provide enantiomerically enriched 1,3-oxathiolane nucleosides with significant antiviral activity and low toxicity.

SUMMARY OF THE INVENTION

A process for the resolution of a racemic mixture of nucleoside enantiomers or their derivatives is disclosed that includes the step of exposing the racemic mixture to an enzyme that preferentially catalyzes a reaction in one of the enantiomers. The process can be used to resolve a wide variety of nucleosides, including pyrimidine and purine nucleosides that are optionally substituted in the carbohydrate moiety or base moiety. The process can also be used to resolve nucleoside derivatives that contain additional heteroatoms in the carbohydrate moiety, for example, FTC and BCH-189. The resolution of nucleosides can be performed on large scale at moderate cost.

It has been discovered that the nucleoside enantiomer (−) 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane ("FTC") exhibits significant activity against HIV (EC_{50} of 0.0077 to 0.02 μM), HBV (hepatitis B virus), and other viruses replicating in a similar manner. The (+)-enantiomer of FTC is also active against HIV (EC_{50} of 0.28–0.84 μM).

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** is an illustration of the chemical structure of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane ("FTC").

FIG. **2** is an illustration of a method for the preparation of enantiomerically enriched cytidine and uridine 1,3-oxathiolane nucleosides.

FIG. **3** is a graph indicating the progress of lipase-catalyzed hydrolysis of the 5'-butyryl ester of FTC over time using the enzymes PS800 (-open square-) and PLE (-open circle with dot-).

FIG. **4** is a graph of the effect of concentration (μM) of racemic and enantiomerically enriched FTC (prepared by the method of Example 3) versus the percent inhibition of human PBM cells infected with HIV-1. ((-darkened circle-, (±)-FTC), (-circle-, (−)-FTC), (-darkened square-,(+)-FTC).

FIG. **5** is a graph of the effect of concentration (μM) of racemic and enantiomerically enriched FTC (prepared by method of Example 2) on the percent inhibition of human PBM cells infected with HIV-1. ((-darkened circle-, (±)-FTC), (-circle -,(−)-FTC), (-darkened square-,(+)-FTC).

FIG. **6** is a graph of the effect of concentration (μM) of racemic and enantiomerically enriched BCH-189 (prepared by the method of Example 3) on the percent inhibition of human PBM cells infected with HIV-1. ((-darkened circle-, (±)-BCH-189), (-circle-, (−)-BCH-189), (-darkened square-, (+)-BCH-189).

FIG. **7** is a graph of the uptake of tritiated (±)-FTC in human PBM cells (average of two determinations) in time (hours) versus pmol/10^6 cells.

FIG. **8** is a graph of the egress of radiolabeled (±)-FTC from human PBM cells, measured in hours versus pmol/10^6 cells ((-darkened square-), (±)-FTC; (-///-), (±)-FTC monophosphate; (-// // //-), (±)-FTC diphosphate; and (-/// ///-), (±)-FTC triphosphate).

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term "enantiomerically enriched nucleoside" refers to a nucleoside composition that includes at least 95% of a single enantiomer of that nucleoside.

As used herein, the term BCH-189 refers to 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane.

As used herein, the term FTC refers to 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane.

As used herein, the term "preferential enzyme catalysis" refers to catalysis by an enzyme that favors one substrate over another.

I. Resolution of Racemic Mixtures of Nucleosides During the Hydrolysis

A method is provided for the resolution of racemic mixtures of nucleoside enantiomers. The method involves the use of an enzyme that preferentially catalyzes a reaction of one enantiomer in a racemic mixture. The reacted enantiomer is separated from the unreacted enantiomer on the basis of the new difference in physical structure. Given the disclosure herein, one of skill in the art will be able to choose an enzyme that is selective for the nucleoside enantiomer of choice (or selective for the undesired enantiomer, as a method of eliminating it), by selecting of one of the enzymes discussed below or by systematic evaluation of other known enzymes. Given this disclosure, one of skill in the art will also know how to modify the substrate as necessary to attain the desired resolution. Through the use of either chiral NMR shift reagents, polarimetry, or chiral HPLC, the optical enrichment of the recovered ester can be determined.

The following examples further illustrate the use of enzymes to resolve racemic mixtures of enantiomers. Other known methods of resolution of racemic mixtures can be used in combination with the method of resolution disclosed herein. All of these modifications are considered within the scope of the invention.

Resolution Based on Hydrolysis of C5'-Nucleoside Esters

In one embodiment, the method includes reacting the C5'-hydroxyl group of a mixture of nucleoside racemates with an acyl compound to form C5'-esters in which the nucleoside is in the "carbinol" end of the ester. The racemic mixture of nucleoside C5'-esters is then treated with an enzyme that preferentially cleaves, or hydrolyses, one of the enantiomers and not the other.

An advantage of this method is that it can be used to resolve a wide variety of nucleosides, including pyrimidine and purine nucleosides that are optionally substituted in the carbohydrate moiety or base moiety. The method can also be used to resolve nucleoside derivatives that contain additional heteroatoms in the carbohydrate moiety, for example, FTC and BCH-189. The broad applicability of this method resides in part on the fact that although the carbinol portion of the ester plays a role in the ability of an enzyme to differentiate enantiomers, the major recognition site for these enzymes is in the carboxylic acid portion of the ester. Further, one may be able to successfully extrapolate the results of one enzyme/substrate study to another, seemingly-different system, provided that the carboxylic acid portions of the two substrates are the same or substantially similar.

Another advantage of this method is that it is regioselective. Enzymes that hydrolyse esters typically do not catalyze extraneous reactions in other portions of the molecule. For example, the enzyme lipase catalyses the hydrolysis of the ester of 2-hydroxymethyl-5-oxo-1,3-oxathiolane without hydrolysing the internal lactone. This contrasts markedly with "chemical" approaches to ester hydrolysis.

Still another advantage of this method is that the separation of the unhydrolysed enantiomer and the hydrolysed enantiomer from the reaction mixture is quite simple. The unhydrolysed enantiomer is more lipophilic than the hydrolysed enantiomer and can be efficiently recovered by simple extraction with one of a wide variety of nonpolar organic solvents or solvent mixtures, including hexane and hexane/ether. The less lipophilic, more polar hydrolysed enantiomer can then be obtained by extraction with a more polar organic solvent, for example, ethyl acetate, or by lyophilization, followed by extraction with ethanol or methanol. Alcohol should be avoided during the hydrolysis because it can denature enzymes under certain conditions.

Enzymes and Substrates

With the proper matching of enzyme and substrate, conditions can be established for the isolation of either nucleoside enantiomer. The desired enantiomer can be isolated by treatment of the racemic mixture with an enzyme that hydrolyses the desired enantiomer (followed by extraction of the polar hydrolysate with a polar solvent) or by treatment with an enzyme that hydrolyses the undesired, enantiomer (followed by removal of the undesired enantiomer with a nonpolar solvent).

Enzymes that catalyze the hydrolysis of esters include esterases, for example pig liver esterase, lipases, including porcine pancreatic lipase and Amano PS-800 lipase, substillisin, and α-chymotrypsin.

The most effective acyl group to be used to esterify the C5'-position of the nucleoside can be determined without undue experimentation by evaluation of a number of homologs using the selected enzyme system. For example, when 1,3-oxathiolane nucleosides are esterified with butyric acid, resolutions with both pig liver esterase and Amano PS-800 proceed with high enantioselectivity (94–100 enantiomeric excess) and opposite selectivity. Non-limiting examples of other acyl groups that can be evaluated for use with a particular nucleoside enantiomeric mixture and particular enzyme include alkyl carboxylic acids and substituted alkyl carboxylic acids, including acetic acid, propionic acid, butyric acid, and pentanoic acid. With certain enzymes, it may be preferred to use an acyl compound that is significantly electron-withdrawing to facilitate hydrolysis by weakening the ester bond. Examples of electron-withdrawing acyl groups include α-haloesters such as 2-chloropropionic acid, 2-chlorobutyric acid, and 2-chloropentanoic acid. α-Haloesters are excellent substrates for lipases.

Resolution Conditions

The enzymatic hydrolyses are typically carried out with a catalytic amount of the enzyme in an aqueous buffer that has a pH that is close to the optimum pH for the enzyme in question. As the reaction proceeds, the pH drops as a result of liberated carboxylic acid. Aqueous base should be added to maintain the pH near the optimum value for the enzyme. The progress of the reaction can be easily determined by monitoring the change in pH and the amount of base needed to maintain pH. The hydrophobic ester (the unhydrolysed enantiomer) and the more polar alcohol (the hydrolysed enantiomer) can be sequentially and selectively extracted from the solution by the judicious choice of organic solvents. Alternatively, the material to be resolved can be passed through a column that contains the enzyme immobilized on a solid support.

Enzymatic hydrolyses performed under heterogeneous conditions can suffer from poor reproducibility. Therefore, it is preferred that the hydrolysis be performed under homogeneous conditions. Alcohol solvents are not preferred because they can denature the enzymes. Homogeneity can be achieved through the use of non-ionic surfactants, such as Triton X-100. However, addition of these surfactants not only assists in dissolving the starting material, they also enhance the aqueous solubility of the product. Therefore, although the enzymatic reaction can proceed more effectively with the addition of a non-ionic surfactant than under heterogeneous conditions, the isolation of both the recovered starting material and the product can be made more difficult. The product can be isolated with appropriate chromatographic and chemical (e.g., selective salt formation) techniques. Diacylated nucleosides can be used but are often quite lipophilic and hard to dissolve in the medium used.

EXAMPLE 1

Enantioselective Lipase-Catalyzed Hydrolysis of FTC Esters

A number of 5'-O-acyl derivatives of FTC were prepared by selective O-acylation of the N-hydrochloride salt (see Table 1) of (±)-FTC. The efficiency of the hydrolysis of the derivatives by lipases was investigated. As shown in Table 1, pig liver esterase (PLE) exhibits a high level of selectivity for the hydrolysis of the ester of the (+)-enantiomer of FTC. In contrast, PS-800 hydrolyses the ester of the (−)-enantiomer of FTC preferentially. The rate of the hydrolysis was also found to be dependent on the nature of the acyl group; the acetyl derivative was significantly slower than the butyryl derivative. It has now been discovered that the rate of the hydrolysis of the propionic acid ester of FTC is even faster than that observed for the butyrate derivative. % Recovery and % ee were both determined using HPLC. Although the enantioselectivity is excellent when employing PLE (typically 97% e.e. or higher), additional enrichment can be accomplished by sequential enzymatic hydrolysis

reactions in which the enantiomerically-enriched butyrate from a PLE-catalyzed hydrolysis is subjected to enzymatic hydrolysis by PS-800.

TABLE 1

Comparison of Effect of Ester on Enzyme Hydrolysis.

substrate	% recovery	% e.e. (s.m.)
FTC Esters with PLE:		
acetate	32.68	N.D.
propionate	39.87	N.D.
butyrate	48.00	98
butyrate	45.71	98.6
FTC Esters with PS800:		
acetate	73.17	N.D.
propionate	52.67	N.D.
butyrate	58.34	N.D.
valerate	41.50	94

EXAMPLE 2

Procedure for the Preparation of (+)- and (−)-FTC via Enantioselective, Lipase-Catalyzed Hydrolysis of FTC Butyrate

The 5'-O-butyrate of (±)-FTC (1) (0.47 mmol, 149 mg) was dissolved in 16 mL of a solution of 4:1 pH 8 buffer:CH_3CN. The clear solution was stirred and treated with 26 mg of pig liver esterase (PLE-A). The progress of the reaction was monitored by HPLC (FIG. **3**). After 20 hours (52% conversion), the reaction mixture was extracted with 2×80 mL of $CHCl_3$ and 80 mL of ethyl acetate. The organic layer extracts were combined, dried over anhydrous $MgSO_4$, filtered, and concentrated by rotary evaporation. The resulting residue was eluted on 2×1000 m pTLC plates using ethyl acetate as eluant (double elution) to give, after isolation, 53 mg (36% based on starting material) of FTC butyrate which was determined to have 98% enantiomeric excess (e.e.) by HPLC analysis. The enantiomerically-enriched butyrate was then treated with 1.6 mL of methanol followed by 0.38 mmol (20 mg) of sodium methoxide. The resulting mixture was stirred at room temperature, and the progress of the reaction was monitored by HPLC. The reaction was completed within 30 minutes. The solvent was removed by rotary evaporation to give a crude white solid (76 mg) that was eluted on a 1000 m pTLC using 5:1 ethyl acetate:ethanol. (−)-FTC was isolated as a white solid (33 mg; 82% yield). HPLC analysis of the FTC as its 5'-O-acetate derivative showed 97% e.e.; $[\alpha]({}^{20}{}_{,D})$ −120.5°(c =0.88; abs. ethanol).

Similarly, 1.2 mmol (375 mg) of the 5'-O-butyrate of (+)-FTC was dissolved in 40 mL of 4:1 pH 8 buffer-CH_3CN. The clear solution was stirred and treated with 58 mg of pig liver esterase (PLE-A). The progress of the reaction was monitored by HPLC. After 90 minutes (38% conversion), the reaction mixture was added to 150 mL of CHCl. The layers were separated and the aqueous layer lyophilized to remove solvent. The white residue from the lyophilization was extracted with 3×10 mL of absolute ethanol. The extracts were filtered, combined, and concentrated in vacuo to yield 179 mg of crude oil. The crude material was eluted on a 45×30 mm column of silica gel using 3×75 mL of ethyl acetate followed by 5:1 ethyl acetate-ethanol. (+)-FTC (a) was isolated as a white solid (109 mg; 37% based on starting butyrate). HPLC analysis of the (+)-FTC as its 5'-O-acetate derivative showed 97.4% e.e.; [a]O($^{20}{}_{,D}$) +113.4° (c=2.53; absolute ethanol)

A similar reaction was performed using 0.12 mmol (37 mg) of the 5'-O-butyrate of FTC and 7 mg of PS-800 in 4.0 mL of 4:1 pH 8 buffer:CH_3CN. The reaction was considerably slower than that with PLE-A and required 74 hours for 59% conversion. The recovered butyrate (11.4 mg; 31% of the initial amount) was found to be 94% e.e. by HPLC.

Resolution of Nucleoside Enantiomers with Cytidine-Deoxycytidine Deaminase

In an alternative embodiment, cytidine-deoxycytidine deaminase is used to resolve racemic mixtures of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane and its derivatives, including 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane. The enzyme catalyses the deamination of the cytosine moiety to a uridine. It has been discovered that one of the enantiomers of 1,3-oxathiolane nucleosides is a preferred substrate for cytidine-deoxycytidine deaminase. The enantiomer that is not converted to a uridine (and therefore is still basic) is extracted from the solution with an acidic solution. Care should be taken to avoid strong acidic solutions (pH below 3.0), that may cleave the oxathiolane ring.

Cytidine-deoxycytidine deaminase can be isolated from rat liver or human liver, or expressed from recombinant sequences in procaryotic system such as in *E. coli.*

The method of resolution of cytidine nucleoside enantiomers using cytidine-deoxycytidine deaminase can be used as the sole method of resolution or can be used in combination with other methods of resolution, including resolution by enzymatic hydrolysis of 5'-O-nucleoside esters as described above.

Combination of Enzymatic Resolution with Classical Resolution Methods

The process described above for resolving racemic mixtures of nucleoside enantiomers can be combined with other classical methods of enantiomeric resolution to increase the optical purity of the final product.

Classical methods of resolution include a variety of physical and chemical techniques. Often the simplest and most efficient technique is recrystallization, based on the principle that racemates are often more soluble than the corresponding individual enantiomers. Recrystallization can be performed at any stage, including on the acylated compounds and or the final enantiomeric product. If successful, this simple approach represents a method of choice.

When recrystallization fails to provide material of acceptable optical purity, other methods can be evaluated. If the nucleoside is basic (for example, a cytidine) one can use chiral acids that form diastereomeric mixtures that may possess significantly different solubility properties. Nonlimiting examples of chiral acids include malic acid, mandelic acid, dibenzoyl tartaric acid, 3-bromocamphor-8-sulfonic acid, 10-camphorsulfonic acid, and di-p-toluoyltartaric acid. Similarly, acylation of the free hydroxyl group with a chiral acid derivative also results in the formation of diastereomeric mixtures whose physical properties may differ sufficiently to permit separation.

Small amounts of enantiomerically enriched nucleosides can be obtained or purified by passing the racemic mixture through an HPLC column that has been designed for chiral separations, including cyclodextrin bonded columns marketed by Rainin Corporation.

EXAMPLE 3

Separation of Racemic Mixtures of Nucleosides by HPLC

The resolutions of the C4'-enantiomers of (±)-BCH-189 and (±)-FTC were performed using a chiral cyclodextrin bonded (cyclobond AC-I) column obtained from Rainin Corporation (Woburn, Mass.). The conditions were as follows: Isocratic 0.5% methanol in water; flow rate 1 ml/min., UV detection at 262 nm. HPLC grade methanol was obtained from J. T. Baker (Phillipsburg, N.J.). The racemic mixtures were injected and fractions were collected. Fractions containing each of the enantiomers were pooled, frozen, and then lyophilized. The compounds were characterized by UV spectroscopy and by their retention times on HPLC. In general, the (−)-enantiomers have lower retention times than the (+)-enantiomers (see *J. Liquid Chromatography* 7:353–376, 1984). The concentrations of the compounds were determined by UV spectroscopy, using a stock solution of known concentration (15 μM) prepared in water for biological evaluation. The retention times for the separated enantiomers are provided in Table 2.

TABLE 2

Retention Times of Enantiomers of BCH-189 and FTC

Compound	R_t (min)
(−)-BCH-189	9.0
(+)-BCH-189	10.0
(−)-FTC	8.3
(+)-FTC	8.7

II. Antiviral Activity of 2-Hydroxymethyl-5-(5-Fluorocytosin-1-yl)-1,3-oxathiolane ("FTC")

It is often desirable to screen a number of racemic mixtures of nucleosides as a preliminary step to determine which warrant further resolution into enantiomerically enriched components and evaluation of antiviral activity. The ability of nucleosides to inhibit HIV can be measured by various experimental techniques. The technique used herein, and described in detail below, measures the inhibition of viral replication in phytohemagglutinin (PHA) stimulated human peripheral blood mononuclear (PBM) cells infected with HIV-1 (strain LAV). The amount of virus produced is determined by measuring the virus-coded reverse transcriptase enzyme. The amount of enzyme produced is proportional to the amount of virus produced. Table 4 provides the EC_{50} values (concentration of nucleoside that inhibits the replication of the virus by 50% in PBM cells) and IC_{50} values (concentration of nucleoside that inhibits 50% of the growth of mitogen-stimulated uninfected human PBM cells) of a number of (±)-1,3-oxathiolane and nucleosides.

EXAMPLE 4

Anti-HIV activity of (±)-1,3-Oxathiolane Nucleosides

A. Three-day-old phytohemagglutinin-stimulated PBM cells (10^6 cells/ml) from hepatitis B and HIV-1 seronegative healthy donors were infected with HIV-1 (strain LAV) at a concentration of about 100 times the 50% tissue culture infectious dose (TICD 50) per ml and cultured in the presence and absence of various concentrations of antiviral compounds.

B. Approximately one hour after infection, the medium, with the compound to be tested (2 times the final concentration in medium) or without compound, was added to the flasks (5 ml; final volume 10 ml). AZT was used as a positive control.

C. The cells were exposed to the virus (about 2×10^5 dpm/ml, as determined by reverse transcriptase assay) and then placed in a CO_2 incubator. HIV-1 (strain LAV) was obtained from the Center for Disease Control, Atlanta, Ga. The methods used for culturing the PBM cells, harvesting the virus and determining the reverse transcriptase activity were those described by McDougal et al. (*J. Immun. Meth.* 76, 171–183, 1985) and Spira et al. (*J. Clin. Meth.* 25, 97–99, 1987), except that fungizone was not included in the medium (see Schinazi, et al., *Antimicrob. Agents Chemother.* 32, 1784–1787 (1988); Id., 34:1061–1067 (1990)).

D. On day 6, the cells and supernatant were transferred to a 15 ml tube and centrifuged at about 900 g for 10 minutes. Five ml of supernatant were removed and the virus was concentrated by centrifugation at 40,000 rpm for 30 minutes (Beckman 70.1 Ti rotor). The solubilized virus pellet was processed for determination of the levels of reverse transcriptase. Results are expressed in dpm/ml of sampled supernatant. Virus from smaller volumes of supernatant (1 ml) can also be concentrated by centrifugation prior to solubilization and determination of reverse transcriptase levels.

The median effective (EC_{50}) concentration was determined by the median effect method (*Antimicrob. Agents Chemother.* 30, 491–498 (1986). Briefly, the percent inhibition of virus, as determined from measurements of reverse transcriptase, is plotted versus the micromolar concentration of compound. The EC_{50} is the concentration of compound at which there is a 50% inhibition of viral growth.

E. Mitogen stimulated uninfected human PBM cells (3.8×10^5 cells/ml) were cultured in the presence and absence of drug under similar conditions as those used for the antiviral assay described above. The cells were counted after 6 days using a hemacytometer and the trypan blue exclusion method, as described by Schinazi et al., *Antimicrobial Agents and Chemotherapy,* 22(3), 499 (1982). The IC_{50} is the concentration of compound which inhibits 50% of normal cell growth.

TABLE 3

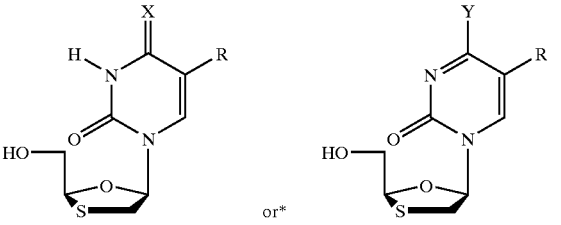
EC_{50} and IC_{50} of Various Analogues of 1,3-Oxathiolane Nucleosides in human PBM cells.

Code	X or Y	R	Antiviral EC_{50}, μM	Cytotoxicity IC_{50}, μM
DLS-009	X = O	H	>100	>100
DLS-010	X = O	Me	64.4	>100
DLS-027	X = O	F	>100	>100
DLS-028	X = O	Cl	60.8	>100
DLS-044	X = O	Br	>100	>100
DLS-029	X = O	I	>100	>100
DLS-020	Y = NH_2	H	0.02	>100
DLS-011	Y = NH_2	Me	>10	>100
DLS-022	Y = NH_2	F	0.011	>100
DLS-023	Y = NH_2	Cl	38.7	>100
DLS-021	Y = NH_2	Br	77.4	>100
DLS-026	Y = NH_2	I	0.72	>100
DLS-058(−)	Y = NH_2	F	0.0077	>100
DLS-059(+)	Y = NH2	F	0.84	>100
DLS-053	Y = NH_2	CF_3	60.7	>100

TABLE 3-continued

EC_{50} and IC_{50} of Various Analogues of 1,3-Oxathiolane Nucleosides in human PBM cells.

Code	X or Y	R	Antiviral EC_{50}, μM	Cytotoxicity IC_{50}, μM



As indicated in Table 3, in general, the substituted cytosine 1,3-oxathiolane nucleosides are more active than the corresponding uracil nucleosides. One of the compounds, (±)-FTC, (referred to as "DLS-022", compound 8) not only exhibited exceptional activity (approximately 10 nM in PBM cells), but also quite low toxicity (>100 μM in PBM, Vero and CEM cells). This activity compares quite favorably with 2',3'-dideoxyadenosine (DDA, EC_{50}=0.91 μM), 3'-azido-2',3'-dideoxyuridine (AZDU, EC_{50}= 0.18–0.46 μM), 3'-dideoxythymidine (DDT, EC_{50}=0.17 μM), and dideoxycytidine (DDC, EC_{50}=0.011 μM).

The IC_{50} of (±)-FTC was measured as over 100 μM, indicating that the compound was not toxic in uninfected PBM cells evaluated up to 100 μM.

EXAMPLE 5

Antiviral Activity of the Enantiomers of FTC Resolved by HPLC

The enantiomers of FTC were isolated by the method of Example 3, and the antiviral activity evaluated by the method of Example 4. The results are provided in Table 4, and illustrated in FIG. **4**.

TABLE 4

Antiviral Activity of the (+) and (−) Enantiomers of FTC

Treatment	Concn., μM	DPM/ml	% Inhibition (Corrected)	EC_{50};μM
FTC (±)	0.0001	73,755	26.6	0.018
	0.005	83,005	16.3	
	0.01	60,465	41.3	
	0.05	34,120	70.4	
	0.1	14,160	92.4	
	0.5	18,095	88.1	
	1	7,555	99.7	
	5	7,940	99.3	
	10	5,810	101.7	
FTC (−)	0.001	76,275	23.8	0.02
	0.005	58,590	43.3	
	0.01	75,350	24.8	
	0.05	28,890	76.2	
	0.1	13,175	93.5	
	0.5	9,485	97.6	
FTC (+)	0.001	94,340	3.8	0.28
	0.005	107,430	−10.6	
	0.01	99,465	−1.8	
	0.05	87,120	11.8	
	0.1	86,340	12.7	
	0.5	33,225	71.4	

As indicated in Table 4, the (−)-enantiomer of FTC is approximately one order of magnitude more potent than the (+)-FTC enantiomer, and has approximately the same anti-HIV activity as the racemic mixture. Neither the enantiomers nor the racemic mixture is toxic up to 100 μM as measured by Trypan Blue exclusion in human PBM cels.

EXAMPLE 6

Antiviral Activity of Enantiomers Resolved by Method of Example 2

The enantiomers of (±)-FTC were also resolved by the method of Example 2, and the antiviral activity evaluated by the method of Example 4. The results are illustrated in FIG. **5**. As indicated in FIG. **5**, the EC_{50} of the racemic mixture of FTC was measured at 0.017 μM, the EC_{50} of (−)-FTC at 0.0077 μM, and the EC_{50} of (+)-FTC at 0.84 μM.

The differences in EC_{50}s as measured in Examples 5 and 6 may be due to a number of factors, including differences in donor PBM cells, the inherent error of the anti-HIV screening procedure (estimated at approximately 10%), and differences in the measurement of concentration of the nucleosides as resolved in the methods of Examples 2 and 3. In the method of Example 2, the FTC enantiomers were isolated as solids and weighed to prepare the testing solution. In the method of Example 3, the concentration of the FTC enantiomers was estimated from UV absorption measurements.

The data indicates that the (+) enantiomer is significantly less potent than the (−) entantiomer or the racemic mixture.

EXAMPLE 7

Antiviral Activity of the Enantiomers of BCH-189 Resolved by HPLC

The enantiomers of BCH-189 were isolated by the method of Example 3, and the antiviral activity evaluated by the method of Example 4. The results are provided in Table 5, and illustrated in FIG. **6**.

TABLE 5

Antiviral Activity of the (+) and (−) Enantiomers of BCH-189

Treatment	Concn., μM	DPM/ml	% Inhibition (Corrected)	EC_{50};μM
Blanks	mean	762		
HIV Std.		158,705		
Uninfected	mean	7,320		
Control	±SD	4,520		
Infected	mean	97,795		
Control	±SD	6,790		
BCH-189 (±)	0.001	65,170	36.1	0.081
	0.005	62,595	38.9	
	0.01	70,875	29.8	
	0.05	77,650	22.3	
	0.1	33,165	71.4	
	0.5	10,765	96.2	
	1	7,745	99.5	
	5	6,800	100.6	
	10	4,470	103.2	
BCH-189 (−)	0.001	76,400	23.6	0.016
	0.005	66,875	34.2	
	0.01	54,170	48.2	
	0.05	57,615	44.4	
	0.1	34,705	69.7	
	0.5	15,250	91.2	
BCH-189 (+)	0.00085	71,795	28.7	0.23
	0.00425	99,710	−2.1	
	0.0085	68,355	32.5	
	0.0415	82,845	16.5	
	0.0825	65,100	36.1	
	0.412	43,260	60.3	

As indicated in Table 6, the (−)-enantiomer of BCH-189 is approximately one order of magnitude more potent than

the (+)-FTC enantiomer, and has approximately the same anti-HIV activity as the racemic mixture. Neither enantiomer exhibited any toxicity in a concentration up to 100 μM as measured by Trypan Blue exclusion in human PBM cells.

EXAMPLE 8

Uptake of (±)-FTC Into Human PBM Cells

Studies were undertaken using radiolabeled agent in order to follow the intracellular profiles of the parent drug and metabolites detected within the cell. All studies were conducted in duplicate. Human peripheral blood mononuclear cells (PBM cells) are suspended in RPMI 1640 medium containing 10% getal calf serum and antibiotics (2×10"cells/ml), 10 ml per timepoint) and incubated with addition of 10 μM FTC (specific ctivity about 700 dpm/pmol). Cells are exposed to the drug for 2, 6, 12 and 24 hours. At these timepoints, the medium is removed and the cells are washed two times with cold Hank's balanced salt solution. Extraction is performed with addition of 0.2 ml of 60% cold methanol/water and stored overnight at −70° C. The following morning, the suspensions are centrifuged and extractions are repeated two times for 0.5 hours at −70° C. The total supernatants (0.6 ml) are lyophilized to dryness. The residues are resuspended in 250 μl of water and aliquots comprising between 50 and 100 μl are analyzed by HPLC. Quantitation of intracellular parent drug and metabolic derivatives are conducted by HPLC. Because of the potential acid lability of some compounds, a buffer system close to physiological pH is used for the separation of the metabolites.

FIG. **7** is a graph of the uptake of tritiated (±)-FTC in human PBM cells (average of two determinations) in time (hours) versus pmol/10^6 cells. The uptake studies indicate that radiolabeled FTC is readily taken up in human lymphocytes, that produce very large amounts of 5'-triphosphate.

EXAMPLE 9

Uptake of (±)-FTC into Human Liver Cells; HVB Activity of FTC

The same procedure was used with human liver cells as with PBM cells to determine uptake of FTC.

The (±)-FTC is taken up by hepG2 cells in large amounts. These human liver cells metabolize a large percentage of the (±)-FTC to (±)-FTC triphosphate.

These data in conjunction with other data indicate that (±)-FTC, as well as its (−) and (+) enantiomers, are effective as antiviral agents against HBV (hepatitis B virus).

EXAMPLE 10

Egress of (±)-FTC from Human PBM Cells

Studies were performed using radiolabeled FTC in order to follow the intracellular profiles of the parent drug and metabolites detected within the cell after removal of drug at different times after pulsing for 24 hours, the time needed for high levels of triphosphates to accumulate. Studies are conducted in duplicate. Uninfected cells (2–106 ml) are suspended in the appropriate medium supplemented with serum (10 ml per timepoint) and incubated at 37° C. in a 5% CO_2 incubator. Radiolabeled FTC concentration is 10 μM. After pulsing the cells with the labeled compound for the desired time, the cells are thoroughly washed and then replenished with fresh medium without the antiviral drugs (0 hr). At 0, 2, 4, 6, 12, 24, and 48 hours (second incubation time), the cells are removed, and immediately extracted with 60% cold methanol/water. The extract is obtained by centrifugation and removal of the cell pellet. The extracts are lyophilized and then stored at −70° C. Prior to analysis, the material is resuspended in 250 τl of HPLC buffer and immediately analyzed. Quantitation of intracellular parent drug and metabolic derivatives are conducted by HPLC, as follows.

Either a Micromeritics or Hewlett-Packard model 1090 PHLC system is used with an anion exchange Partisil 10 SAX column (Whatman, Inc.), at a flow rate of 1 ml/min, 1 kpsi pressure, using uv detection at 262 nm.

The mobile phase consists of:

a. deionized water

b. 2 mM NaH_2PO_4/16 mM NaOAc (pH 6.6)

c. 15 mM NaH_2PO_4/120.2 mM NaOAc (pH 6.6)

d. 100 mM NaH_2PO_4/800 mM NaOAc (pH 6.6)

Separation method: isocratic for 5 min with A, followed by a 15 min linear gradient to 100% B, followed by a 20 min linear gradient to 100% C, followed by 10 min linear gradient to 100% D, followed by 30 min isocratic with 100% D.

Retention times (minutes) in human cells:

Compound	Unchanged	Mono-phosphate	Diphosphate	Triphosphate
DLS-022	5.0	39.0	55.0	68.0
BCH-189	3.5	40.0	55.0	69.0

FIG. **8** is a graph of the egress of radiolabeled (±)-FTC from human PBM cells, measured in hours after drug removal versus concentration (pmol/10^6 cells). As indicated in the Figure, FTC-triphosphate has an intracellular half-life of approximately 12 hours and can be easily detected intracellularly at concentrations of 1–5 μM 48 hours after the removal of the extracellular drug, which is well above the EC_{50} for the compound. Further, the affinity (K') for (±)-FTC triphosphate against HIV RT is 0.2 μM, below the 48 hour concentration level.

III. Preparation of Pharmaceutical Compositions

Humans suffering from diseases caused by HIV infection can be treated by administering to the patient an effective amount of (±)-FTC, or its (−) or (+) enantiomer or a pharmaceutically acceptable salt thereof in the presence of a pharmaceutically acceptable carrier or diluent. The active materials can be administered by any appropriate route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, or topically, in liquid or solid form.

Pharmaceutically acceptable salts are known to those in the art and include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acids include hydrochloric, hydrobromic, sulfuric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, lactic, salicyclic, succinic, toluene-p-sulfonic, tartaric, acetic, citric, methanesulfonic, formic, benzoic, malonic, naphthalene-2-sulfonic and benzenesulfonic acids. Salts derived from appropriate bases include alkali metal (e.g., sodium), alkaline earth metal (e.g., magnesium), ammonium and quaternary amine.

The active compound is included in the pharmaceutically acceptable carrier or diluent in an amount sufficient to deliver to a patient a therapeutically effective amount of

compound to inhibit viral replication in vivo, especially HIV and HBV replication, without causing serious toxic effects in the patient treated. By "HIV inhibitory amount" is meant an amount of active ingredient sufficient to exert an HIV inhibitory effect as measured by, for example, an assay such as the ones described herein.

A preferred dose of (−) or (±)-FTC will be in the range from about 1 to 20 mg/kg of bodyweight per day, more generally 0.1 to about 100 mg per kilogram body weight of the recipient per day.

The compound is conveniently administered in unit dosage form: for example containing 7 to 7000 mg, preferably 70 to 1400 mg of active ingredient per unit dosage form.

Ideally the active ingredient should be administered to achieve peak plasma concentrations of the active compound of from about 0.2 to 70 μM, preferably about 1.0 to 10 μM. This may be achieved, for example, by the intravenous injection of a 0.1 to 5% solution of the active ingredient, optionally in saline, or administered as a bolus of the active ingredient.

The concentration of active compound in the drug composition will depend on absorption, inactivation, and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at varying intervals of time.

A preferred mode of administration of the active compound is oral. Oral compositions will generally include an inert diluent or an edible carrier. They may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition.

The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar, shellac, or other enteric agents. (±)-FTC, or its (−) or (+)-enantiomer or pharmaceutically acceptable salts thereof can be administered as a component of an elixir, suspension, syrup, wafer, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors. (±)-FTC, or its (−) or (+)-enantiomers, or pharmaceutically acceptable salts thereof can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action, such as antibiotics, antifungals, antiinflamnatories, or other antivirals, including other nucleoside anti-HIV compounds.

Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

The pharmaceutical composition can also include antifungal agents, chemotherapeutic agents, and other antiviral agents such as interferon, including α, β, and gamma interferon.

If administered intravenously, preferred carriers are physiological saline or phosphate buffered saline (PBS).

In a preferred embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc.

Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) are also preferred as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811 (which is incorporated herein by reference in its entirety). For example, liposome formulations may be prepared by dissolving appropriate lipid(s) (such as stearoyl phosphatidyl ethanolamine, stearoyl phosphatidyl choline, aractiadoyl phosphatidyl choline, and cholesterol) in an inorganic solvent that is then evaporated, leaving behind a thin film of dried lipid on the surface of the container. An aqueous solution of the active compound or its monophosphate, diphosphate, and/or triphosphate derivatives are then introduced into the container. The container is then swirled by hand to free lipid material from the sides of the container and to disperse. lipid aggregates, thereby forming the liposomal suspension.

IV. Preparation of Phosphate Derivatives of FTC

Mono, di, and triphosphate derivative of FTC can be prepared as described below.

The monophosphate can be prepared according to the procedure of Imai et al., *J. Org. Chem.,* 34(6), 1547–1550 (June 1969). For example, about 100 mg of FTC and about 280 μl of phosphoryl chloride are reacted with stirring in about 8 ml of dry ethyl acetate at about 0° C. for about four hours. The reaction is quenched with ice. The aqueous phase is purified on an activated charcoal column, eluting with 5% ammonium hydroxide in a 1:1 mixture of ethanol and water. Evaporation of the eluant gives ammonium FTC-5'-monophosphate.

The diphosphate can be prepared according to the procedure of Davisson et al., *J. Orq. Chem.,* 52(9), 1794–1801 (1987). FTC diphosphate can be prepared from the corresponding tosylate, that can be prepared, for example, by

reacting the nucleoside with tosyl chloride in pyridine at room temperature for about 24 hours, working up the product in the usual manner (e.g., by washing, drying, and crystallizing it).

The triphosphate can be prepared according to the procedure of Hoard et al., *J. Am. Chem. Soc.,* 87(8), 1785–1788 (1965). For FTC is activated (by making a imidazolide, according to methods known to those skilled in the art) and treating with tributyl ammonium pyrophosphate in DMF. The reaction gives primarily the triphosphate of the nucleoside, with some unreacted monophosphate and some diphosphate. Purification by anion exchange chromatography of a DEAE column is followed by isolation of the triphosphate, e.g., as the tetrasodium salt.

This invention has been described with reference to its preferred embodiments. Variations and modifications of the invention, a method of resolution and antiviral activity of nucleoside enantiomers, will be obvious to those skilled in the art from the foregoing detailed description of the invention. It is intended that all of these variations and modifications be included within the scope of the appended claims.

We claim:

1. The (−)-enantiomer of cis-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolane-5-yl)-(1H)-pyrimidin-2-one that is at least 95% free of the corresponding (+)-enantiomer.

2. (−)-Cis-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolane-5-yl)-(1H)-pyrimidin-2-one or a pharmaceutically acceptable salt, ester or salt of an ester thereof.

3. The substantially pure (−)-enantiomer of cis-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one or a pharmaceutically acceptable salt, ester, or salt of an ester thereof, wherein the (+) enantiomer is present in an amount of no more than 5% w/w.

4. The compound of claim **3** wherein the (+)-enantiomer is present in an amount of no more than about 2% w/w.

5. The compound of claim **3** wherein the (+)-enantiomer is present in an amount of less than 1% w/w.

6. A pharmaceutical composition comprising a compound as claimed in any one of claims **2**–**5** in combination with a pharmaceutically acceptable carrier.

7. (−)-Cis-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolane-5-yl)-(1H)-pyrimidin-2-one or a pharmaceutically acceptable salt thereof.

8. The 5'-O-alkyl derivative of the (−)-enantiomer of cis-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolane-5-yl)-(1H)-pyrimidin-2-one.

9. The 5'-O-alkylC(O)-derivative of the (−)-enantiomer of cis-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolane-5-yl)-(1H)-pyrimidin-2-one.

10. The derivative of claim **9**, wherein alkylC(O)— is selected from the group consisting of acetic, propionic, butyric, and pentanoic.

11. The monophosphate, diphosphate, or triphosphate of the (−)-enantiomer of cis-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolane-5-yl)-(1H)-pyrimidin-2-one.

12. A pharmaceutically acceptable salt of the (−)-enantiomer of cis-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolane-5-yl)-(1H)-pyrimidin-2-one that is at least 95% free of the corresponding (+)-enantiomer.

13. A pharmaceutical composition comprising an effective HIV treatment amount for humans of the (−)-enantiomer of cis-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolane-5-yl)-(1H)-pyrimidin-2-one that is at least 95% free of the corresponding (+)-enantiomer, in combination with a pharmaceutically acceptable carrier or diluent.

14. A pharmaceutical composition comprising an effective HIV treatment amount for humans of the (−)-enantiomer of a pharmaceutically acceptable salt of a compound of cis-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolane-5-yl)-(1H)-pyrimdin-2-one that is at least 95% free of the corresponding (+)-enantiomer, in combination with a pharmaceutically acceptable carrier or diluent.

15. The pharmaceutical composition of claim **13**, in a form for oral administration.

16. The pharmaceutical composition of claim **15**, wherein the composition is in tablet form.

17. The pharmaceutical composition of claim **15**, wherein the composition is in capsule form.

18. The pharmaceutical composition of claim **13**, wherein the composition is a liquid.

19. The pharmaceutical composition of claim **13**, in a form for intravenous administration.

20. The pharmaceutical composition of claim **19**, wherein the carrier comprises a sterile diluent for injection.

21. The pharmaceutical composition of claim **13**, in a form for topical administration.

22. The pharmaceutical composition of claim **14**, in a form for oral administration.

23. The pharmaceutical composition of claim **22**, wherein the composition is in tablet form.

24. The pharmaceutical composition of claim **22**, wherein the composition is in capsule form.

25. The pharmaceutical composition of claim **14**, wherein the composition is a liquid.

26. The pharmaceutical composition of claim **14**, in a form for intravenous administration.

27. The pharmaceutical composition of claim **17**, wherein the carrier comprises a sterile diluent for injection.

28. The pharmaceutical composition of claim **14**, in a form for topical administration.

* * * * *

EXHIBIT C

US008592397B2

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

(10) **Patent No.: US 8,592,397 B2**
(45) **Date of Patent: Nov. 26, 2013**

(54) **COMPOSITIONS AND METHODS FOR COMBINATION ANTIVIRAL THERAPY**

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(60) Provisional application No. 60/440,246, filed on Jan. 14, 2003, provisional application No. 60/440,308, filed on Jan. 14, 2003.

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(52) **U.S. Cl.**
USPC **514/81**

(58) **Field of Classification Search**
USPC 514/45; 424/400, 408
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,524,846	A	8/1970	Moffatt et al.
3,622,677	A	11/1971	Short et al.
3,682,930	A	8/1972	Bourquin et al.
3,994,974	A	11/1976	Murakami et al.
4,003,878	A	1/1977	Skaar et al.
4,258,062	A	3/1981	Jonas et al.
4,355,032	A	10/1982	Verheyden et al.
4,384,005	A	5/1983	McSweeney
4,430,343	A	2/1984	Iemura et al.
4,476,248	A	10/1984	Gordon et al.
4,724,233	A	2/1988	De Clercq et al.
4,808,716	A	2/1989	Holy et al.
4,816,570	A	3/1989	Farquhar
4,879,288	A	11/1989	Warawa et al.
4,957,924	A	9/1990	Beauchamp
4,968,788	A	11/1990	Farquhar
5,047,407	A	9/1991	Belleau et al.
5,075,445	A	12/1991	Jarvest et al.
5,142,051	A	8/1992	Holy et al.
5,151,426	A	9/1992	Belleau et al.
5,155,268	A	10/1992	Hester
5,177,064	A	1/1993	Bodor
5,179,104	A	1/1993	Chu et al.
5,204,466	A	4/1993	Liotta et al.
5,208,221	A	5/1993	Kim et al.
5,210,085	A	5/1993	Liotta et al.
5,386,030	A	1/1995	Kim et al.
5,466,806	A	11/1995	Belleau et al.
5,476,938	A	12/1995	Vemishetti et al.
5,486,520	A	1/1996	Belleau et al.
5,506,347	A	4/1996	Erion et al.
5,512,596	A	4/1996	Kim et al.
5,514,798	A	5/1996	Bischofberger et al.
5,538,975	A	7/1996	Dionne
5,587,480	A	12/1996	Belleau et al.
5,618,820	A	4/1997	Dionne
5,618,964	A	4/1997	Cheng et al.
5,627,186	A	5/1997	Cameron et al.
5,663,159	A	9/1997	Starrett, Jr. et al.
5,696,254	A	12/1997	Mansour et al.
5,733,788	A	3/1998	Bischofberger
5,744,596	A	4/1998	Mansour et al.
5,756,706	A	5/1998	Mansour et al.
5,763,606	A	6/1998	Mansour et al.
5,792,756	A	8/1998	Starrett, Jr. et al.
5,798,340	A	8/1998	Bischofberger et al.
5,814,639	A	9/1998	Liotta et al.

(Continued)

FOREIGN PATENT DOCUMENTS

EP	0 182 024	A2	5/1986
EP	0 206 459	A2	12/1986

(Continued)

OTHER PUBLICATIONS

Gilead Sciences Conference Call to Discuss Triangle Pharmaceuticals Acquisition dated Dec. 4, 2002, Ristig et al. (Tenofovir Disoproxil Fumarate, TDF) Therapy for Chronic Hepatitis B in Human Immunodeficiency Virus/Hepatitis B Virus—Coinfected Individuals for Whom Interferon-alpha and Lamivudine Therapy Have Failed, JID 2002; 186 pp. 1844-1847.*

Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc. Case No. 08-CV-10838 (Dec. 12, 2008).

Answer and Counterclaim against Gilead Sciences, Inc. filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Feb. 5, 2009).

Plaintiff's Reply to Teva's Counterclaim, filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Feb. 25, 2009).

First Amended Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Jul. 1, 2009).

(Continued)

Primary Examiner — Alton Pryor

(57) **ABSTRACT**

The present invention relates to therapeutic combinations of [2-(6-amino-purin-9-yl)-1-methyl-ethoxymethyl]-phosphonic acid diisopropoxycarbonyloxymethyl ester (tenofovir disoproxil fumarate, Viread®) and (2R, 5S, cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one(emtricitabine, Emtriva™, (-)-cis FTC) and their physiologically functional derivatives. The combinations may be useful in the treatment of HIV infections, including infections with HIV mutants bearing resistance to nucleoside and/or non-nucleoside inhibitors. The present invention is also concerned with pharmaceutical compositions and formulations of said combinations of tenofovir disoproxil fumarate and emtricitabine, and their physiologically functional derivatives, as well as therapeutic methods of use of those compositions and formulations.

26 Claims, No Drawings

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,859,021	A	1/1999	Cameron et al.
5,905,082	A	5/1999	Roberts et al.
5,914,331	A	6/1999	Liotta et al.
5,922,695	A	7/1999	Arimilli et al.
5,935,946	A	8/1999	Munger, Jr. et al.
5,977,089	A	11/1999	Arimilli et al.
6,043,230	A	3/2000	Arimilli et al.
6,057,305	A	5/2000	Holy et al.
6,069,249	A	5/2000	Arimilli et al.
6,113,920	A	9/2000	Maye et al.
6,114,343	A	9/2000	Liotta et al.
6,121,315	A	9/2000	Nair et al.
6,194,391	B1	2/2001	Schinazi et al.
6,312,662	B1	11/2001	Erion et al.
RE38,333	E	11/2003	Arimilli et al.
7,094,413	B2	8/2006	Buelow et al.
2001/0012518	A1	8/2001	Makool-Morehead et al.
2001/0014352	A1	8/2001	Batra et al.
2003/0203969	A1	10/2003	Bevec et al.
2004/0180089	A1	9/2004	Plachetka et al.
2004/0224917	A1	11/2004	Dahl et al.
2004/0253218	A1	12/2004	Eisenbach-Schwartz et al.
2005/0197320	A1	9/2005	Chen et al.
2006/0128692	A1	6/2006	Chen et al.
2006/0246130	A1	11/2006	Dahl et al.
2007/0036861	A1	2/2007	Oury et al.
2007/0077295	A1	4/2007	Dahl et al.
2007/0099902	A1	5/2007	Dahl et al.
2009/0036408	A1	2/2009	Dahl et al.

FOREIGN PATENT DOCUMENTS

EP	0 269 947	A1	6/1988
EP	0 369 409	A1	5/1990
EP	0 481 214	A1	4/1992
EP	0 482 657	A2	4/1992
EP	0 632 048	A1	1/1995
EP	0 647 649	A1	4/1995
EP	0 694 547	A2	1/1996
EP	1332757		8/2003
GB	942 152	A	11/1963
GB	1 523 865	A	9/1978
GB	2 111 043	A	6/1983
WO	WO 88/05438		7/1988
WO	WO 91/19721	A1	12/1991
WO	WO 92/01698		2/1992
WO	WO 92/09611	A1	6/1992
WO	WO 92/13869		8/1992
WO	WO-9214743	*	9/1992
WO	WO 94/03466		2/1994
WO	WO 94/03467	A2	2/1994
WO	WO 95/07919		3/1995
WO	WO 95/07920	A1	3/1995
WO	WO 95/32957	A1	12/1995
WO	WO 96/18605	A1	6/1996
WO	WO 98/04569	A1	2/1998
WO	WO 99/25352	A1	5/1999
WO	WO 99/61026		12/1999
WO	WO 00/25797	A1	5/2000
WO	WO-0208241	*	1/2002
WO	WO 02/062123	A2	8/2002
WO	WO 02/068058	A2	9/2002
WO	WO 02/070518	A1	9/2002
WO	WO 03/045327		6/2003
WO	WO 03/059327		7/2003
WO	WO 2004/064845	A1	8/2004
WO	WO 2005/021001		3/2005
WO	WO 2006/135933		12/2006
WO	WO 2006/135993	A1	12/2006

OTHER PUBLICATIONS

Amended Answer and Counterclaim against Gilead Sciences, Inc. filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd.•Case No. 08-CV-10838 (Jul. 20, 2009).

Plaintiff's Reply to Teva USA's Amended Counterclaim, filed by Gilead Sciences, Emory University, Case No. 08-CV-10838 (Aug. 10, 2009).

Second Amended Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Sep. 25, 2009).

Second Amended Answer and Counterclaim against Gilead Sciences, Inc. filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Filed Oct. 9, 2009).

Plaintiff's Reply to Teva USA's Second Amended Counterclaim, filed by Gilead Sciences, Emory University,Case No. 08-CV-10838 (Oct. 15, 2009).

Third Amended Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Sep. 25, 2009.

Third Amended Answer filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Filed Aug. 6, 2010).

Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Mar. 5, 2010).

Answer filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (May 10, 2010).

Declaration of Colleen Tracy in Support of a Motion for Leave to File Amended Complaint Case No. 10-CV-01796 (Apr. 11, 2011).

Memorandum of Law in Opposition of a Motion for Leave to File Amended Complaint Case No. 10-CV-01796 (Apr. 25, 2011).

Declaration of James Galbraith in Opposition of a Motion for Leave to File Amended Complaint Case No. 10-CV-01796 (Apr. 25, 2011).

Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc. and Emory University Case No. 10-CV-01798 (Mar. 5, 2010).

Answer filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01798 (May 10, 2010).

Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc. and Teva Pharmaceutials Industries, Ltd. filed by Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company. Case No. 10-CV-01851 (Mar. 11, 2010).

Answer filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case. No. 10-CV-01851 (May 17, 2010).

Beauchamp et al., "Amino acid ester prodrugs of acyclovir," *Antivir. Chem. and Chemoth.* 3(3):157-64, 1992.

Colla et al., "Synthesis and antiviral activity of water-soluble esters of acyclovir [9-[(2-Hydroxyethoxy)methyl]guanine]," *J. Med. Chem.* 26:602-04, 1983.

Davidsen et al., "N-(Acyloxyalkyl)pyridinium salts as soluble prodrugs of a potent platelet activating factor antagonist," J. Med. Chem. 37(26):4423-4429, 1994.

Engel, R., "Phosphonates as analogues of natural phosphates," Chem. Rev. 77(3):349-367, 1977.

Farquhar et al., "Synthesis and antitumor evaluation of Bis[(pivaloxy)methyl] 2'-deoxy-5-fluorouridine 5'-monophosphate (FdUMP): a strategy to introduce nucleotides into cells," *J. Med. Chem.* 37(23):3902-03, 1994.

Folkmann et al., "Acyloxymethyl carbonochloridates. New intermediates in prodrug synthesis," Synthesis, pp. 1159-1166, 1990.

McIntee et al., "Probing the mechanism of action and decomposition of amino acid phosphomonoester amidates of antiviral nucleoside prodrugs," *J. Med. Chem.* 40(2):3323-31, 1997.

Naesens et al., "Antiretroviral activity and pharmacokinetics in mice of oral Bis(Pivaloyloxymethyl)-9-(2-phosphonylmethoxyethyl)adenine, the Bis(Pivaloyloxymethyl)ester prodrug of 9-(2-Phosphonylmethoxyethyl)adenine," Antimicro AG & Chemo. 40(1)22-28, 1996.

Notari, "Prodrug Design," *Pharmaceutical Therapy*, 14:25-53, 1981.

Serafinowska et al., "Synthesis and in vivo Evaluation of prodrug of 9-{2-(Phosphonomethoxy) ethoxy} adenine," J. Med. Chem. 38:1372-1379, 1995.

(56) **References Cited**

OTHER PUBLICATIONS

Srinivas et al., "Metabolism and in vitro antiretroviral activities of Bis(Pivaloyloxymethyl) prodrugs of acyclic nucleoside phosphonates," Antimicro AG & Chemo. 37(10):2247-2250, 1993.
Thornber "Isosterism and Molecular Modification in Drug design" Chem. Soc. Reviews 18: 563-580, 1979.
Weller et al., "Orally active Fibrinogen receptor antagonists. 2. Amidoximes a prodrug of amidines," J. Med. Chem. 39:3139-3147, 1995.
Office Action for Patent Application No. 2,261,619 issued by the Canadian Patent Office (Dec. 22, 2004).
First Office Action for Patent Application No. 97197460.8 issued by the Chinese Patent Office (Jan. 16, 2004) (translation).
Second Office Action for Patent Application No. 97197460.8 issued by the Chinese Patent Office (Aug. 5, 2005) (translation).
Third Office Action for Patent Application No. 97197460.8 issued by the Chinese Patent Office (Jun. 9, 2006) (translation).
First Examination Report for Patent Application No. 602/DEL/2007 issued by the Indian Patent Office (Nov. 18, 2009).
Official Action for Patent Application No. 10-508318 issued by the Japanese Patent Office (Apr. 10, 2007) (translation).
Official Action for Patent Application No. 10-1999-7000806 issued by the Korean Intellectual Property Office (Apr. 28, 2005) (translation).
Decision of Rejection for Application No. 7000806/1999 issued by the Korean Intellectual Property Office (Jan. 20, 2006) (translation).
Official Action for Patent Application No. 333687 issued by the Intellectual Property Office of New Zealand (Mar. 2, 1999).
Decision of Rejection for Patent Application No. 86110757 issued by the Intellectual Property Office of Taiwan (Nov. 11, 1999) (translation).
Final Office Action for Patent Application No. 86110757 issued by the Intellectual Property Office of Taiwan (Sep. 5, 2000) (translation).
Final Office Action for Patent Application. No. 89123708 issued by the Intellectual Property Office of Taiwan (Apr. 12, 2001) (translation).
International Search Report for PCT/US1997/013244 (Oct. 20, 1997).
Request for Ex Parte Reexamination of U.S. Patent No. 5,922,695 (submitted Apr. 30, 2007).
Order Granting Request for Ex Parte Reexamination of U.S. Patent No. 5,922,695 (Jul. 13, 2007).
Official Action for Ex Parte Reexamination of U.S. Patent No. 5,922,695 (Dec. 11, 2007).
Request for Ex Parte Reexamination of U.S. Patent No. 5,977,089 (submitted Apr. 30, 2007).
Order Granting Request for Ex Parte Reexamination of U.S. Patent No. 5,977,089 (Jul. 13, 2007).
Official Action for Ex Parte Reexamination of U.S. Patent No. 5,977,089 (Jan. 16, 2008).
Request for Ex Parte Reexamination of U.S. Patent No. 6,043,230 (submitted Apr. 30, 2007).
Order Granting Request for Ex Parte Reexamination of U.S. Patent No. 6,043,230 (Jul. 13, 2007).
Official Action for Ex Parte Reexamination of U.S. Patent No. 6,043,230 (Dec. 11, 2007).
Alexander et al., "Investigation of (Oxodioxolenyl)methyl carbamates as nonchiral bioreversible prodrug moieties for chiral amines," J. Med. Chem. 39:480-486, 1996.
Arimilli et al., "Synthesis, in vitro biological evaluation and oral bioavailability of 9-[2-(phosphonomethoxy)propyl]adenine (PMPA) prodrugs," Antiviral Chem. & Chemo. 8(6):557-567, 1997.
Arimilli et al., "Orally bioavailable acylic nucleoside phosphonate prodrugs: Adefovir, Dipivoxil and Bis(POC)PMPA," vol. 3 (accepted for publication), Adv. Antiviral Drug Design, 1998.
Balzarini et al, "Differential antiherpesvirus and antiretrovirus effects of the (S) and (R) enantiomers of acyclic nucleoside phosphonates: potent and selective in vitro and in vivo antiretrovirus activities of (R)-9-(2-phosphonomethoxypropyl)-2,6-diaminopurine." Antimicrob Agents Chemother. Feb. 1993; 37(2): 332-338.
Benzaria et al., "New prodrugs of 9-(2-phosphonomethoxyethyl)adenine (PMEA): Synthesis and stability studies," Nucls. & Nuclt. 14(3-5):563-565, 1995.
Berge et al., "Pharmaceutical salts," J. Pharm. Sci. 66(1):1-19, 1977.
De Clercq et al., "(S)-9-(2,3-dihydroxypropyl)adenine: An aliphatic nucleoside analog with broad spectrum antiviral activity," Science 200:563-565, 1978.
Flaherty et al., "Synthesis and selective monoamine oxidase B-inhibiting properties of 1-Methy1-1,2,3,6-tetrahydropyrid-4-y1 carbamate derivatives: potential prodrugs of (R)-and (S)-Nordeprenyl," J. Med. Chem. 39:4759-4761, 1996.
Hammer et al., "Ether, carbonate and urethane deoxynucleoside derivatives as prodrugs," Acta Chemica Scandinavia 50:609-622, 1996.
Ikeda et al., "Studies of prodrugs III. A convenient and practical preparation of Amphicillin prodrugs," Chem. Pharm. Bull. 32:4316-4322, 1984.
Iyer et al., "Synthesis of acyloxyalkyl acylphosphonates as potential prodrugs of the antiviral Trisodium phosphonoformate (Foscarnet sodium)," Tet. Lett. 30(51): 7141-7144, 1989.
Krise et al., "Prodrugs of phosphates, phosphonates, and phosphinates," Advanced Drug Delivery Reviews 19:287-310, 1996.
Landgrebe, J. A., "Crystallization and filtration," Theory and Practice in the Organic Laboratory, 3rd Edition, pp. 65-77, 1982.
Lindahl et al., "Synthesis of an acyloxymethyl prodrug of the Inositol phosphate alpha-Trinositol," J. Carbohydrate Chemistry 15(5):549-554, 1996.
Maillard et al., "Adenosine receptor prodrugs: Synthesis and biological activity of derivatives of potent A1-selective agonists," J. Pharm. Sci. 83(1):46-53, 1994.
Osol et al., Editor, Remington's Pharmaceutical Sciences, Sixteenth Edition, pp. 1554-1557, 1980.
Robinson et al., "Discovery of the Hemifumarate and (alpha-L-Alanyloxy)methylester as prodrugs of an antirheumatic oxindole: Prodrugs for enolic OH group," J. Med. Chem. 39:10-18, 1996.
Safadi et al., "Phosphoryloxymethyl carbamates and carbonates—Novel water soluble prodrugs for amines and hindered alcohols," Pharm. Res. 10(9):1350-1355, 1993.
Sakamoto et al., "Studies on prodrugs II. Preparation and characterization of (5-substituted 2-oxo-1, 3-dioxolen-4-yl)methyl esters of Ampicillin," Chem. Pharm. Bull. 32(6):2241-2248, 1983.
Samara et al., "Pharmacokinetic analysis of Diethylcarbonate prodrugs of Ibuprofen and Naproxen," Biopharmaceutics & Drug Disposition 16:201-210, 1995.
Shaw et al., "Metabolism and pharmacokinetics of novel oral prodrugs of 9-[(R)-2-(phosphonomethoxy)propyl]adenine (PMPA) in dogs," Pharm. Res. 14(12):1824-1829, 1997.
Srivastva et al., "Bioreversible phosphate protective groups: Synthesis and stability of model acyloxymethyl phosphates," Bioorg. Chem. 12:118-129, 1984.
Starret et al., "Synthesis and in vitro evaluation of a phosphonate prodrug:bis(pivaloyloxymethyl) 9-(2-phophonylmethoxyethyl)adenine," Antiviral Res. 19:267-273, 1992.
Starret et al., "Synthesis, oral bioavailability determination, and in vitro evaluation of prodrugs of the Antiviral agent 9-[2-(phosphonomethoxy)ethyl)adenine (PMEA)," J. Med. Chem. 37:1857-1864, 1994.
Sueoka et al., "Pharmacokinetics of Alkoxycarbonyloxy ester prodrugs of PMPA in dogs," Abstract, American Association of Pharmaceutical Science, Western Regional Meeting, Apr. 24-25, 1997.
Tsai et al., "Prevention of SIV infection in macaques by (R)-9-(2-phosphonylmethoxypropyl)adenine," Science 270:1197-1199, 1995.
Tsai et al., "Effects of (R)-9-(2-phophonylmethoxypropyl)adenine monotherapy on chronic SIV infection in macaques," Aids Res. & Hum. Retro. 13(8):707-712, 1997.
Ueda et al., "Vinyl compounds of nucleic acid bases I. Synthesis of N-vinyluracil, N-vinylthymine, and N-vinyladenine," Die Makromolekulare Chemie 120:13-20, 1968.
Examiner's First Report on Patent Application No. 85827/98 issued by the Australian Patent Office (Feb. 28, 2001).

(56) **References Cited**

OTHER PUBLICATIONS

Examiner's Second Report on Patent Application No. 85827/98 issued by the Australian Patent Office (Mar. 27, 2002).
Office Action for Patent Application No. 2,298,059 issued by the Canadian Intellectual Property Office (Apr. 25, 2007).
First Office Action for Patent Application No. 200410046290.X issued by the Chinese Patent Office (Jun. 17, 2005) (translation).
First Office Action for Patent Application No. 200510099916.8 issued by the Chinese Patent Office (Jun. 15, 2007) (translation).
Second Office Action for Patent Application No. 200510099916.8 issued by the Chinese Patent Office (Nov. 16, 2007) (translation).
Office Action for Patent Application No. 11-510067 issued by the Japanese Patent Office (Dec. 11, 2007) (translation) (translation).
Office Action for Patent Application No. 10-2000-7000636 issued by the Korean Intellectual Property Office (Aug. 19. 2005) (translation).
Decision of Rejection for Patent Application No. 7000636/2000 issued by the Korean Intellectual Property Office (May 10, 2006) (translation).
Office Action for Patent Application No. 87112168 issued by the Taiwanese Intellectual Property Office (Oct. 20, 2000) (translation).
Decision of Rejection for Patent Application No. 87112168 issued by the Taiwanese Intellectual Property Office (Apr. 6, 2001) (translation).
Office Action for Patent Application No. 87112168 issued by the Taiwanese Intellectual Property Office (May 6, 2002) (translation).
Office Action for Patent Application No. 87112168 issued by the Taiwanese Intellectual Property Office (Mar. 1, 2004) (translation).
Office Action for Patent Application No. 93112403 issued by the Taiwanese Intellectual Property Office (Apr. 27, 2005) (translation).
Office Action for U.S. Appl. No. 08/900,752 issued by the United States Patent and Trademark Office (Apr. 16, 1998).
International Search Report for PCT/US1998/015254 (Nov. 25, 1998).
Request for Ex Parte Reexamination of U.S. Patent No. 5,935,946 (submitted Apr. 30, 2007).
Order Granting Request for Ex Parte Reexamination of U.S. Patent No. 5,935,946 (Jul. 13, 2007).
Official Action for Ex Parte Reexamination of U.S. Patent No. 5,935,946 (Jan. 16, 2008).
"Atripla Fact Sheet", www.fda.gov, [Online], Jul. 12, 2006, pp. 1-2 retrieved from the internet www.fda.gov/cder/drug/inforpage/atripla/factsheet.htm [retrieved on Jan. 31, 2007.
Banker, Modern Pharmaceutics, Drugs and the Pharmaceutical Sciences, p. 340, Mercel Dekker, Inc. 1996.
European Medicines Agency: "Scientific discussion (Truvada)," EMEA, [on line], Feb. 2005, pp. 1-28, (retrieved from http:// www.emea.eu.int/humandocs/PDFs/EPAR/truvada/2832505en6.pdf [retrieved on Jan. 31, 2007].
FDA: "Guidance for industry fixed dose combination and co-packaged drug products for treatment of HIV," www.fda.org. [on line], May 2004, pp. 1-17, (retrieved from http://www.fda.gov/oc/initiatives/hiv/hivguidance.html>[retrieved on Jan. 31, 2007].
Gilead: "Bristol-Myers Squibb and Gilead announce data supporting bioequivalence for single PIII fixed dose regimen of Sustiva® (efavirenz) and Truvada® (emtricitabine and tenofovir fumarate)" Gilead Press Release (online Jan. 9, 2006), pp. 1-5, retrieved from http://www.gilead.com/press.
Gilead: "Gilead provides update on development of fixed-dose regimen of Truvada (emtricitabine and tenofovir disoproxil fumarate) and Sustiva (efavirenz)," Gilead Press Release, [on line], Apr. 26, 2005, pp. 1-3, retrieved from http://www.gilead.com/press.
Gilead: "Gilead provides update on development of fixed-dose regimen of Truvada (emtricitabine and tenofovir disoproxil fumarate) and Sustiva (efavirenz)," Gilead Press Release, [on line], Aug. 9 2005, pp. 1-3, retrieved from http://www.gilead.com/press.
"HIV Treatment Information," Project Inform, (on line), Jan. 2006, pp. 1-3, retrieved from http://www.projinf.org/bn/news_013006.html [retrieved on Jan. 31, 2007].
Ibbotson et al., Drugs 2003, 63(11), 1089-1096.
Kleinebudde et al. European journal of Pharmaceutics and Biopharmaceutics, 2004, 58, 317-326.
Lachman, et al. (1987) "The Theory and Practice of Industrial Pharmacy", Varghese Publishing House, Dadar Bombay, pp. 330-331.
Parikh, Handbook of Pharmaceutical Granulation Tech., NY, Marcel Dekker Inc., 1996.
Pharmaceutical Technology (2005), Big Pharma Companies Team Up to Develop Once-Daily Triple-Combination HIV Drug,vol. 29, No. 4.
Pujari et al., "Safety and long term effectiveness of generic fixed-dose formulations of nevirpine-based HAART amongst antiretroviral-naïve HIV-infected patients in India," World Health Organization, [on line], Dec. 16, 2003, pp. 99-116; (Retrieved from: http://libdoc.who.int/publications/2003/a86263.pdf.
Staszewski et al., NEJM, 1999, 341 5,1865-1873.
U.S. Department of Health and Human Services (2004) "Guidance for Industry Fixed Dose Combination and Co-Packaged Drug Products for Treatment of HIV—Draft Guidance" pp. 1-21.
Examiners First Report Patent Application No. 20026257795 issued by the Australian Patent Office (Sep. 29, 2009).
Official Action for Patent Application. No. 200800033/27 issued by the Eurasian Patent Office (2010) (translation).
Communication from the Examining Division of the EPO for Appln No. 06773195.3 (May 13, 2009).
Official Action for Patent Application No. 7001077/2008 issued by the Korean Intellectual Property Office (Sep. 13, 2010).
First Examination Report for Application No. 564102 Issued by the Intellectual Property Office of New Zealand (Oct. 6, 2009).
International Search Report for PCT/US2006/023223 (Feb. 23, 2007).
Written Opinion issued by the ISA for PCT/US2006/023223 (Feb. 23, 2007).
Response to the Written Opinion of the ISA (May 10, 2007).
International Preliminary Report on Patentability for PCT/US2006/023223 (Oct. 8, 2007).
International Preliminary Report on Patentability for PCT/US2006/023222 (Oct. 8, 2007).
Byrn (editor), Solid State Chemistry of Drugs, 2cd Edition, p. 22, 1999.
Drugs and the Pharmaceutical Sciences, vol. 1999, p. 60 (Mark Gibson, ed), 2009.
Newman and Byrn, "Solid-state analysis of the active pharmaceutical ingredient in drug products" Drug Discovery Today, 8(19) 898-905 (2003).
Zhang et al. "Phase transformation considerations during process development and manufacture of solid oral dosage forms" Adv Drug Del Reviews 56(30), 371-390 (2004).
Office Action for Patent Application No. 2,611,520 issued by the Canadian Patent Office (Jun. 7, 2010).
Communication of Intent to Grant Patent Application No. 06773194.3 (EP1890681) and Druckexemplar issued by the European Patent Office (Jul. 15, 2008).
Decision to Grant Patent Application No. 06773194.3 (EP 1890681B1) issued by the European Patent Office (Dec. 11, 2008).
Teva Pharmaceuticals Industries Ltd., Notice of Opposition of EP 189068181 (Application No. 06773194.3) (Oct. 7, 2009).
Communication of notices of opposition pursuant to Rule 79(2) EPC for EP 1890681B1 (Application No. 06773194.3) and Request to File Observations (Nov. 12, 2009).
Reply of the Patent Proprietor to the Notice of Opposition of EP 1890681B1 (Application No. 06773194.3) (Jun. 22, 2010).
Summons to Attend Oral Proceedings and Annex to the Communication for the Opposition of EP 1890681B1 (Application No. 06773194.3) (Oct. 14, 2010).
Gilead Sciences Inc., Written Submission in preparation for Oral Proceedings for the Opposition of EP 1890681B1 (Application No. 06773194.3) (Feb. 4, 2011).
Gilead Sciences Inc., Written Submission in preparation for Oral Proceedings for the Opposition of EP 1890681B1 (Application No. 06773194.3) (Mar. 2 & 4, 2011).
Teva Pharmaceuticals Industries Ltd., Written Submission in preparation for Oral Proceedings for the Opposition of EP 1890681B1 (Application No. 06773194.3) (Mar. 17, 2011).

(56) **References Cited**

OTHER PUBLICATIONS

Information About the Results of the Oral Proceedings for EP 1890681B1 (Application No. 06773194.3) (Apr. 5, 2011).
Examination Report Patent Application No. 504045 issued by the Intellectual Property Office of New Zealand (Oct. 6, 2009).
International Search Report for PCT/US2006/023222 (Feb. 23, 2007).
Written Opinion of the ISA for PCT/US2006/023222 (Feb. 23, 2007).
"AIDS," Monthly Index of Medical Specialties, pp. 194-198 (2002).
"Anti-HIV Drug Updates—Three Drugs on the Near Horizon," Project Inform Perspective 35:4-7 (2003).
"Gilead Buys Triangle in $464M Deal" Pharma Marketletter, 1 page (Dec. 9, 2002).
"Gilead Captures Triangle for $464 Million," Chemical Market Reporter 262(21):1 page (Dec. 9, 2002).
"Gilead set to acquire Triangle for $464m," BT Catalyst 17(1):1 page (Jan. 1, 2003).
"New Uses for Tenofovir; More Questions about d4T," Project Inform Perspective 35:15-16 (2003).
"Pill" Encarta Dictionary, 2 pages (2009).
"Rescriptor," Patient Prescribing Information Leaflet, 7 pages (2001).
"Scientific Discussion," EMEA, pp. 1/28-3/28, European Medicines Agency: (Feb. 2005).
"Time-Release," Compact Oxford English Dictionary, 1 page (2009).
Anderson, "Antiviral dynamics and sex differences of zidovudine and lamivudine triphosphate concentrations in HIV-infected individuals," AIDS 17:2159-2168 (2003).
Arribas et al., "Tenofovir Disoproxil Fumarate, Entricitabine and Efavirenz Compared with Zidovudine/Lamivudine and Efavirenz in Treatment-Naive Patients 144-Week Analysis," JAIDS 47(1):74-78 (2008).
Bartlett et al., "Overview of the effectiveness of triple combination therapy in antiretroviral-naive HIV-1 infected adults," AIDS 15:1369-1377 (2001).
Benzaria et al., "Synthesis, in Vitro Antiviral Evaluation, and Stability Studies of Bis(S-acy1-2-thioethyl) Ester Derivatives of 9-[2-(Phosphonometyoxy)ethyl]adenine (PMEA) as Potential PMEA Prodrugs with Improved Oral Bioavailability," J. Med. Chem. 39:4958-4965 (1996).
Blackburn et al., "DNA and RNA structure," pp. 15-81, Nucleic Acids in Chemistry and Biology, 1996.
Bundgaard et al., "Design and Application of Prodrugs," pp. 113-191, Textbook of Drug Design and Development, 1991.
Conference Call Transcript—Gilead Sciences Conference call to Discuss Triangle Pharmaceuticals Acquisition. Event Date/Time Dec. 4, 2002/ 9:00 AM ET (11 pages).
Dando et al., "Emtricitabine/Tenofovir Disoproxil Fumarate," Drugs 64(18):2075-2082 (2004).
De Clercq et al., "New Developments in Anti-HIV Chemotherapy," Curr. Med. Chem. 8(13):1543-1572 (2001).
De Clercq et al., "New developments in anti-HIV chemotherapy," Farmaco 56(1-2):3-12 (2001).
De Clercq, "Antiviral drugs: current state of the art," J. Clin. Virol. 22:73-89 (2001).
De Clercq, "Highlights in the Development of New Antiviral Agents," Mini-Rev. Med. Chem. 2(2):163-175 (2002).
De Clercq, "New developments in anti-HIV chemotherapy," Biochem Biophys Acta 1587(2-3):158-175 (2002).
De Lombaert et al., "N-Phosphonomethyl Dipeptides and their Phosphonate Prodrugs, a New Generation of Neutral Endopeptidase (NEP, EC 3.4.24.11) Inhibitor," J. Med. Chem. 37:498-511 (1994).
Delehanty et al. Slides from the oral presentation for "A Randomized Study of Three Doses of FTC Versus 3TC in HIV-Infected Patients," 6th CROI (Jan. 31-Feb. 4, 1999) Chicago.
Delehanty et al., "A Phase I/II Randomized, Controlled Study of FTC Versus 3TC in HIV-Infected Patients," 6th CROI (Jan. 31-Feb. 4, 1999) Chicago, Session 5, Abstract 16.
Farquhar et al., "Biologically Reversible Phosphate-Protective Groups," J. Pharm. Sci. 72:324-325 (1983).
Fasman et al., pp. 385-394, Practical Handbook of Biochem. and Molec. Biol., 1989.
Fell et al., "The tensile strength of lactose tablets" J. Pharm. Pharmacol. 20:657-659 (1968).
Feng et al. 2009 "The triple combination of tenofovir, emtricitabine and efavirenz show synergistic anti-HIV-1 activity in vitro: a mechanism of action study," Retrovirology 6:44, http://www.retrovirology.com/content/6/1/44.
Feng, J. et al, "Mechanistic studies show that 9-)-FTC-TP is a better inhibitor of HIV-1 reverse transcriptase than 3TC-TP," FASEB 13:1511-1517 (1999).
Fiske et al., "Pharmacokinetics, safety and tolerability of single escalating doses of DMP 266, an HIV non-nucleoside reverse transcriptase inhibitor, in healthy volunteers," Pharm. Res. 14(11 Suppl.):S609 (1997).
Frampton et al., "Emtricitabine: A Review of Its Use in the Management of HIV Inspection," Drugs 65(10):1427-1448 (2005).
Freeman et al.., "3 Prodrug Design for Phosphate and Phosphonates," Progress in Medicinal Chemistry 34:112-147 (1997).
Fridland, "Tenofovir," Curr. Opin. Anti-Infect. Invest. Drugs 2(3):295-301 (2000).
Fung et al., "Tenofovir Disoproxil Fumarate: A Nucleotide Reverse Transcriptase Inhibitor for the Treatment of HIV Infection," Clin. Therapeutics 24(10):1515-1548 (2002).
Gilead Sciences, Inc., "Data Comparing Viread (R) and Emtriva (R) to Combivir (R) as Part of Combination HIV Therapy Published in New England Journal of Medicine," p. 1-5, Press Release, Jan. 18, 2006.
Gilead Sciences, Inc., "Gilead Sciences to Acquire Triangle Pharmaceuticals for $464 Million; Gilead to Launch Coviracil in 2003; Will Develop Co-Formulation of Viread and Coviracil," 2 pages, Press Release Dec 2, 2002.
Gilead Sciences, Inc., "U.S. FDA Approves Gilead Sciences' Emtriva A one-capsule, Once-Daily Medication for The Treatment of HIV," pp. 3-7, Press Release, Jul. 2, 2003.
Gilead Sciences, Inc., Physician Insert for Truvada, pp. 1-30 (2007).
Harris et al., "Genotypic Analysis of HIV-1 Infected Art Naive Patients Receiving Emtricitabine (FTC) or Lamivudine (3TC) in a Double Blind Equivalence Trial," 5th International Workshop on Drug Resistance and Treatment Strategies, Jun. 4-8, No. 104 (2001).
Havlir et al., "In Vivo Antagonism with Zidovudine plus Stavudine Combination Therapy," J. Infect. Disease 182:321-325 (2000).
Hazen et al., "Relative Anti-HIV-I Efficacy of Lamivudine and Emtricitabine In Vitro Is Dependent on Cell Type," J. AIDS 32:255-258 (2003).
Hostetler et al., "Greatly Enhanced Inhibition of Human Immunodeficiency Virus Type I Replication in CEM and HT 4-6C Cells by 3'-Deoxythymidine Diphosphate Dimyristoylglycerol, a Lipid Prodrug of 3'—Deoxythymidine," Antimicro. Agent Chemo. 36(9):2025-2029 (1992).
Hostetler et al.., "Synthesis and Antiretroviral Activity of Phospholipid Analogs of Azidothymidine and Other Antiviral Nucleosides," J. Biol. Chem. 265(11):6112-6117 (1990).
Ishida and Asao, "Self-association and unique DNA binding properties of the anti-cancer agent TAS-103, a dual inhibitor of topoisomerases I and II," Biochem. Biophys. Acta 1587(2-3):155-163 (2002).
Jones et al.., "Minireview: nucleotide prodrugs," Antiviral Res. 27:1-17 (1995).
Kearney et al., "Effect of Demographic Variables on the Pharmacokinetics of Tenofovir DF in HIV-Infected Patients and Healthy Subjects," 410 ICAAC Abstracts, Chicago, IL, Sep. 22-25, 2001, Abstract A-504.
Khamnei et al., "Neighboring Group Catalysis in the Design of Nucleotide Prodrugs," J. Med. Chem. 39:4109-4115 (1996).
King et al. "Potency of Nonnucleoside Reverse Transcriptase Inhibitors (NNRTIs) Used in Combination with Other Human Immunodeficiency Virus NNRTIs, NRTIs, or Protease Inhibitors," Antimicrobial Agents and Chemotherapy 46(6):1640-1646 (2002).

(56) **References Cited**

OTHER PUBLICATIONS

Kucera et al., "Novel Membrane-Interactive Ether Lipid Analogs That Inhibit Infectious HIV-1 Production and Induce Defective Virus Formation," AIDS Res. & Hum. Retro. 6:491-501 (1990).
Lieberman et al., "," Pharmaceutical Dosage Forms 1:177-178 (1989).
Liu et al., "Thymidylate synthase as a translational regulator of cellular gene expression," Biochem. Biophys. Acta 1587(2-3):174-182 (2002).
Loveday, "Nucleoside reverse transcriptase inhibitor resistance," JAIDS 26:S10-S24 (2001).
Margot et al., "Development of HIV-1 Drug Resistance Through 144 Weeks in Antiretroviral-Naive Subjects on Emtricitabine, Tenofovir Disoproxil Fumarate, and Efavirenz Compared with Lamivudine/Zidovudine and Efavirenz in Study GS-01-934," JAIDS 52(2):209-221 (2009).
Margot et al., "Genotypic and phenotypic analyses of HIV-1 in antiretroviral-experienced patients treated with tenofovir DF," AIDS 16:1227-1235 (2002).
Margot et al., "Resistance development over 144 weeks in treatment-naive patients receiving tenofovir disoproxil fumarate or stavudine with lamivudine and efavirenz in Study 903," HIV Medicine 7:442-450 (2006).
Masho et al., "Review of Tenofovir-Emtricitabine," Ther. Clin. Risk Manag. 3(6):1097-1104 (2007).
McColl et al., "Pooled Analysis of Recent Emtricitabine and Lamivudine Clinical Trials Reveals Differences in Rates of Development of the M184V/I Mutation," Poster No. PE7.3/17, 10th European AIDS Conference (EACS) Nov. 17-20, 2005, Dublin Ireland.
Miller et al., Sixth International Congress on Drug Therapy in HIV Infection, Nov. 17-21, 2002 (1 page).
Mills et al., "Artemis: Efficacy and Safety of Darunavir/ritonavir (DRV/r) 800/100mg Once-daily vs Lopinavir/ritonavir (LPV/r) in Treatment-naive, HIV-1-infected Patients at 96 wks," 48th Annual ICAAC/IDSA, 46th Annual Meeting, Washington, D.C. Oct. 25-28, 2008, Presentation No. H-1250c.
Molina et al., "A Lopinavir/Ritonavir-Based Once-Daily Regimen Results in Better Compliance and Is Non-inferior to a Twice-Daily Regimen Through 96 Weeks," AIDS Research and Human Retroviruses 23(12):1505-1514 (2007).
Molina et al., "Once-Daily Combination Therapy with Emtricitabine, Didanosine, and Efavirenz in Human Immunodeficiency Virus-Infected Patients," J. Infect. Dis. 182:599-602 (2000).
Mulato et al., "Anti-HIV activity of adefovir (PMEA) and PMPA in combination with antiretroviral compounds: in vitro analyses," Antiviral Res. 36(2):91-97 (1997).
Murry et al., "Reversion of the M184V Mutation in Simian Immunodeficiency Virus Reverse Transcriptase is Selected by Tenofovir, Even in the Presence of Lamivudine," J. Virol. 77(2):1120-1130 (2003).
Pallella et al., J. Med. Chem. 338:853-860 (1998).
Pharmaceutical Dosage Forms and Drug Delivery Systems, 6th Ed. pp. 171-174 (1995).
Piantadosi et al., "Synthesis and Evaluation of Novel Ether Lipd Nucleoside Conjugates for Anti-HIV-I Activity," J. Med. Chem. 34:1408-1414 (1991).
Pozniak et al., "Tenofovir Disoproxil Fumarate, Emtricitabine, and Efavirenz Versus Fixed-Dose Zidovudine/Lamivudine and Efavirenz in Antiretroviral-Naive Patients," JAIDS 43(5):535-540 (2006).
Puech et al., "Intracellular delivery of nucleoside monophosphates through a reductase-mediated activation process," Antiviral Res. 22:155-174 (1993).
Richman, "Antiretroviral activity of emtricitabine, a potent nucleoside reverse transcriptase inhibitor," Antivir. Ther. 6(2):83-88 (2001).
Richman, "HIV Chemotherapy" Nature 410:995-1001 (2001).
Ristig et al., "Tenofovir Disoproxil Fumarate Therapy for Chronic Hepatitis B in Human Immunodeficiency Virus/Hepatitis B Virus—Coinfected Individuals for Whom Interferon-α and Lamivudine Therapy Have Failed," J. Infect. Dis.186:1844-1847 (2002).
Rousseau et al., "Prototype trial design for rapid dose selection of antiretroviral drugs: an example using emtricitabine (Coviracil)," Journal of Antimicrobial Chemotherapy 48:507-513 (2001).
Sanne et al., "Two Randomized, Controlled, Equivalence Trials of Emtricitabine (FTC) to Lamivudine (3TC)," Poster 4432 presented at the XIV International AIDS Conference, Jul. 7-12, 2002, Barcelona, Spain.
Sanne et al., "Genotypic Analysis of HIV-1 Infected ART-Naive Patients Receiving Emtricitabine (FTC) or Lamivudine (3TC) in a Double Blind Equivalence Trial," Poster No. 4433, presented at the XIV International AIDS Conference Jul. 7-12, 2002, Barcelona, Spain.
Schinazi et al., "Characterization of Human Immunodeficiency Viruses Resistant to Oxathiolane-Cytosine Nucleosides," Antimicrobial Agents and Chemotherapy 374:875-881 (1993).
Schinazi et al., "Selective Inhibition of Human Immunodeficiency Viruses by Racemates and Enantiomers of cis-5-Fluoro-1-[2-(Hydroxymethyl)-1,3-Oxathiolan-5-yl]Cytosine," Antimicrobial Agents and Chemotherapy 36(11):2423-2431(1992).
Schinazi et al., Letter to the Editor "Assessment of the Relative Potency of Emtricitabine and Lamivudine," J. AIDS 34(2)243-245 (2003).
Siddiqui et al., "Design and Synthesis of Lipophilic Phosphoramidate D4T-MP Prodrugs Expressing High Potency Against HIV in Cell Culture: Structural Determinants for In Vitro Activity and QSAR," J. Med. Chem. 42(20):4122-4128 (1999).
Smith et al., "Randomized, double-blind, placebo-matched, multicenter trial of abavacir/lamivudine or tenofovir/emtricitabine with lopinavir/ritonavir for inital HIV treatment," AIDS 23:1547-1556 (2009).
Tamari, "A Decade in HIV Treatment: What Is the State of the Art and How Did We Arrive," Clinical Excellence for Nurse Practitioners 5(1):4-12 (2001).
Tisdale et al., "Rapid in vitro selection of human immunodeficiency virus type 1 resistant to 3'—thiacytidine inhibitors due to a mutation in the YMDD region of reverse transcriptase," Proc. Natl. Acad. Sci. USA 90:5653-5656 (1993).
Wainberg et al. "In vitro selection and characterization of HIV-1 with reduced susceptibility to PMPA," Antiviral Therapy 4:87-94 (1999).
Walmsley et al., "Gemini: A Noninferiority Study of Saquinavir/Ritonavir Versus Lopinavir/Ritonavir as Initial HIV-1 Therapy in Adults," J. Acquir. Immune Defic. Syndr. 50(4):367-374 (2009).
Wang et al. "Lack of Significant Pharmacokinetic Interactions between Emtricitabine and Other Nucleoside Antivirals in Healthy Volunteers," 41st ICAAC Abstracts, Chicago, IL, Sep. 22-25, 2001, Abstract A-505.
Wang et al. "Pharmacokinetic and pharmacodynamic characteristics of emtrictabine support its once daily dosing," Int. Conf. AIDS, Jul. 7-12 14:abstract TUPeB4546 (2002).
Yeni et al., "Antiretroviral Treatment for Adult HIV Infection in 2002," JAMA 288(2):222-235 (2002).
Yuan et al., "Degradation Kinetics of Oxycarbonyloxymethyl Prodrugs of Phosphonates in Solution," Pharm. Res. 18(2):234-237 (2001).
Search and Examination Report for Application No. AP/P/2005/003348 issued by the African Regional Intellectual Property Organization (Apr. 10, 2008).
Examiner's First Report on Patent Application No. 2004206821 issued by the Australian Patent Office (Aug. 28, 2007).
Examiner's Second Report on Patent Application No. 2004206821 issued by the Australian Patent Office (Aug. 20, 2008).
Examiner's First Report on Patent Application No. 2009200414 issued by the Australian Patent Office (Feb. 24, 2010).
Pre-Grant Opposition Petition against Brazilian Patent Application PI 0406760-6 (Aug. 20, 2010) (translation).
Office Action for Patent Application No. 2,512,475 issued by the Canadian Patent Office (Jan. 10, 2008).
First Office Action for Patent Application No. 200480002190.5 issued by the Patent Office of the People's Republic of China (Aug. 4, 2006) (translation).
Rejection Decision for Patent Application No. 200480002190.5 issued by the Patent Office of the People's Republic of China (Jan. 15, 2010) (translation).

(56) **References Cited**

OTHER PUBLICATIONS

Official Action for Patent Application No. 200501134/28 issued by the Eurasian Patent Office (Oct. 15, 2006) (translation).
Official Action for Patent Application No. 200501134/28 issued by the Eurasian Patent Office (Dec. 25, 2008) (translation).
Examiner's Remarks for Patent Application No. 200501134/28 issued by the Eurasian Patent Office (translation).
Communication and Annex for Patent Publication EP1583542 (Application No. 04701819.7) issued by the European Patent Office (Oct. 24, 2005).
Communication and Annex for Patent Publication EP1583542 (Application No. 04701819.7) issued by the European Patent Office (Jul. 26, 2006).
Communication and Annex for Patent Publication EP1583542 (Application No. 04701819.7) issued by the European Patent Office (Jul. 17, 2007).
Decision to Grant Patent Publication EP1583542 (Application No. 04701819.7) issued by the European Patent Office and Druckexemplar (May 23, 2008).
Teva Pharmaceutical Industries Ltd., Notice of Opposition of EP Patent EP1583542B1 (Application No. 04701819.7) (Mar. 13, 2009).
Generics [UK] Limited, Notice of Opposition of EP Patent EP1583542B1 (Application No. 04701819.7) (Mar. 18, 2009).
Communication of further notices of opposition pursuant to Rule 79(2) EPC for EP Patent EP1583542B1 (Application No. 04701819.7) and Request to File Observations (Apr. 23, 2009).
Reply of the Patent Proprietor to the Notices of Opposition of EP Patent EP1583542B1 (Application No. 04701819.7) (Jan. 4, 2010).
Letter Regarding the Opposition Procedure for EP Patent EP1583542B1 (Application No. 04701819.7) (Mar. 11, 2010).
Summons to Attend Oral Proceedings and Annex to the Communication for EP Patent EP1583542B1 (Application No. 04701819.7) (May 21, 2010).
Teva Pharmaceutical Industries Ltd., Written Submission in preparation for Oral Proceedings for EP Patent EP1583542B1 (Application No. 04701819.7) (Sep. 16, 2010).
Generics [UK] Limited, Written Submission in preparation for Oral Proceedings for EP Patent EP1583542B1 (Application No. 04701819.7) (Sep. 17, 2010).
Gilead Sciences Inc., Written Submission in preparation for Oral Proceedings for EP Patent EP1583542B1 (Application No. 04701819.7) (Sep. 17, 2010).
Information about the Results of Oral Proceedings for EP Patent EP1583542B1 (Application No. 04701819.7), Claims, Amended Claims and Minutes of the Oral Proceeding (Nov. 19, 2010).
Decision of the Opposition Division for EP Patent EP1583542B1 (Application No. 04701819.7), Claims, Grounds for the Decision and Provision of the minutes (Jan. 31 and Feb. 14, 2011).
Notice of Appeal of the Decision of the Opposition Division for EP Patent EP1583542B1 (Application No. 04701819.7) (Mar. 29, 2011).
Extended European Search Report for Patent Publication EP1923063 (Application No. 08152527.1) issued by the European Patent Office (Mar. 10, 2009).
Communication pursuant to Article 94(3) EPC for Patent Publication EP1923063 (Application No. 08152527.1) issued by the European Patent Office (Sep. 4, 2009).
Substantive Examination Report for Patent Application No. W0-200502145 issued by the Indonesian Patent Office (2010).
First Examination Report for Patent Application No. 3383/DELNP/2005 issued by the Indian Patent Office (Jul. 31, 2007).
Opponents Comments to the Reply Statement by the Applicant relating to Patent Application No. 3383/DELNP/2005 (Aug. 14, 2008).
Decision of Hearing of the Indian Patent Office for Patent Application No. 3383/DELNP/2005 (Mar. 25, 2009).
Office Action for Patent Application No. 2006-500939 issued by the Japanese Patent Office (Nov. 9, 2009) (translation).
Office Action for Patent Application No. 2006-500939 issued by the Japanese Patent Office (Mar. 25, 2010) (translation).
Office Action for Korean Patent Application No. 7013069/2005 issued by the Korean Intellectual Property Office (May 22, 2007) (translation).
Office Action for Patent Application No. 7007002/2008 issued by the Korean Intellectual Property Office (Jun. 11, 2008) (translation).
Decision of Rejection for Patent Application No. 7007002/2008 issued by the Korean Intellectual Property Office (Jan. 7, 2009) (translation).
Office Action for Patent Application No. 7009376/2009 issued by the Korean Intellectual Property Office (Oct. 9, 2009) (translation).
Examination Report for Patent Application No. 540728 issued by the Intellectual Property Office of New Zealand (Apr. 24, 2007).
Further Examination Report for Patent Application No. 540728 issued by the Intellectual Property Office of New Zealand (Feb. 11, 2008).
Further Examination Report for Patent Application No. 540728 issued by the Intellectual Property Office of New Zealand (Jun. 18, 2008).
Official Action for Patent Application No. 93100813 issued by the Taiwanese Intellectual Property Office (Jun. 22, 2005) (translation).
Rejection of Patent Application No. 93100813 issued by the Taiwanese Intellectual Property Office (Feb. 22, 2006) (translation).
Second Official Action for Patent Application No. a 2005 07947 issued by the Ukrainian Patent Office (2006) (translation).
Third Official Action for Patent Application No. a 2005 07947 issued by the Ukrainian Patent Office (2007) (translation).
First Official Action for Patent Application No. a 2008 00555 issued by the Ukrainian Patent Office (2011) (translation).
Opinion on Patent Application No. 1-2005-00812 issued by the Vietnamese Patent Office (Jul. 27, 2008).
International Search Report for PCT/US2004/000832 (Jul. 12, 2004).
Revised International Search Report for PCT/US2004/000832 (Aug. 5, 2004).
Written Opinion issued by the ISA for PCT/US2004/000832 (Jul. 12, 2004).
International Preliminary Report on Patentability for PCT/US2004/000832 (Dec. 29, 2004).
Fourth Amended Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. and Exhibits filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Oct. 3, 2011).
Forth Amended Answer filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Oct. 17, 2011).
Teva's Opening Claim Construction Brief filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Nov. 4, 2011).
Declaration of Michael J. Freno in Support of Teva's Opening Claim Construction Brief and Exhibits filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Nov. 4, 2011).
Plaintiffs' Opening Claim Construction Brief filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Nov. 4, 2011).
Declaration of Adam C. LaRock in Support of Plaintiffs' Claim Constructions and Exhibits filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Nov. 4, 2011).
Declaration of Paul A. Bartlett in Support of Plaintiffs' Claim Constructions and Exhibits filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Nov. 4, 2011).
Teva's Rebuttal Claim Construction Brief filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Dec. 16, 2011).
Declaration of Daniel P. Margolis in Support of Teva's Rebuttal Claim Construction Brief and Exhibits filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Dec. 16, 2011).
Plaintiffs' Rebuttal Claim Construction Brief filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Dec. 16, 2011).
Declaration of Adam C. LaRock in Support of Plaintiffs' Rebuttal Claim Constructions and Exhibits filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Dec. 16, 2011).

(56) **References Cited**

OTHER PUBLICATIONS

Order signed by Judge Richard J. Sullivan Case No. 08-CV-10838 (Dec. 19, 2011).
Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc. Case No. 09-CV-04463 (May 8, 2009).
Answer and Counterclaim filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 09-CV-04463 (Aug. 10, 2009).
Plaintiffs' Reply to Teva USA's Counterclaim filed by Gilead Sciences, Inc., Emory University Case No. 09-CV-04463 (Aug. 31, 2009).
Order signed by Judge Richard J. Sullivan Case No. 08-CV-10838 (May 26, 2010).
First Amended Complaint for Patent Infringement filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Jun. 15, 2011).
Answer to Amended Complaint filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jun. 29, 2011).
Plaintiff's Opening Claim Construction Brief filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Dec. 2, 2011).
Teva's Opening Claim Construction Brief filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Dec. 2, 2011).
Declaration of Daniel P. Margolis in Support of Teva's Opening Claim Construction Brief and Exhibits filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Dec. 2, 2011).
Declaration of Natalie Lieber and Exhibits filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Dec. 2, 2011).
Declaration of Allan S. Myerson in Support of Plaintiffs' Claim Constructions and Exhibits filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Dec. 2, 2011).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01796 (Dec. 5, 2011).
Teva's Rebuttal Claim Construction Brief filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jan. 13, 2012).
Declaration of Daniel P. Margolis in Support of Teva's Rebuttal Claim Construction Brief and Exhibits filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jan. 13, 2012).
Plaintiffs' Rebuttal Claim Construction Brief filed by Gilead Sciences, Inc., Emory University Case No. 10-CV-01796 (Jan. 13, 2012).
Declaration of Natalie Lieber in Support of Plaintiffs' Rebuttal Claim Constructions and Exhibits filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Jan. 13, 2012).
Endorsed Letter to Judge Richard J. Sullivan Case No. 10-CV-01796 (Jan. 13, 2012).
Transcript of Proceedings held on Apr. 26, 2012 Case No. 10-CV-01796.
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01796 (Apr. 26, 2012).
Stipulation and Agreement Regarding U.S. Patent Nos. 5,922,965, 5,935,946, 5,977,089, and 6,043,230 Case No. 10-CV-01796 (Oct. 9, 2012).
Transcript of Proceedings held on Oct. 3, 3012, 2012 Case No. 10-CV-01796.
Plaintiff's Pretrial Memorandum filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Jan. 14, 2013).
Plaintiff's Proposed Findings of Fact and Conclusions of Law filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Jan. 14, 2013).
Defendants' Pretrial Memorandum filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jan. 14, 2013).
Defendants' Proposed Findings of Fact and Conclusions of Law filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jan. 14, 2013).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01796 (Jan. 18, 2013).
Defendants' Memorandum in Opposition to Plaintiff's Pretrial Memorandum filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jan. 28, 2013).
Plaintiff's Opposition to Defendants' Pretrial Memorandum by Gilead Sciences, Inc. Case No. 10-CV-01796 (Jan. 28, 2013).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01796 (Jan. 30, 2013).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01798 (May 26, 2010).
Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co's Opening Claim Construction Brief Case No. 10-CV-01851 (Dec. 2, 2011).
Affirmation of Andrew W. Williams in Support of Plaintiffs Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co's Opening Claim Construction Brief and Exhibits Case No. 10-CV-01851 (Dec. 2, 2011).
Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd.'s Opening Claim Construction Brief Case No. 10-CV-01851 (Dec. 2, 2011).
Declaration of Karen C. Shen in Support of Teva's Opening Claim Construction Brief and Exhibits Case No. 10-CV-01851 (Dec. 2, 2011).
Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd.'s Rebuttal Claim Construction Brief Case No. 10-CV-01851 (Jan. 13, 2012).
Declaration of Slaven Jesic in Support of Teva's Rebuttal Claim Construction Brief and Exhibits Case No. 10-CV-01851 (Jan. 13, 2012).
Declaration of Robin D. Rogers, Ph.D. in Support of Teva's Claim Construction and Exhibits Case No. 10-CV-01851 (Jan. 13, 2012).
Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co's Rebuttal Claim Construction Brief Case No. 10-CV-01851 (Jan. 13, 2012).
Declaration of Andrew W. Williams in Support of Plaintiffs Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co's Rebuttal Claim Construction Brief and Exhibits Case No. 10-CV-01851 (Jan. 13, 2012).
Ait-Khaled, et al., "Zidovudine appears to prevent selection of K65R and L74V mutations normally selected by abacavir mono- or combination therapies not containing zidovudine" *Antiviral Therapy*, 2002, 7:S107 (Abstract).
Borroto-Esoda, et al. "In Vitro evaluation of the anti-HIV activity and metabolic interactions of tenofovir and emtricitabine" *Antiviral Therapy*, 2006, vol. 11, pp. 377-384.
Gallant, et al., "Early Non-Response to Tenofovir DF (TDF) + Abacavir (ABC) and Lamivudine (3TC) in a Randomized Trial Compared to Efavirenz (EFV) + ABC and 3TC: ESS30009 Unplanned Interim Analysis" *Abstr Intersci Conf Antimicrob Agents Chemother Intersci Conf Antimicrob Agents Chemother*, 2003, Abstract No. H-1722a.
Jamsek, et al. "Poor Virological Responses and Early Emergence of Resistance in Treatment Naïve, HIV-infected Patients Receiving a Once Daily Triple Nucleoside Regimen of Didanosine, Lamivudine, and Tenofovir DF" *11th Conf Retrovir Oppor Infect*, 2004, Abstract No. 51.
Lanier, et al. "Prediction of NRTI Optins by Linking Reverse Transcriptase Genotype to Phenotypic Breakpoints" *10th Conf Retrovir Oppor Infect* , 2003, Abstract No. 586.
Lu, et al., "Determination of Clinical Cut-Offs for Reduced Response to Tenofovir DF therapy in Antiretroviral-Experienced Patients" *Antiviral Therapy*, 2002, vol. 7(Suppl 1), 5104, Abstract No. 125.
Quan, et al., "Endogenous Reverse Transcriptase Assays Reveal Synergy between Combinations of the M184V and other Drug Resistance-conferring Mutations in Interactions with Nucleoside Analog Triphosphates" *J. Mol. Bio.*, 1998, vol. 227, pp. 237-247.

* cited by examiner

COMPOSITIONS AND METHODS FOR COMBINATION ANTIVIRAL THERAPY

This non-provisional application is a continuation of U.S. patent application Ser. No. 10/540,794, filed Mar. 20, 2006, which is a national stage entry of PCT/US04/00832, filed Jan. 13, 2004 which claims the benefit of Provisional Application Nos. 60/440,246 and 60/440,308, both filed Jan. 14, 2003, which is incorporated herein by reference.

FIELD OF THE INVENTION

The invention relates generally to combinations of compounds with antiviral activity and more specifically with anti-HIV properties. In particular, it relates to chemically stable combinations of structurally diverse anti-viral agents.

BACKGROUND OF THE INVENTION

Human immunodeficiency virus (HIV) infection and related diseases are a major public health problem worldwide. Human immunodeficiency virus type 1 (HIV-1) encodes at least three enzymes which are required for viral replication: reverse transcriptase (RT), protease (Prt), and integrase (Int). Although drugs targeting reverse transcriptase and protease are in wide use and have shown effectiveness, particularly when employed in combination, toxicity and development of resistant strains have limited their usefulness (Palella, et al *N. Engl. J. Med*. (1998) 338:853-860; Richman, D. D. *Nature* (2001) 410:995-1001). Human immunodeficiency virus type 1 (HIV-1) protease (Prt) is essential for viral replication and is an effective target for approved antiviral drugs. The HIV Prt cleaves the viral Gag and Gag-Pol polyproteins to produce viral structural proteins (p17, p24, p7 and p6) and the three viral enzymes. Combination therapy with RT inhibitors has proven to be highly effective in suppressing viral replication to unquantifiable levels for a sustained period of time. Also, combination therapy with RT and Prt inhibitors (PI) have shown synergistic effects in suppressing HIV replication. Unfortunately, a high percentage, typically 30 to 50% of patients currently fail combination therapy due to the development of drug resistance, non-compliance with complicated dosing regimens, pharmacokinetic interactions, toxicity, and lack of potency. Therefore, there is a need for new HIV-1 inhibitors that are active against mutant HIV strains, have distinct resistance profiles, fewer side effects, less complicated dosing schedules, and are orally active. In particular, there is a need for a less onerous dosage regimen, such as once per day oral dosing, optimally with as few pills as possible.

The use of combinations of compounds can yield an equivalent antiviral effect with reduced toxicity, or an increase in drag efficacy. Lower overall drug doses can reduce the frequency of occurrence of drug-resistant variants of HIV. Many different methods have been used to examine the effects of combinations of compounds acting together in different assay systems (Furman WO 02/068058). Lower doses predict better patient compliance when pill burden decreases, dosing schedules are simplified and, optionally, if synergy between compounds occurs (Loveday, C. "Nucleoside reverse transcriptase inhibitor resistance" (2001) *JAIDS Journal of Acquired Immune Deficiency Syndromes* 26:S10-S24). AZT (Zidovudine™, 3'-azido, 3'-deoxythymidine) demonstrates synergistic antiviral activity in vitro in combination with agents that act at HIV-1 replicative steps other than reverse transcription, including recombinant soluble CD4 castanospermine and recombinant interferon-α. However, it must be noted that combinations of compounds can give rise to increased cytotoxicity. For example, AZT and recombinant interferon-α have an increased cytotoxic effect on normal human bone marrow progenitor cells.

Chemical stability of combinations of antiviral agents is an important aspect of co-formulation success and the present invention provides examples of such combinations.

There is a need for new combinations of orally-active drugs for the treatment of patients infected with certain viruses, e.g. HIV, that provide enhanced therapeutic safety and efficacy, impart lower resistance, and predict higher patient compliance.

SUMMARY OF THE INVENTION

The present invention provides combinations of antiviral compounds, in particular compositions and methods for inhibition of HIV. In an exemplary aspect, the invention includes a composition including tenofovir disoproxil fumarate and emtricitabine which has anti-HIV activity. The composition of tenofovir DF and emtricitabine is both chemically stable and either synergistic and/or reduces the side effects of one or both of tenofovir DF and emtricitabine. Increased patient compliance is likely in view of the lower pill burden and simplified dosing schedule.

The present invention relates to therapeutic combinations of [2-(6-amino-purin-9-yl)-1-methyl-ethoxymethyl]-phosphonic acid diisopropoxycarbonyloxymethyl ester fumarate (tenofovir disoproxil fumarate, tenofovir DF, TDF, Viread®) and (2R, 5S, cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine, Emtriva™, (-)-cis FTC) and their use in the treatment of HIV infections including infections with HIV mutants bearing resistance to nucleoside and/or non-nucleoside inhibitors. The present invention is also concerned with pharmaceutical compositions and formulations of said combinations of tenofovir disoproxil fumarate and emtricitabine. Another aspect of the invention is a pharmaceutical formulation comprising a physiologically functional derivative of tenofovir disoproxil fumarate or a physiologically functional derivative of emtricitabine.

Therapeutic combinations and pharmaceutical compositions and formulations of the invention include the combination of PMEA or PMPA (tenofovir) compounds with emtricitabine or (2R, 5S, cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (3TC, lamivudine, Epivir™), and their use in the treatment of HIV infections.

One aspect of the invention is a method for the treatment or prevention of the symptoms or effects of an HIV infection in an infected animal which comprises administering to, i.e. treating, said animal with a therapeutically effective amount of a combination comprising [2-(6-ammo-purin-9-yl)-1-methyl-ethoxymethyl]-phosphonic acid diisopropoxycarbonyloxymethyl ester fumarate (tenofovir DF, TDF) or a physiologically functional derivative thereof, and (2R, 5S, cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine) or a physiologically functional derivative thereof.

Another aspect of the invention is a unit dosage form of a therapeutic combination comprising tenofovir disoproxil fumarate and emtricitabine, or physiological functional derivatives thereof. The unit dosage form may be formulated for administration by oral or other routes and is unexpectedly chemically stable in view of the properties of the structurally diverse components.

Another aspect of the invention is directed to chemically stable combination antiviral compositions comprising tenofovir disoproxil fumarate and emtricitabine. In a further

aspect of the invention, the chemically stable combinations of tenofovir disoproxil fumarate and emtricitabine further comprise a third antiviral agent. In this three-component mixture, the unique chemical stability of tenofovir disoproxil fumarate and emtricitabine is taken advantage of in order to enable the combination with the third antiviral agent. Particularly useful third agents include, by way of example and not limitation, those of Table A. Preferably, the third component is an agent approved for antiviral use in humans, more preferably, it is an NNRTI or a protease inhibitor (PI), more preferably yet, it is an NNRTI. In a particularly preferred embodiment, the invention is directed to a combination of the chemically stable mixture of tenofovir disoproxil fumarate and emtricitabine together with efavirenz.

Another aspect of the invention is a patient pack comprising at least one, typically two, and optionally, three active ingredients and other antiviral agents selected from tenofovir disoproxil fumarate and emtricitabine, and an information insert containing directions on the use of tenofovir disoproxil fumarate and emtricitabine together in combination.

Another aspect of the invention is a process for preparing the combinations hereinbefore described, which comprises bringing into association tenofovir DF and emtricitabine of the combination in a medicament to provide an antiviral effect. In a further aspect of the present invention, there is provided the use of a combination of the present invention in the manufacture of a medicament for the treatment of any of the aforementioned viral infections or conditions.

DETAILED DESCRIPTION OF THE INVENTION

While the invention will be described in conjunction with the enumerated claims, it will be understood that they are not intended to limit the invention to those claims. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the scope of the present invention as defined by the claims.

Definitions

Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings:

When tradenames are used herein, applicants intend to independently include the tradename product and the active pharmaceutical ingredients) of the tradename product.

The term "chemical stability" means that the two primary antiviral agents in combination are substantially stable to chemical degradation. Preferably, they are sufficiently stable in physical combination to permit commercially useful shelf life of the combination product. Typically, "chemically stable" means that a first component of the mixture does not act to degrade a second component when the two are brought into physical combination to form a pharmaceutical dosage form. More typically, "chemically stable" means that the acidity of a first component does not catalyzes or otherwise accelerate the acid decomposition of a second component. By way of example and not limitation, in one aspect of the invention, "chemically stable" means that tenofovir disoproxil fumarate is not substantially degraded by the acidity of emtricitabine. "Substantially" in this context means at least about less than 10%, preferably less than 1%, more preferably less than 0.1%, more preferably yet, less than 0.01% acid degradation of tenofovir disoproxil fumarate over a 24-hour period when the products are in a pharmaceutical dosage form.

The terms "synergy" and "synergistic" mean that the effect achieved with the compounds used together is greater than the sum of the effects that results from using the compounds separately, i.e. greater than what would be predicted based on the two active ingredients administered separately. A synergistic effect may be attained when the compounds are: (1) co-formulated and administered or delivered simultaneously in a combined formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation therapy, a synergistic effect may be attained when the compounds are administered or delivered sequentially, e.g. in separate tablets, pills or capsules, or by different injections in separate syringes. In general, during alternation therapy, an effective dosage of each active ingredient is administered sequentially, i.e. serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together. A synergistic antiviral effect denotes an antiviral effect which is greater than the predicted purely additive effects of the individual compounds of the combination.

The term "physiologically functional derivative" means a pharmaceutically active compound with equivalent or near equivalent physiological functionality to tenofovir DF or emtricitabine when administered in combination with another pharmaceutically active compound in a combination of the invention. As used herein, the term "physiologically functional derivative" includes any: physiologically acceptable salt, ether, ester, prodrug, solvate, stereoisomer including enantiomer, diastereomer or stereoisomerically enriched or racemic mixture, and any other compound which upon administration to the recipient, is capable of providing (directly or indirectly) such a compound or an antivirally active metabolite or residue thereof.

"Bioavailability" is the degree to which the pharmaceutically active agent becomes available to the target tissue after the agent's introduction into the body. Enhancement of the bioavailability of a pharmaceutically active agent can provide a more efficient and effective treatment for patients because, for a given dose, more of the pharmaceutically active agent will be available at the targeted tissue sites.

The compounds of the combinations of the invention may be referred to as "active ingredients" or "pharmaceutically active agents."

The term "prodrug" as used herein refers to any compound that when administered to a biological system generates the drug substance, i.e. active ingredient, as a result of spontaneous chemical reaction(s), enzyme catalyzed chemical reaction(s), and/or metabolic chemical reaction(s).

"Prodrug moiety" means a labile functional group which separates from the active inhibitory compound during metabolism, systemically, inside a cell, by hydrolysis, enzymatic cleavage, or by some other process (Bundgaard, Hans, "Design and Application of Prodrugs" in *Textbook of Drug Design and Development* (1991), P. Krogsgaard-Larsen and H. Bundgaard, Eds. Harwood Academic Publishers, pp. 113-191). Prodrug moieties can serve to enhance solubility, absorption and lipophilicity to optimize drug delivery, bioavailability and efficacy. A "prodrug" is thus a covalently modified analog of a therapeutically-active compound.

"Alkyl" means a saturated or unsaturated, branched, straight-chain, branched, or cyclic hydrocarbon radical derived by the removal of one hydrogen atom from a single carbon atom of a parent alkane, alkene, or alkyne. Typical alkyl groups consist of 1-18 saturated and/or unsaturated carbons, such as normal, secondary, tertiary or cyclic carbon atoms. Examples include, but are not limited to: methyl, Me (—CH_3), ethyl, Et (—CH_2CH_3), acetylenic (—C≡CH), ethylene, vinyl (—CH=CH_2), 1-propyl, n-Pr, n-propyl (—$CH_2CH_2CH_3$), 2-propyl, i-Pr, i-propyl (—$CH(CH_3)_2$), allyl (—CH_2CH=CH_2), propargyl (—CH_2C≡CH), cyclopropyl (—C_3H_5), 1-butyl, n-Bu, n-butyl

(—$CH_2CH_2CH_2CH_3$), 2-methyl-1-propyl, i-Bu, i-butyl (—$CH_2CH(CH_3)_2$), 2-butyl, s-Bu, s-butyl (—$CH(CH_3)CH_2CH_3$), 2-methyl-2-propyl, t-Bu, t-butyl (—$C(CH_3)_3$), 1-pentyl, n-pentyl, (—$CH_2CH_2CH_2CH_2CH_3$), 2-pentyl (—$CH(CH_3)CH_2CH_2CH_3$), 3-pentyl (—$CH(CH_2CH_3)_2$), 2-methyl-2-butyl (—$C(CH_3)_2CH_2CH_3$), cyclopentyl (—C_5H_9), 3-methyl-2-butyl (—$CH(CH_3)CH(CH_3)_2$), 3-methyl-1-butyl (—$CH_2CH_2CH(CH_3)_2$), 2-methyl-1-butyl (—$CH_2CH(CH_3)CH_2CH_3$), 1-hexyl (—$CH_2CH_2CH_2CH_2CH_2CH_3$), 5-hexenyl (—$CH_2CH_2CH_2CH_2CH{=}CH_2$) 1-hexyl (—$CH(CH_3)CH_2CH_2CH_2CH_3$), 3-hexyl (—$CH(CH_2CH_3)(CH_2CH_2CH_3)$), cyclohexyl (—$C_6H_{11}$), 2-methyl-2-pentyl (—$C(CH_3)_2CH_2CH_2CH_3$), 3-methyl-2-pentyl (—$CH(CH_3)CH(CH_3)CH_2CH_3$), 4-methyl-2-pentyl (—$CH(CH_3)CH_2CH(CH_3)_2$), 3-methyl-3-pentyl (—$C(CH_3)(CH_2CH_3)_2$), 2-methyl-3-pentyl (—$CH(CH_2CH_3)CH(CH_3)_2$), 2,3-dimethyl-2-butyl (—$C(CH_3)_2CH(CH_3)_2$), and 3,3-dimethyl-2-butyl (—$CH(CH_3)C(CH_3)_3$.

"Aryl" means a monovalent aromatic hydrocarbon radical of 6-20 carbon atoms derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system. Typical aryl groups include, but are not limited to, radicals derived from benzene, substituted benzene, naphthalene, anthracene, biphenyl, and the like.

"Arylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with an aryl radical. Typical arylalkyl groups include, but are not limited to, benzyl, 2-phenylethan-1-yl, 2-phenylethen-1-yl, naphthylmethyl, 2-naphthylethan-1-yl, 2-naphthylethen-1-yl, naphthobenzyl, 2-naphthophenylethan-1-yl and the like. The arylalkyl group 6 to 20 carbon atoms e.g., the alkyl moiety, including alkanyl, alkenyl or alkynyl groups, of the arylalkyl group is 1 to 6 carbon atoms and the aryl moiety is 5 to 14 carbon atoms.

"Substituted alkyl", "substituted aryl", and "substituted arylalkyl" mean alkyl, aryl, and arylalkyl respectively, in which one or more hydrogen atoms are each independently replaced with a substituent. Typical substituents include, but are not limited to, —X, —R, —O^-, —OR, —SR, —S^-, —NR_2, —NR_3, ═NR, —CX_3, —CN, —OCN, —SCN, —N═C═O, —NCS, —NO, —NO_2, ═N_2, —N_3, NC(═O)R, —C(═O)R, —C(═O)NRR —$S(═O)_2O^-$, —$S(═O)_2$OH, —$S(═O)_2$R, —$OS(═O)_2$OR, —$S(═O)_2$NR, —S(═O)R, —$OP(═O)O_2RR$, —$P(═O)O_2RR$ —P(═O)$(O^-)_2$, —$P(═O)(OH)_2$, —C(═O)R, —C(═O)X, —C(S)R, —C(O)OR, —$C(O)O^-$, —C(S)OR, —C(O)SR, —C(S)SR, —C(O)NRR, —C(S)NRR, —C(NR)NRR, where each X is independently a halogen: F, Cl, Br, or I; and each R is independently —H, alkyl, aryl, heterocycle, or prodrug moiety.

"Heteroaryl" and "Heterocycle" refer to a ring system in which one or more ring atoms is a heteroatom, e.g. nitrogen, oxygen, and sulfur. Heterocycles are described in: Katritzky, Alan R., Rees, C. W., and Scriven, E. *Comprehensive Heterocyclic Chemistry* (1996) Pergamon Press; Paquette, Leo A.; *Principles of Modern Heterocyclic Chemistry* W. A. Benjamin, New York, (1968), particularly Chapters 1, 3, 4, 6, 7, and 9; "The Chemistry of Heterocyclic Compounds, A series of Monographs" (John Wiley & Sons, New York, 1950 to present), in particular Volumes 13, 14, 16, 19, and 28. Exemplary heterocycles include but are not limited to substituents, i.e. radicals, derived from pyrrole, indole, furan, benzofuran, thiophene, benzothiophene, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-quinolyl, 3-quinolyl, 4-quinolyl, 2-imidazole, 4-imidazole, 3-pyrazole, 4-pyrazole, pyridazine, pyrimidine, pyrazine, purine, cinnoline, pthalazine, quinazoline, quinoxaline, 3-(1,2,4-N)-triazolyl, 5-(1,2,4-N)-triazolyl, 5-tetrazolyl, 4-(1-O 3-N)-oxazole, 5-(1-O, 3-N)-oxazole, 4-(1-S, 3-N)-thiazole, 5-(1-S, 3-N)-thiazole, 2-benzoxazole, 2-benzothiazole, 4-(1,2,3N)-benzotriazole, and benzimidazole.

Stereochemical definitions and conventions used herein generally follow S. P. Parker, Ed., *McGraw-Hill Dictionary of Chemical Terms* (1984) McGraw-Hill Book Company, New York; and Eliel, E. and Wilen, S., *Stereochemistry of Organic Compounds* (1994) John Wiley & Sons, Inc., New York. Many organic compounds exist in optically active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L or R and S are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (−) are employed to designate the sign of rotation of plane-polarized light by the compound, with (−) or l meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, these compounds, called stereoisomers, are identical except that they are mirror images of one another. A specific stereoisomer is also referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture or a racemate. The terms "racemic mixture" and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.

The term "chiral" refers to molecules which have the property of non-superimposability of the mirror image partner, while the term "achiral" refers to molecules which are superimposable on their mirror image partner.

The term "stereoisomers" refers to compounds which have identical chemical constitution, but differ with regard to the arrangement of the atoms or groups in space.

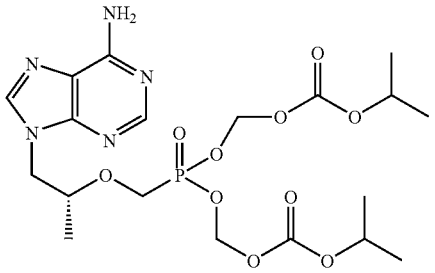
"Diastereomer" refers to a stereoisomer with two or more centers of chirality and whose molecules are not mirror images of one another. Diastereomers have different physical properties, e.g. melting points, boiling points, spectral properties, and reactivities. Mixtures of diastereomers may separate under high resolution analytical procedures such as electrophoresis and chromatography.

"Enantiomers" refer to two stereoisomers of a compound which are non-superimposable mirror images of one another.

Active Ingredients of the Combinations

The present invention provides novel combinations of two or more active ingredients being employed together. In some embodiments, a synergistic antiviral effect is achieved. In other embodiments, a chemically stable combination is obtained. The combinations include at least one active ingredient selected from (1) tenofovir disoproxil fumarate and physiologically functional derivatives, and at least one active ingredient selected from (2) emtricitabine and physiologically functional derivatives. The term "synergistic antiviral effect" is used herein to denote an antiviral effect which is greater than the predicted purely additive effects of the individual components (a) and (b) of the combination.

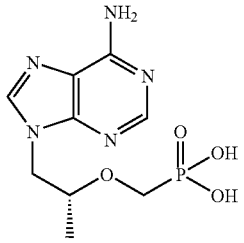
Tenofovir disoproxil fumarate (also known as Viread®, Tenofovir DF, Tenofovir disoproxil, TDF, Bis-POC-PMPA (U.S. Pat. Nos. 5,935,946, 5,922,695, 5,977,089, 6,043,230, 6,069,249) is a prodrug of tenofovir, and has the structure:



and including fumarate salt ($HO_2CCH_2CH_2CO_2^-$).

The chemical names for Tenofovir disoproxil include: [2-(6-amino-purin-9-yl)-1-methyl-ethoxymethyl]-phosphonic acid diisopropoxycarbonyloxymethyl ester; 9-[(R)-2-[[bis [[(isopropoxycarbonyl)oxy]methoxy]phosphinyl]methoxy] propyl]adenine; and 2,4,6,8-tetraoxa-5-phosphanonanedioic acid, 5-[[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy] methyl]-, bis(1-methylethyl)ester, 5-oxide. The CAS Registry numbers include: 201341-05-1; 202138-50-9; 206184-49-8. It should be noted that the ethoxymethyl unit of tenofovir has a chiral center. The R (rectus, right handed configuration) enantiomer is shown. However, the invention also includes the S isomer. The invention includes all enantiomers, diastereomers, racemates, and enriched stereoisomer mixtures of tenofovir (PMPA) and physiologically functional derivatives thereof.

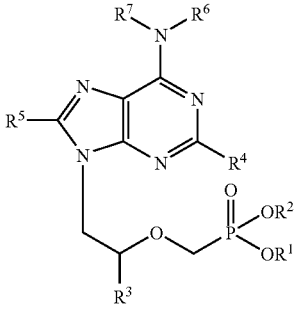
PMPA or tenofovir (U.S. Pat. Nos. 4,808,716, 5,733,788, 6,057,305) has the structure:



The chemical names of PMPA, tenofovir include: (R)-9-(2-phosphonylmethoxypropyl)adenine; and phosphonic acid, [[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy] methyl]. The CAS Registry number is 147127-20-6.

Tenofovir disoproxil fumarate (DF) is a nucleotide reverse transcriptase inhibitor approved in the United States in 2001 for the treatment of HIV-1 infection in combination with other antiretroviral agents. Tenofovir disoproxil fumarate or Viread® (Gilead Science, Inc.) is the fumarate salt of tenofovir disoproxil. Viread® may be named as: 9-[(R)-2-[[bis [[(isopropoxycarbonyl)oxy]methoxy]phosphinyl]methoxy] propyl]adenine fumarate (1:1); or 2,4,6,8-tetraoxa-5-phosphanonanedioic acid, 5-[[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy]methyl]-, bis(1-methylethyl) ester, 5-oxide, (2E)-2-butenedioate (1:1). The CAS Registry number is 202138-50-9.

Physiologically functional derivatives of tenofovir disoproxil fumarate include PMEA (adefovir, 9-((R)-2-(phosphonomethoxy)ethyl)adenine) and PMPA compounds. Exemplary combinations include a PMEA or PMPA compound in combination with emtricitabine or 3TC. PMEA and PMPA compounds have the structures:



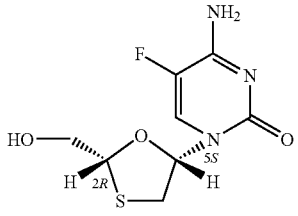
where PMEA (R^3 is H) and PMPA (R^3 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, or CH_2OR^8 where R^8 is C_1-C_6 alkyl, C_1-C_6 hydroxyalkyl or C_1-C_6 haloalkyl. R^6 and R^7 are independently H or C_1-C_6 alkyl. R^4 and R^5 are independently H, NH_2, NHR or NR_2 where R is C_1-C_6 alkyl. R^1 and R^2 are independently H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_6-C_{20} arylalkyl, C_6-C_{20} substituted arylalkyl, acyloxymethyl esters $—CH_2OC(=O)R^9$ (e.g. POM) or acyloxymethyl carbonates $—CH_2OC(=O)OR^9$ (e.g. POC) where R^9 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl or C_6-C_{20} substituted aryl. For example, R_1 and R_2 may be pivaloyloxymethoxy, POM, $—CH_2OC(=O)C(CH_3)_3$; $—CH_2OC(=O)OC(CH_3)_3$; or POC, $—CH_2OC(=O)OCH(CH_3)_2$. Also for example, tenofovir has the structure where R^3 is CH_3, and R^1, R^2, R^4, R^5, R^6 and R^7 are H. Dialkyl phosphonates may be prepared according to the methods of: Quast et al (1974) *Synthesis* 490; Stowell et al (3990) *Tetrahedron Lett.* 3261; U.S. Pat. No. 5,663,159.

The PMPA compound may be enantiomerically-enriched or purified (single stereoisomer) where the carbon atom bearing R^3 may be the R or S enantiomer. The PMPA compound may be a racemate, i.e. a mixture of R and S stereoisomers.

Adefovir (9-(2-phosphonomethoxyethyl)adenine where R_1-R_7=H) is an exemplary PMEA compound (U.S. Pat. Nos. 4,808,716, 4,724,233). As the bis-pivalate prodrug, Adefovir dipivoxil, also known as bis-POM PMEA, (R_3-R_7=H, R_1 and R_2=$CH_2OC(=O)C(CH_3)_3$, pivoxil, POM, pivaloyloxymethoxy), is effective against HIV and Hepatitis B infections (U.S. Pat. Nos. 5,663,159, 6,451,340). Adefovir dipivoxil has demonstrated minor to moderate synergistic inhibition of HIV replication in combination with other compounds with anti-HIV activity including PMPA, d4T, ddC, nelfinavir, ritonavir, and saquinavir (Mulato et al (1997) *Antiviral Research* 36:91-97).

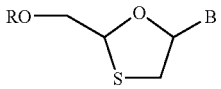
The invention includes all enantiomers, diastereomers, racemates, and enriched stereoisomer mixtures of PMEA and PMPA, and physiologically functional derivatives thereof.

Emtricitabine ((-)-cis-FTC, Emtriva™), a single enantiomer of FTC, is a potent nucleoside reverse transcriptase inhibitor approved for the treatment of HIV (U.S. Pat. Nos. 5,047,407, 5,179,104, 5,204,466, 5,210,085, 5,486,520, 5,538,975, 5,587,480, 5,618,820, 5,763,606, 5,814,639, 5,914,331, 6,114,343, 6,180,639, 6,215,004; WO 02/070518). The single enantiomer emtricitabine has the structure:



The chemical names for emtricitabine include: (-)-cis-FTC; β-L-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane; (2R,5S)-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine; and 4-amino-5-fluoro-1-(2-hydroxymethyl-[1,3]-(2R,5S)-oxathiolan-5-yl)-1H-pyrimidin-2-one. The CAS Registry numbers include: 143491-57-0; 143491-54-7. It should be noted that FTC contains two chiral centers, at the 2 and 5 positions of the oxathiolane ring, and therefore can exist in the form of two pairs of optical isomers (i.e. enantiomers) and mixtures thereof including racemic mixtures. Thus, FTC may be either a cis or a trans isomer or mixtures thereof. Mixtures of cis and trans isomers are diastereomers with different physical properties. Each cis and trans isomer can exist as one of two enantiomers or mixtures thereof including racemic mixtures. The invention includes all enantiomers, diastereomers, racemates, and enriched stereoisomer mixtures of emtricitabine and physiologically functional derivatives thereof. For example, the invention includes physiological functional derivatives such as the 1:1 racemic mixture of the enantiomers (2R, 5S, cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine) and its mirror image (2S, 5R, cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one, or mixtures of the two enantiomers in any relative amount. The invention also includes mixtures of cis and trans forms of FTC.

Physiologically functional derivatives of emtricitabine include 1,3 oxathiolane nucleosides having the structure:

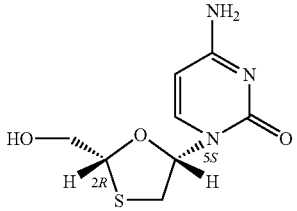


In the 1,3 oxathiolane nucleoside structure above, B is a nucleobase including any nitrogen-containing heterocyclic moiety capable of forming Watson-Crick hydrogen bonds in pairing with a complementary nucleobase or nucleobase analog, e.g. a purine, a 7-deazapurine, or a pyrimidine. Examples of B include the naturally occurring nucleobases: adenine, guanine, cytosine, uracil, thymine, and minor constituents and analogs of the naturally occurring nucleobases, e.g. 7-deazaadenine, 7-deazaguanine, 7-deaza-8-azaguanine, 7-deaza-8-azaadenine, inosine, nebularine, nitropyrrole, nitroindole, 2-aminopurine, 2-amino-6-chloropurine, 2,6-diaminopurine, hypoxanthine, pseudouridine, 5-fluorocytosine, 5-chlorocytosine, 5-bromocytosine, 5-iodocytosine, pseudocytosine, pseudoisocytosine, 5-propynylcytosine, isocytosine, isoguanine, 7-deazaguanine, 2-thiopyrimidine, 6-thioguanine, 4-thiothymine, 4-thiouracil, O^6-methylguanine, N^6-methyladenine, O^4-methylthymine, 5,6-dihydrothymine, 5,6-dihydrouracil, 4-methylindole, pyrazolo[3,4-D]pyrimidines (U.S. Pat. Nos. 6,143,877 and 6,127,121; WO 01/38584), and ethenoadenine (Fasman (1989) in *Practical Handbook of Biochemistry and Molecular Biology*, pp. 385-394, CRC Press, Boca Raton, Fla.).

Nucleobases B may be attached in the configurations of naturally-occurring nucleic acids to the 1,3 oxathiolane moiety through a covalent bond between the N-9 of purines, e.g. adenin-9-yl and guanin-9-yl, or N-1 of pyrimidines, e.g. thymin-1-yl and cytosin-1-yl (Blackburn, G. and Gait, M. Eds. "DNA and RNA structure" in *Nucleic Acids in Chemistry and Biology*, 2nd Edition, (1996) Oxford University Press, pp. 15-81).

Also in the 1,3 oxathiolane nucleoside structure above, R is H, C_1-C_{18} is alkyl, C_1-C_{18} substituted alkyl, C_2-C_{18} alkenyl, C_2-C_{18} substituted alkenyl, C_2-C_{18} alkynyl, C_2-C_{18} substituted alkynyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_2-C_{20} heterocycle, C_2-C_{20} substituted heterocycle, phosphonate, phosphophosphonate, diphosphophosphonate, phosphate, diphosphate, triphosphate, polyethyleneoxy, or a prodrug moiety.

Physiologically functional derivatives of emtricitabine also include 3TC (lamivudine, Epivir®), a reverse transcriptase inhibitor approved in the United States for the treatment of HIV-1 infection in combination with AZT as Combivir® (GlaxoSmithKline). U.S. Pat. Nos. 5,859,021; 5,905,082; 6,177,435; 5,627,186; 6,417,191. Lamivudine (U.S. Pat. Nos. 5,587,480, 5,696,254, 5,618,820, 5,756,706, 5,744,596, 568,164, 5,466,806, 5,151,426) has the structure:



For example and for some therapeutic uses, 3TC may be a physiologically functional derivative of emtricitabine in combination with tenofovir DF or a physiologically functional derivative of tenofovir DF.

It will be appreciated that tenofovir DF and emtricitabine, and their physiologically functional derivatives may exist in keto or enol tautomeric forms and the use of any tautomeric form thereof is within the scope of this invention. Tenofovir DF and emtricitabine will normally be utilized in the combinations of the invention substantially free of the corresponding enantiomer, that is to say no more than about 5% w/w of the corresponding enantiomer will be present.

Prodrugs

The invention includes all prodrugs of tenofovir and emtricitabine. An exemplary prodrug of tenofovir is tenofovir disoproxil fumarate (TDF, Viread®). A large number of structurally-diverse prodrugs have been described for phosphonic acids (Freeman and Ross in *Progress in Medicinal Chemistry* 34:112-147 (1997). A commonly used prodrug class is the acyloxyalkyl ester, which was first used as a prodrug strategy for carboxylic acids and then applied to phosphates and phosphonates by Farquhar et al (1983) *J. Pharm. Sci.* 72:324; also U.S. Pat. Nos. 4,816,570, 4,968,788, 5,663,159 and 5,792,756. Subsequently, the acyloxyalkyl ester was used to deliver phosphonic acids across cell membranes and to enhance oral bioavailability. A close variant of the acyloxyalkyl ester strategy, the alkoxycarbonyloxyalkyl ester, may also enhance oral bioavailability as a prodrug moiety in the compounds of the combinations of the invention. Aryl esters of phosphorus

groups, especially phenyl esters, are reported to enhance oral bioavailability (DeLambert et al (1994) *J. Med. Chem.* 37:498). Phenyl esters containing a carboxylic ester ortho to the phosphate have also been described (Khamnei and Torrence, (1996) *J. Med. Chem.* 39:4109-4115). Benzyl esters are reported to generate the parent phosphonic acid. In some cases, substituents at the ortho- or para-position may accelerate the hydrolysis. Benzyl analogs with an acylated phenol or an alkylated phenol may generate the phenolic compound through the action of enzymes, e.g. esterases, oxidases, etc., which in turn undergoes cleavage at the benzylic C—O bond to generate the phosphoric acid and the quinone methide intermediate. Examples of this class of prodrugs are described by Mitchell et al (1992) *J. Chem. Soc. Perkin Trans. I*2345; Brook et al WO 91/19721. Still other benzylic prodrugs have been described containing a carboxylic ester-containing group attached to the benzylic methylene (Glazier et al WO 91/19721). Thio-containing prodrugs are reported to be useful for the intracellular delivery of phosphonate drugs. These proesters contain an ethylthio group in which the thiol group is either esterified with an acyl group or combined with another thiol group to form a disulfide. Deesterification or reduction of the disulfide generates the free thio intermediate which subsequently breaks down to the phosphoric acid and episulfide (Puech et al (1993) *Antiviral Res.*, 22:155-174; Benzaria et al (1996) *J. Med. Chem.* 39:4958). Cyclic phosphonate esters have also been described as prodrugs of phosphorus-containing compounds.

Prodrug esters in accordance with the invention are independently selected from the following groups: (1) mono-, di-, and tri-phosphate esters of tenofovir or emtricitabine or any other compound which upon administration to a human subject is capable of providing (directly or indirectly) said mono-, di, or triphosphate ester; (2) carboxylic acid esters (3) sulphonate esters, such as alkyl- or aralkylsulphonyl (for example, methanesulphonyl); (4) amino acid esters (for example, alanine, L-valyl or L-isoleucyl); (5) phosphonate; and (6) phosphonamidate esters.

Ester groups (1)-(6) may be substituted with; straight or branched chain C_1-C_{18} alkyl (for example, methyl, n-propyl, t-butyl, or n-butyl); C_3-C_{12} cycloalkyl; alkoxyalkyl (for example, methoxymethyl); arylalkyl (for example, benzyl); aryloxyalkyl (for example, phenoxymethyl); C_5-C_{20} aryl (for example, phenyl optionally substituted by, for example, halogen, C_1-C_4 alkyl, C_1-C_4 alkoxy, or amino; acyloxymethyl esters —$CH_2OC(=O)R^9$ (e.g. POM) or acyloxymethyl carbonates —$CH_2OC(=O)OR^9$ (e.g. POC) where R^9 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl or C_6-C_{20} substituted aryl. For example, ester groups may be: —$CH_2OC(=O)C(CH_3)_3$, —$CH_2OC(=O)OC(CH_3)_3$ or —$CH_2OC(=O)OCH(CH_3)_2$.

An exemplary aryl moiety present in such esters comprises a phenyl or substituted phenyl group. Many phosphate prodrug moieties are described in U.S. Pat. No. 6,312,662; Jones et al (1995) *Antiviral Research* 27:1-17; Kucera et al (1990) *AIDS Res. Hum. Retro Viruses* 6:491-501; Piantadosi et al (1991) *J. Med. Chem.* 34:1408-14; Hosteller et al (1992) *Antimicrob. Agents Chemother.* 36:2025-29; Hostetler et al (1990) *J. Biol. Chem.* 265:611127; and Siddiqui et al (1999) *J. Med. Chem.* 42:4122-28.

Pharmaceutically acceptable prodrugs refer to a compound that is metabolized in the host, for example hydrolyzed or oxidized, by either enzymatic action or by general acid or base solvolysis, to form an active ingredient. Typical examples of prodrugs of the active ingredients of the combinations of the invention have biologically labile protecting groups on a functional moiety of the active compound. Prodrugs include compounds that can be oxidized, reduced, aminated, deaminated, esterified, deesterified, alkylated, dealkylated, acylated, deacylated, phosphorylated, dephosphorylated, or other functional group change or conversion involving forming or breaking chemical bonds on the prodrug.

Chemical Stability of a Pharmaceutical Formulation

The chemical stability of the active ingredients in a pharmaceutical formulation is of concern to minimize the generation of impurities and ensure adequate shelf-life. The active ingredients, tenofovir disoproxil fumarate and emtricitabine, in the pharmaceutical formulations of the invention have relatively low pKa values, indicative of the potential to cause acidic hydrolysis of the active ingredients. Emtricitabine, with a pKa of 2.65 (Emtriva™ Product Insert, Gilead Sciences, Inc. 2003, available at gilead.com) is subject to hydrolytic deamination of the 5-fluoro cytosine nucleobase to form the 5-fluoro uridine nucleobase. Tenofovir disoproxil fumarate, with a pKa of 3.75 (Yuan L. et al "Degradation Kinetics of Oxycarbonyloxymethyl Prodrugs of Phosphonates in Solution", Pharmaceutical Research (2001) Vol. 18, No. 2, 234-237), is subject also to hydrolytic deamination of the exocyclic amine of the adenine nucleobase, and to hydrolysis of one or both of the POC ester groups (U.S. Pat. No. 5,922, 695). It is desirable to formulate a therapeutic combination of tenofovir disoproxil fumarate and emtricitabine, and the physiological functional derivatives thereof, with a minimum of impurities and adequate stability.

The combinations of the present invention provide combination pharmaceutical dosage forms which are chemically stable to acid degradation of: (1) a first component (such as tenofovir disoproxil fumarate, and physiological functional derivatives; (2) a second component (such as emtricitabine, and physiological functional derivatives; and (3) optionally a third component having antiviral activity. The third component includes anti-HIV agents and include: protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), and integrase inhibitors. Exemplary third active ingredients to be administered in combination with first and second components are shown in Table A. First and second components are as defined in the above section entitled: ACTIVE INGREDIENTS OF THE COMBINATIONS.

Salts

Any reference to any of the compounds in the compositions of the invention also includes any physiologically acceptable salt thereof. Examples of physiologically acceptable salts of tenofovir DF, emtricitabine and their physiologically functional derivatives include salts derived from an appropriate base, such as an alkali metal (for example, sodium), an alkaline earth (for example, magnesium), ammonium and NX_4^+ (wherein X is C_1-C_4 alkyl), or an organic acid such as fumaric acid, acetic acid, succinic acid. Physiologically acceptable salts of an hydrogen atom or an amino group include salts of organic carboxylic acids such as acetic, benzoic, lactic, fumaric, tartaric, maleic, malonic, malic, isethionic, lactobionic and succinic acids; organic sulfonic acids, such as methanesulfonic, ethanesulfonic, benzenesulfonic and p-toluenesulfonic acids; and inorganic acids, such as hydrochloric, sulfuric, phosphoric and sulfamic acids. Physiologically acceptable salts of a compound of an hydroxy group include the anion of said compound in combination with a suitable cation such as Na^+ and NX_4^+ (wherein X is independently selected from H or a C_1-C_4 alkyl group).

For therapeutic use, salts of active ingredients of the combinations of the invention will be physiologically acceptable, i.e. they will be salts derived from a physiologically accept-

able acid or base. However, salts of acids or bases which are not physiologically acceptable may also find use, for example, in the preparation or purification of a physiologically acceptable compound. All salts, whether or not derived from a physiologically acceptable acid or base, are within the scope of the present invention.

Administration of the Formulations

While it is possible for the active ingredients of the combination to be administered alone and separately as monotherapies, it is preferable to administer them as a pharmaceutical co-formulation. A two-part or three-part combination may be administered simultaneously or sequentially. When administered sequentially, the combination may be administered in one, two, or three administrations.

Preferably, two-part or three-part combinations are administered in a single pharmaceutical dosage form. More preferably, a two-part combination is administered as a single oral dosage form and a three-part combination is administered as two identical oral dosage forms. Examples include a single tablet of tenofovir disoproxil fumarate and emtricitabine, or two tablets of tenofovir disoproxil fumarate, emtricitabine, and efavirenz.

It will be appreciated that the compounds of the combination may be administered: (1) simultaneously by combination of the compounds in a co-formulation or (2) by alternation, i.e. delivering the compounds serially, sequentially, in parallel or simultaneously in separate pharmaceutical formulations. In alternation therapy, the delay in administering the second, and optionally a third active ingredient, should not be such as to lose the benefit of a synergistic therapeutic effect of the combination of the active ingredients. By either method of administration (1) or (2), ideally the combination should be administered to achieve peak plasma concentrations of each of the active ingredients. A one pill once-per-day regimen by administration of a combination co-formulation may be feasible for some HIV-positive patients. Effective peak plasma concentrations of the active ingredients of the combination will be in the range of approximately 0.001 to 100 μM. Optimal peak plasma concentrations may be achieved by a formulation and dosing regimen prescribed for a particular patient. It will also be understood that tenofovir DF and emtricitabine, or the physiologically functional derivatives of either thereof, whether presented simultaneously or sequentially, may be administered individually, in multiples, or in any combination thereof. In general, during alternation therapy (2), an effective dosage of each compound is administered serially, where in co-formulation therapy (1), effective dosages of two or more compounds are administered together.

Formulation of the Combinations

When the individual components of the combination are administered separately they are generally each presented as a pharmaceutical formulation. The references hereinafter to formulations refer unless otherwise stated to formulations containing either the combination or a component compound thereof. It will be understood that the administration of the combination of the invention by means of a single patient pack, or patient packs of each formulation, within a package insert diverting the patient to the correct use of the invention is a desirable additional feature of this invention. The invention also includes a double pack comprising in association for separate administration, formulations of tenofovir disoproxil fumarate and emtricitabine, or a physiologically functional derivative of either or both thereof.

The combination therapies of the invention include: (1) a combination of tenofovir DF and emtricitabine or (2) a combination containing a physiologically functional derivative of either or both thereof.

The combination may be formulated in a unit dosage formulation comprising a fixed amount of each active pharmaceutical ingredient for a periodic, e.g. daily, dose or subdose of the active ingredients.

Pharmaceutical formulations according to the present invention comprise a combination according to the invention together with one or more pharmaceutically acceptable carriers or excipients and optionally other therapeutic agents. Pharmaceutical formulations containing the active ingredient may be in any form suitable for the intended method of administration. When used for oral use for example, tablets, troches, lozenges, aqueous or oil suspensions, dispersible powders or granules, emulsions, hard or soft capsules, syrups or elixirs may be prepared (*Remington's Pharmaceutical Sciences* (Mack Publishing Co., Easton, Pa.). Compositions intended for oral use may be prepared according to any method known to the art for die manufacture of pharmaceutical compositions and such compositions may contain one or more agents including antioxidants, sweetening agents, flavoring agents, coloring agents and preserving agents, in order to provide a palatable preparation. Tablets containing the active ingredient in admixture with non-toxic pharmaceutically acceptable excipient which are suitable for manufacture of tablets are acceptable. These excipients may be, for example, inert diluents, such as calcium or sodium carbonate, lactose, lactose monohydrate, croscarmellose sodium, povidone, calcium or sodium phosphate; granulating and disintegrating agents, such as maize starch, or alginic acid; binding agents, such as cellulose, microcrystalline cellulose, starch, gelatin or acacia; and lubricating agents, such as magnesium stearate, stearic acid or talc. Tablets may be uncoated or may be coated by known techniques including microencapsulation to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate alone or with a wax may be employed.

Formulations for oral use may be also presented as hard gelatin capsules where the active ingredient is mixed with an inert solid diluent, for example pregelatinized starch, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, such as peanut oil, liquid paraffin or olive oil.

Aqueous suspensions of the invention contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients include a suspending agent, such as sodium carboxymethylcellulose, methylcellulose, hydroxypropyl methylcelluose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as a naturally occurring phosphatide (e.g., lecithin), a condensation product of an alkylene oxide with a fatty acid (e.g., polyoxyethylene stearate), a condensation product of ethylene oxide with a long chain aliphatic alcohol (e.g., heptadecaethyleneoxycetanol), a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol anhydride (e.g., polyoxyethylene sorbitan monooleate). The aqueous suspension may also contain one or more preservatives such as ethyl or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents, such as sucrose, sucralose or saccharin.

Oil suspensions may be formulated by suspending the active ingredient in a vegetable oil, such as arachis oil, olive

oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oral suspensions may contain a thickening agent, such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents, such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid, BHT, etc.

Dispersible powders and granules of the invention suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, a suspending agent, and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those disclosed above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions or liposome formulations. The oily phase may be a vegetable oil, such as olive oil or arachis oil, a mineral oil, such as liquid paraffin, or a mixture of these. Suitable emulsifying agents include naturally-occurring gums, such as gum acacia and gum tragacanth, naturally occurring phosphatides, such as soybean lecithin, esters or partial esters derived from fatty acids and hexitol anhydrides, such as sorbitan monooleate, and condensation products of these partial esters with ethylene oxide, such as polyoxyethylene sorbitan monooleate. The emulsion may also contain sweetening and flavoring agents. Syrups and elixirs may be formulated with sweetening agents, such as glycerol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, a flavoring or a coloring agent.

The pharmaceutical compositions of the invention may be in the form of a sterile injectable preparation, such as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, such as a solution in 1,3-butane-diol or prepared as a lyophilized powder. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile fixed oils may conventionally be employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid may likewise be used in the preparation of injectables.

The pharmaceutical compositions of the invention may be injected parenterally, for example, intravenously, intraperitoneally, intrathecally, intraventricularly, intrasternally, intracranially, intramuscularly or subcutaneously, or they may be administered by infusion techniques. They are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art.

The pharmaceutical compositions of the invention may also be administered intranasally or by inhalation and are conveniently delivered in the form of a dry powder inhaler or an aerosol spray presentation from a pressurized container or a nebuliser with the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFC 134a), carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurized container or nebuliser may contain a solution or suspension of the composition, e.g. using a mixture of ethanol and the propellant as the solvent, which may additional contain a lubricant, e.g. sorbitan trioleate. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated to contain a powder mix of a compound of the formula (I) and a suitable powder base such as lactose or starch. Aerosol or dry powder formulations are preferably arranged so that each metered dose or "puff" contains from 20 μg to 20 mg of a composition for delivery to the patient. The overall daily dose with an aerosol will be in the range of from 20 μg to 20 mg which may be administered in a single dose or, more usually, in divided doses throughout the day.

The amount of active ingredient that may be combined with the carrier material to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a time-release formulation intended for oral administration to humans may contain approximately 1 to 1000 mg of active material compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95% of the total compositions (weight:weight). The pharmaceutical composition can be prepared to provide easily measurable amounts for administration. For example, an aqueous solution intended for intravenous infusion may contain from about 3 to 500 μg of the active ingredient per milliliter of solution in order that infusion of a suitable volume at a rate of about 30 mL/hr can occur. As noted above, formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be administered as a bolus, electuary or paste.

The combinations of the invention may conveniently be presented as a pharmaceutical formulation in a unitary dosage form. A convenient unitary dosage formulation contains the active ingredients in any amount from 1 mg to 1 g each, for example but not limited to, 10 mg to 300 mg. The synergistic effects of tenofovir DF in combination with emtricitabine may be realized over a wide ratio, for example 1:50 to 50:1 (tenofovir DF:emtricitabine). In one embodiment, the ratio may range from about 1:10 to 10:1. In another embodiment, the weight/weight ratio of tenofovir to emtricitabine in a co-formulated combination dosage form, such as a pill, tablet, caplet or capsule will be about 1, i.e. an approximately equal amount of tenofovir DF and emtricitabine. In other exemplary co-formulations, there may be more or less tenofovir than FTC. For example, 300 mg tenofovir DF and 200 mg emtricitabine can be co-formulated in a ratio of 1.5:1 (tenofovir DF:emtricitabine). In one embodiment, each compound will be employed in the combination in an amount at which it exhibits antiviral activity when used alone. Exemplary Formulations A, B, C, D, E, and F (Examples) have ratios of 12:1 to 1:1 (tenofovir DF:emtricitabine). Exemplary Formulations A, B, C, D, E, and F use amounts of tenofovir DF and emtricitabine ranging from 25 mg to 300 mg. Other ratios and amounts of the compounds of said combinations are contemplated within the scope of the invention.

A unitary dosage form may further comprise tenofovir DF and emtricitabine, or physiologically functional derivatives of either thereof, and a pharmaceutically acceptable carrier.

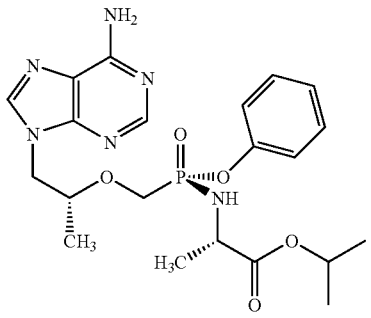
It will be appreciated by those skilled in the art that the amount of active ingredients in the combinations of the invention required for use in treatment will vary according to a variety of factors, including the nature of the condition being treated and the age and condition of the patient, and will ultimately be at the discretion of the attending physician or health care practitioner. The factors to be considered include the route of administration and nature of the formulation, the animal's body weight, age and general condition and the nature and severity of the disease to be treated. For example, in a Phase I/II monotherapy study of emtricitabine, patients received doses ranging from 25 mg to 200 mg twice-a-day for two weeks. At each dose regimen greater or equal to 200 mg, a 98-percent (1.75 log 10) or greater viral suppression was observed. A once-a-day dose of 200 mg of emtricitabine reduced the viral load by an average of 99 percent (1.92 log 10). Viread® (tenofovir DF) has been approved by the FDA for the treatment and prophylaxis of HIV infection as a 300 mg oral tablet. Emtriva™ (emtricitabine) has been approved by the FDA for the treatment of HIV as a 200 mg oral tablet.

It is also possible to combine any two of the active ingredients in a unitary dosage form for simultaneous or sequential administration with a third active ingredient. The three-part combination may be administered simultaneously or sequentially. When administered sequentially, the combination may be administered in two or three administrations. Third active ingredients have anti-HIV activity and include protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), and integrase inhibitors. Exemplary third active ingredients to be administered in combination with tenofovir DF, emtricitabine, and their physiological functional derivatives, are shown in Table A.

TABLE A

5,6 dihydro-5-azacytidine
5-aza 2'deoxycytidine
5-azacytidine
5-yl-carbocyclic 2'-deoxyguanosine (BMS200, 475)
9 (arabinofuranosyl)guanine; 9-(2' deoxyribofuranosyl)guanine
9-(2'-deoxy 2'fluororibofuranosyl)-2,6-diaminopurine
9-(2'-deoxy 2'fluororibofuranosyl)guanine
9-(2'-deoxyribofuranosyl)-2,6 diaminopurine
9-(arabinofuranosyl)-2,6 diaminopurine
Abacavir, Ziagen ®
Acyclovir, ACV; 9-(2-hydroxyethoxylmethyl)guanine
Adefovir dipivoxil, Hepsera ®
amdoxivir, DAPD
Amprenavir, Agenerase ®
araA; 9-β-D-arabinofuranosyladenine (Vidarabine)
atazanivir sulfate (Reyataz ®)
AZT; 3'-azido-2',3'-dideoxythymdine, Zidovudine, (Retrovir ®)
BHCG; (.+−.)-(1a,2b,3a)-9-[2,3-bis(hydroxymethyl)cyclobutyl]guanine
BMS200,475; 5-yl-carbocyclic 2'-deoxyguanosine
Buciclovir; (R) 9-(3,4-dihydroxybutyl)guanine
BvaraU; 1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil (Sorivudine)
Calanolide A
Capravirine
CDG; carbocyclic 2'-deoxyguanosine
Cidofovir, HPMPC; (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine
Clevudine, L-FMAU; 2'-Fluoro-5-methyl-β-L-arabino-furanosyluracil
Combivir ® (lamivudine/zidovudine)
Cytallene; [1-(4'-hydroxy-1',2'-butadienyl)cytosine]
d4C; 3'-deoxy-2',3'-didehydrocytidine
DAPD; (−)-β-D-2,6-diaminopurine dioxolane
ddA; 2',3'-dideoxyadenosine
ddAPR; 2,6-diaminopurine-2',3'-dideoxyriboside
ddC; 2',3'-dideoxycytidine (Zalcitabine)
ddI; 2',3'-dideoxyinosine, didanosine, (Videx ®, Videx ® EC)
Delavirdine, Rescriptor ®
Didanosine, ddI, Videx ®; 2',3'-dideoxyinosine
DXG; dioxolane guanosine
E-5-(2-bromovinyl)-2'-deoxyuridine
Efavirenz, Sustiva ®
Enfuvirtide, Fuzeon ®
F-ara-A; fluoroarabinosyladenosine (Fludarabine)
FDOC; (−)-β-D-5-fluoro-1-[2-(hydroxymethyl)-1,3-dioxolane]cytosine
FEAU; 2'-deoxy-2'-fluoro-1-β-D-arabinofuranosyl-5-ethyluracil
FIAC; 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine
FIAU; 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouridine
FLG; 2',3'-dideoxy-3'-fluoroguanosine
FLT; 3'-deoxy-3'-fluorothymidine
Fludarabine; F-ara-A; fluoroarabinosyladenosine
FMAU; 2'-Fluoro-5-methyl-β-L-arabino-furanosyluracil
FMdC
Foscarnet; phosphonoformic acid, PFA
FPMPA; 9-(3-fluoro-2-phosphonylmethoxypropyl)adenine
Gancyclovir, GCV; 9-(1,3-dihydroxy-2-propoxymethyl)guanine
GS-7340; 9-[R-2-[[(S)-[[(S)-1-(isopropoxycarbonyl)ethyl]amino]-phenoxyphosphinyl]methoxy]propyl]adenine
HPMPA; (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine
HPMPC; (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (Cidofovir)
Hydroxyurea, Droxia ®
Indinavir, Crixivan ®
Kaletra ® (lopinavir/ritonavir)
Lamivudine, 3TC, Epivir ™; (2R,5S,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one
L-d4C; L-3'-deoxy-2',3'-didehydrocytidine
L-ddC; L-2',3'-dideoxycytidine
L-Fd4C; L-3'-deoxy-2',3'-didehydro-5-fluorocytidine
L-FddC; L-2',3'-dideoxy-5-fluorocytidine
Lopinavir
Nelfinavir, Viracept ®
Nevirapine, Viramune ®
Oxetanocin A; 9-(2-deoxy-2-hydroxymethyl-β-D-erythro-oxetanosyl)adenine
Oxetanocin G; 9-(2-deoxy-2-hydroxymethyl-β-D-erythro-oxetanosyl)guanine
Penciclovir
PMEDAP; 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine
PMPA, tenofovir; (R)-9-(2-phosphonylmethoxypropyl)adenine
PPA; phosphonoacetic acid
Ribavirin; 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide
Ritonavir, Norvir ®
Saquinavir, Invirase ®, Fortovase ®
Sorivudine, BvaraU; 1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil
Stavudine, d4T, Zerit ®; 2',3'-didehydro-3'-deoxythymidine
Trifluorothymidine, TFT; Trifluorothymidine
Trizivir ® (abacavir sulfate/lamivudine/zidovudine)
Vidarabine, araA; 9-β-D-arabinofuranosyladenine
Zalcitabine, Hivid ®, ddC; 2',3'-dideoxycytidine
Zidovudine, AZT, Retrovir ®; 3'-azido-2',3'-dideoxythymdine
Zonavir; 5-propynyl-1-arabinosyluracil

Another aspect of the present invention is a three-part combination comprising tenofovir DF, FTC, and 9-[(R)-2-[[(S)—[[(S)-1-(isopropoxycarbonyl)ethyl]amino]phenoxy-phosphinyl]methoxy]propyl]adenine, also designated herein as GS-7340, which has the structure:



GS-7340 is a prodrug of tenofovir and the subject of commonly owned, pending application, U.S. Ser. No. 09/909,560, filed Jul. 20, 2001 and Becker et al WO 02/08241.

For example, a ternary unitary dosage may contain 1 mg to 1000 mg of tenofovir disoproxil fumarate, 1 mg to 1000 mg of emtricitabine, and 1 mg to 1000 mg of the third active ingredient. As a further feature of the present invention, a unitary dosage form may further comprise tenofovir DF, emtricitabine, the third active ingredient, or physiologically functional derivatives of the three active ingredients thereof, and a pharmaceutically acceptable carrier.

Combinations of the present invention enable patients greater freedom from multiple dosage medication regimens and ease the needed diligence required in remembering and complying with complex daily dosing times and schedules. By combining tenofovir disoproxil fumarate and emtricitabine into a single dosage form, the desired daily regimen may be presented in a single dose or as two or more sub-doses per day. The combination of co-formulated tenofovir DF and emtricitabine may be administered as a single pill, once per day.

A further aspect of the invention is a patient pack comprising at least one active ingredient: tenofovir disoproxil fumarate, emtricitabine, or a physiologically functional derivative of either of the combination and an information package or product insert containing directions on the use of the combination of the invention.

Segregation of active ingredients in pharmaceutical powders and granulations is a widely recognized problem that can result in inconsistent dispersions of the active ingredients in final dosage forms. Some of the main factors contributing to segregation are particle size, shape and density. Segregation is particularly troublesome when attempting to formulate a single homogenous tablet containing multiple active ingredients having different densities and different particle sizes. Glidants are substances that have traditionally been used to improve the flow characteristics of granulations and powders by reducing interparticulate friction. See Lieberman, *Lachman, & Schwartz, Pharmaceutical Dosage Forms: Tablets*, Volume 1, p. 177-178 (1989), incorporated herein by reference. Glidants are typically added to pharmaceutical compositions immediately prior to tablet compression to facilitate the flow of granular material into the die cavities of tablet presses. Glidants include: colloidal silicon dioxide, asbestos free talc, sodium aluminosilicate, calcium silicate, powdered cellulose, microcrystalline cellulose, corn starch, sodium benzoate, calcium carbonate, magnesium carbonate, metallic stearates, calcium stearate, magnesium stearate, zinc stearate, stearowet C, starch, starch 1500, magnesium lauryl sulfate, and magnesium oxide. Exemplary Tablet Formulation A has colloidal silicon dioxide (Examples). Glidants can be used to increase and aid blend composition homogeneity in formulations of anti-HIV drugs (U.S. Pat. No. 6,113,920). The novel compositions of the present invention may contain glidants to effect and maintain homogeneity of active ingredients during handling prior to tablet compression.

The present invention provides pharmaceutical formulations combining the active ingredients tenofovir DF and emtricitabine, or physiologically functional derivatives thereof, in a sufficiently homogenized form, and a method for using this pharmaceutical formulation. An object of the present invention is to utilize glidants to reduce the segregation of active ingredients in pharmaceutical compositions during pre-compression material handling. Another object of the present invention is to provide a pharmaceutical formulation combining the active ingredients tenofovir DF and emtricitabine, or physiologically functional derivatives thereof, with a pharmaceutically acceptable glidant, resulting in a mixture characterized by a pharmaceutically acceptable measure of homogeneity.

Formulations include those suitable for oral, rectal, nasal, topical (including transdermal, buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods represent a further feature of the present invention and include the step of bringing into association the active ingredients with the carrier, which constitutes one or more accessory ingredients, and maintaining chemical stability. In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, caplets, cachets or tablets each containing a predetermined amount of the active ingredients; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. povidone, gelatin, hydroxypropyl methylcellulose), lubricant, inert diluent, preservative, disintegrant (e.g. sodium starch glycollate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose) surface-active or dispersing agent. Molded tablets may be made by molding a mixture of the powdered compound moistened with an inert liquid diluent in a suitable machine. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredients therein using, for example, cellulose ether derivatives (e.g., hydroxypropyl methylcellulose) or methacrylate derivatives in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredients in a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier. Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylates. Topical administration may also be by means of a transdermal iontophoretic device.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for penile administration for prophylactic or therapeutic use may be presented in condoms, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such earners as are known in the art to be appropriate.

Pharmaceutical formulations suitable for rectal administration wherein the carrier is a solid are most preferably presented as unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art. The suppositories may be conveniently formed by admixture of the active combination with the softened or melted carrier(s) followed by chilling and shaping in moulds.

Formulations suitable for parenteral administration include aqueous and nonaqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and nonaqueous sterile suspensions which may include suspending agents and thickening agents; and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Exemplary unit dosage formulations are those containing a daily dose or daily subdose of the active ingredients, as hereinbefore recited, or an appropriate fraction thereof. It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include such further agents as sweeteners, thickeners and flavoring agents.

The compounds of the combination of the present invention may be obtained in a conventional manner, known to those skilled in the art. Tenofovir disoproxil fumarate can be prepared, for example, as described in U.S. Pat. No. 5,977,089. Methods for the preparation of FTC are described in WO 92/14743, incorporated herein by reference.

Composition Use

Compositions of the present invention are administered to a human or other mammal in a safe and effective amount as described herein. These safe and effective amounts will vary according to die type and size of mammal being treated and the desired results of the treatment. Any of the various methods known by persons skilled in the art for packaging tablets, caplets, or other solid dosage forms suitable for oral administration, that will not degrade the components of the present invention, are suitable for use in packaging. The combinations may be packaged in glass and plastic bottles. Tablets, caplets, or other solid dosage forms suitable for oral administration may be packaged and contained in various packaging materials optionally including a desiccant, e.g. silica gel. Packaging may be in the form of unit dose blister packaging. For example, a package may contain one blister tray of tenofovir DF and another blister tray of emtricitabine pills, tablets, caplets, or capsule. A patient would take one dose, e.g. a pill, from one tray and one from the other. Alternatively, the package may contain a blister tray of the co-formulated combination of tenofovir DF and emtricitabine in a single pill, tablet, caplet or capsule. As in other combinations and packaging thereof, the combinations of the invention include physiological functional derivatives of tenofovir DF and FTC.

The packaging material may also have labeling and information related to the pharmaceutical composition printed thereon. Additionally, an article of manufacture may contain a brochure, report, notice, pamphlet, or leaflet containing product information. This form of pharmaceutical information is referred to in the pharmaceutical industry as a "package insert." A package insert may be attached to or included with a pharmaceutical article of manufacture. The package insert and any article of manufacture labeling provides information relating to the pharmaceutical composition. The information and labeling provides various forms of information utilized by health-care professionals and patients, describing the composition, its dosage and various other parameters required by regulatory agencies such as the United States Food and Drug Agency.

Assays of the Combinations

The combinations of the inventions may be tested for in vitro activity against HIV and sensitivity, and for cytotoxicity in laboratory adapted cell lines, e.g. MT2 and in peripheral blood mononuclear cells (PBMC) according to standard assays developed for testing anti-HIV compounds, such as WO 02/068058 and U.S. Pat. No. 6,475,491. Combination assays may be performed at varying concentrations of the compounds of the combinations to determine EC_{50} by serial dilutions.

Exemplary Formulations

The following examples further describe and demonstrate particular embodiments within the scope of the present invention. Techniques and formulations generally are found in *Remington's Pharmaceutical Sciences* (Mack Publishing Co., Easton, Pa.). The examples are given solely for illustration and are not to be construed as limitations as many variations are possible without departing from spirit and scope of the Invention. The following examples are intended for illustration only and are not intended to limit the scope of the invention in any way. "Active ingredient" denotes tenofovir disoproxil fumarate, emtricitabine, or a physiologically functional derivative of either thereof.

Tablet Formulation

The following exemplary formulations A, B, C, D, E, and F are prepared by wet granulation of the ingredients with an aqueous solution, addition of extragranular components and then followed by addition of magnesium stearate and compression.

Formulation A:

	mg/tablet
Tenofovir Disoproxil Fumarate	300
emtricitabine	200
Microcrystalline Cellulose	200
Lactose Monohydrate	175
Croscarmellose Sodium	60
Pregelatinized Starch	50
Colloidal silicon dioxide	5
Magnesium Stearate	10
total:	1000

Formulation B:

	mg/tablet
Tenofovir Disoproxil fumarate	300
emtricitabine	100
Microcrystalline Cellulose	200
Lactose Monohydrate	180
Sodium Starch Glycollate	60
Pregelatinized Starch	50
Magnesium Stearate	10
total:	900

Formulation C:

	mg/tablet
Tenofovir Disoproxil fumarate	200
emtricitabine	200
Microcrystalline Cellulose	200
Lactose Monohydrate	180
Sodium Starch Glycollate	60
Pregelatinized Starch	50
Magnesium Stearate	10
total:	900

Formulation D:

	mg/tablet
Tenofovir Disoproxil fumarate	300
emtricitabine	25
Microcrystalline Cellulose	200
Lactose Monohydrate	180
Sodium Starch Glycollate	60
Pregelatinized Starch	50
Magnesium Stearate	10
total:	825

Formulation E:

	mg/tablet
Tenofovir Disoproxil fumarate	200
emtricitabine	25
Microcrystalline Cellulose	200
Lactose Monohydrate	180
Sodium Starch Glycollate	60
Pregelatinized Starch	50
Magnesium Stearate	10
total:	725

Formulation F:

	mg/tablet
Tenofovir Disoproxil fumarate	100
emtricitabine	100
Microcrystalline Cellulose	200
Lactose Monohydrate	180
Sodium Starch Glycollate	60

-continued

	mg/tablet
Pregelatinized Starch	50
Magnesium Stearate	10
total:	700

Formulation G (Controlled Release Formulation):

This formulation is prepared by wet granulation of the ingredients with an aqueous solution, followed by the addition of magnesium stearate and compression.

	mg/tablet
Tenofovir Disoproxil fumarate	300
emtricitabine	200
Hydroxypropyl Methylcellulose	112
Lactose B.P.	53
Pregelatinized Starch B.P.	28
Magnesium Stearate	7
total:	700

Drug release takes place over a period of about 6-8 hours and is complete after 12 hours.

Capsule Formulations

Formulation H:

A capsule formulation is prepared by admixing the ingredients and filling into a two-part hard gelatin or hydroxypropyl methylcellulose capsule.

	mg/capsule
Active Ingredient	500
Microcrystalline Cellulose	143
Sodium Starch Glycollate	25
Magnesium Stearate	2
total:	670

Formulation I (Controlled Release Capsule):

The following controlled release capsule formulation is prepared by extruding ingredients a, b, and c using an extruder, followed by spheronization of the extrudate and drying. The dried pellets are then coated with release-controlling membrane (d) and filled into a two-piece, hard gelatin or hydroxypropyl methylcellulose capsule.

	mg/capsule
(a) Active Ingredient	500
(b) Microcrystalline Cellulose	125
(c) Lactose B.P.	125
(d) Ethyl Cellulose	13
total:	763

Formulation J (Oral Suspension):

The active ingredients are admixed with the ingredients and filling them as dry powder. Purified water is added and mixed well before use.

Active Ingredient	500 mg
Confectioner's Sugar	2000 mg
Simethicone	300 mg
Methylparaben	30 mg
Propylparaben	10 mg
Flavor, Peach	500 mg
Purified Water q.s. to	5.00 ml

Formulation K (Suppository):

One-fifth of the Witepsol H15 is melted in a steam-jacketed pan at 45° C. maximum. The active ingredients are sifted through a 200 micron sieve and added to the molten base with mixing, using a Silverson fitted with a cutting head, until a smooth dispersion is achieved. Maintaining the mixture at 45° C., the remaining Witepsol H15 is added to the suspension and stirred to ensure a homogenous mix. The entire suspension is passed through a 250 micron stainless steel screen and, with continuous stirring, is allowed to cool to 40° C. At a temperature of 38° C. to 40° C., 2.02 g of the mixture is filled into suitable, 2 ml plastic molds. The suppositories are allowed to cool to room temperature.

	mg/Suppository
Active Ingredient	500
Hard Fat, B.P. (Witepsol H15 - Dynamit Nobel)	1770
total	2270

Fixed Dose Combination Tablet

A fixed dose combination tablet of tenofovir disoproxil fumarate (TDF) 300 mg/emtricitabine 200 mg was formulated using a wet granulation/fluid-bed drying process using conventional methods. See: U.S. Pat. No. 5,935,946; L. Young (editor). Tableting Specification Manual 5th ed., American Pharmaceutical Association, Washington, D.C., (2001); L. Lachman, H. Lieberman (editors). Pharmaceutical Dosage Forms: Tablets (Vol 2), Marcel Dekker Inc., New York, 185-202 (1981); J. T. Fell and J. M. Newton, J. Pharm. Pharmacol. 20, 657-659 (1968); US Pharmacopeia 24-National Formulary 19, "Tablet Friability", Chapter <1216>, Page 2148 (2000).

The effects of granulation water level (ranging from 40% to 50% w/w) and wet massing time were studied on the physicochemical properties of the final powder blend and its performance with respect to blend uniformity and compressibility (tablet compactibility). In addition, content uniformity, assay, stability and dissolution performance was evaluated for the TDF/emtricitabine fixed dose combination tablets.

Formulation Equipment

Equipment included a high shear mixer equipped with a pressure tank and spray nozzle tip to add the granulating water, a fluid-bed dryer, a mill, a tumble blender, a rotary tablet press, and a tablet deduster.

Formulation Process

The dried, milled powder was blended with the extragranular microcrystalline cellulose and croscarmellose sodium and then blended with magnesium stearate. Powder samples were removed after mixing with die magnesium stearate. The blend samples were evaluated for, bulk density, mesh analysis and compressibility. The powder blend mixed with the magnesium stearate was compressed into tablets on a press setup.

Materials

The following Table 1 lists the quantitative composition of the TDF/emtricitabine tablet formulation.

TABLE 1

Ingredient	% w/w	Unit Formula for tablet cores (mg/tablet)	Quantity per 12 kg Batch (kg)
Tenofovir Disoproxil Fumarate [a]	30.0	300.0	3.60
Emtricitabine [a]	20.0	200.0	2.40
Pregelatinized Starch, NF/EP	5.0	50.0	0.60
Croscarmellose Sodium, NF/EP	6.0	60.0	0.72
Lactose Monohydrate, NF/EP [a]	8.0	80.0	0.96
Microcrystalline Cellulose, NF/EP [c]	30.0	300.0	3.60
Magnesium Stearate, NF/EP	1.0	10.0	0.12
Purified Water, USP/EP	[b]	[b]	[b]
Totals	100.0	1000.0	12.00

[a] Actual weight is adjusted based on the Drug Content Factor (DCF) of tenofovir disoproxil fumarate and emtricitabine.
[b] Water removed during drying.

Characterization Equipment

Moisture content was measured by loss on drying using a heat lamp/balance system. The powder blend was sampled with a sampling thief fitted with chambers to determine powder blend uniformity. Duplicate samples were removed from each of several locations in the blender. Blend uniformity analysis was performed on one sample from each location.

Particle size analysis of the final powder blend was determined by sifting a multi-gram sample through a screen using a sonic sifter. The quantity of final powder blend retained on each sieve and the fines collector was determined by calculating the difference in weight between the sieves and fines collector before and after the test. The geometric mean diameter particle size was calculated by logarithmic weighting of the sieved distribution.

Bulk density was determined by filling a graduated cylinder with the final powder blend and measuring the weight differential between the empty and filled graduate cylinder per unit volume.

Tablets were characterized for friability using a friabilator, a hardness tester, a thickness micrometer equipped with a printer, and a weighing balance.

Compression characteristics were determined using a rotary tablet press equipped with a flat-faced, beveled edged punch to a target weight of 400 mg. The powder blends were compressed using target upper punch pressures ranging from approximately 100 to 250 MPa. The apparent normalized ejection force was determined and normalized for tablet thickness and diameter.

Tablet hardness was determined using a hardness tester. Tablet thickness was determined using a micrometer, and tablet weights were determined using a top loading balance.

Wet Granulation

The powders were blended in a granulator and then granulated using water. The impeller and chopper speeds were kept constant in the blender at a low setting during the granulation and wet massing operations. After water addition, the impeller and chopper were stopped and the granulator bowl was opened to observe the granulation consistency and texture. The lid was closed and the wet massing phase was performed. Acceptable granules had 40% w/w and 60% w/w water, respectively.

Wet Milling

To facilitate a uniform drying process, each wet granulation was deagglomerated with a mill fitted with a screen and an impeller. The milled wet granules were charged into a fluid-bed dryer immediately following wet milling.

Fluid-Bed Drying

Milled wet granules were dried using an inlet air setpoint temperature of about 70° C. and airflow of approximately 100 cfm. The target LOD was about 1.0% with a range of not more than (NMT) 1.5%. The total fluid-bed drying time ranged from 53 to 75 minutes. Final LOD ranged from 0.4% to 0.7% for all of the batches dried. The final exhaust temperatures for all the batches ranged from 47° C. to 50° C.

Dry Milling

All dried granules were milled through a perforated screen. The mill was equipped with a square impeller and operated. The lots were milled and manually transferred to the V-blender.

Blending

Each lot was blended using the V-blender. In one set of three formulations, starting with 12 kg materials, final powder blend yield available for compression after blending ranged from 10.5 kg (87.5%) to 11.1 kg (92.5%). The final powder blend bulk density ranged from 0.48 to 0.58 g/cc and the geometric mean diameter particle size ranged from 112 to 221 μm. Percent water and wet massing time affect final powder blend particle size and bulk density.

The powder blending for both tenofovir DF and emtricitabine gave a mean (n=10) strength value for tenofovir DF ranged from 100.6% to 102.8% of target strength for the lots and the relative standard deviation (RSD) was from 0.5% to 1.7%. The mean (n=10) strength value for emtricitabine ranged from 101.3% to 104.1% of target strength for the lots with the relative standard deviation (RSD) ranged from 0.6% to 1.7%. The final powder blend moisture level ranged from 0.8% to 1.1% LOD.

Tablet Compression

The final blends were compressed using a rotary tablet press and the tablets were film-coated.

Three 300 gm formulations (Table 2) were granulated in a granulator equipped with a 1-L bowl. The quantities of intragranular components were based on a 300 g total batch size. The formulations in lots 1 and 2 differed in the amount of microcrystalline cellulose 30% vs. 20% w/w, respectively. Lots 2 and 3 were identical except for the type of binder. Lot 2 contained 5% w/w of pregelatinized starch and lot 3 contained 5% w/w povidone as binder.

TABLE 2

Ingredient	Lot 1 % w/w	Lot 2 % w/w	Lot 3 % w/w
Tenofovir Disoproxil Fumarate	30.0	30.0	30.0
Emtricitabine	20.0	20.0	20.0
Pregelatinized Starch, NF/EP	5.0	5.0	N/A
Povidone, USP/NF (C-30)	N/A	N/A	5.0
Croscarmellose Sodium, NF/EP	6.0	6.0	6.0
Lactose Monohydrate, NF/EP	8.0	18.0	18.0
Microcrystalline Cellulose, NF/EP [a]	30.0	20.0	20.0
Magnesium Stearate, NF/EP	1.0	1.0	1.0
Purified Water, USP/EP	[a]	[a]	[a]
Total	100.0	100.0	100.0

[a] Water removed during drying.

After water addition, the impeller and chopper were stopped and the granulator bowl was opened to observe the granulation consistency and texture. To achieve similar granulation consistency, lots 1, 2, and 3 were granulated with 45%, 40%, and 30% w/w water, respectively. The lid was closed and the wet massing phase was performed. All lots had a 30 sec wet massing resulting in acceptable granulations. The wet granulations from all batches were hand screened through a sieve to deagglomerate. The resulting granulations were tray dried in a convection oven set at 60° C. for approximately 20 hours to an LOD<1.0%. The dried granulations from all batches were hand screened through a sieve. In order to fit the granulation into the small scale (300 mL) V-blender, the final blend batch size was adjusted to 100 g. A portion, 81 g of the resulting blend from Lot 1 was blended with 15 g microcrystalline cellulose, 3 g croscarmellose sodium and 1 g magnesium stearate. 86 g of the resulting granulation from Lot 2 and Lot 3 were each blended with 10 g microcrystalline cellulose, 3 g croscarmellose sodium and 1 g magnesium stearate.

Purity analysis was conducted by reverse-phase HPLC (high performance liquid chromatography). Impurities related to tenofovir disoproxil fumarate and emtricitabine were characterized and measured in the bulk API (active pharmaceutical ingredient) before formulation in the three lots of Table 2, and again after formulation in the resulting tablets. The impurities include by-products from hydrolysis of the exocyclic amino groups of tenofovir disoproxil fumarate and emtricitabine, and the hydrolysis of the disoproxil (POC) esters of tenofovir disoproxil fumarate. In each lot, the sum total of impurities related to tenofovir disoproxil fumarate and emtricitabine was less than 1% after formulation and tablet manufacture.

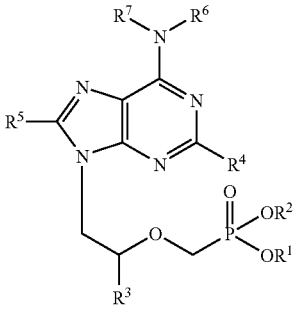
The physicochemical properties of tenofovir disoproxil fumarate and emtricitabine tablets were evaluated by visual appearance, water content, label strength, impurity and degradation product contents, and tablet dissolution. Stability studies were conducted on drug product packaged in container-closure systems that are identical to the intended clinical and commercial container-closure system. There was no sign of discoloration or tablet cracking during the course of the stability study. Film-coated tenofovir disoproxil fumarate and emtricitabine tablets exhibited satisfactory stability at 40° C./75% RH (relative humidity) for up to six months when packaged and stored with silica gel desiccant. No significant loss (defined as ≥ 5% degradation) in % label strength of tenofovir DF or emtricitabine was observed after six months at 40° C./75% RH. when packaged and stored with desiccant. The increase in the total degradation products was 1.5% for tenofovir DF and 0.6-0.7% for emtricitabine after six months at 40° C./75% RH when packaged and stored with 3 grams of desiccant.

All publications and patent applications cited herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although certain embodiments are described in detail above, those having ordinary skill in the art will clearly understand that many modifications are possible in the claims without departing from the teachings thereof. All such modifications are intended to be encompassed within the claims of the invention.

Embodiments of the Invention

A1. A pharmaceutical composition comprising an effective amount of a compound of the formula:



(1)

wherein R^1 and R^2 are independently selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_6-C_{20} arylalkyl, C_6-C_{20} substituted arylalkyl, acyloxymethyl esters $CH_2OC(=O)R^9$ and acyloxymethyl carbonates $—CH_2OC(=O)OR^9$ where R^9 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl and C_6-C_{20} substituted aryl;

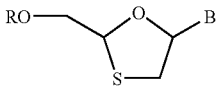
R^3 is selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, or CH_2OR^8 where R^8 is C_1-C_6 alkyl, C_1-C_6 hydroxyalkyl and C_1-C_6 haloalkyl;

R^4 and R^5 are independently selected from H, NH_2, NHR and NR_2 where R is C_1-C_6 alkyl; and

R^6 and R^7 are independently selected from H and C_1-C_6 alkyl;

or a physiologically functional derivative thereof;

in combination with an effective amount of a compound of the formula



(2)

wherein B is selected from adenine, guanine, cytosine, uracil, thymine, 7-deazaadenine, 7-deazaguanine, 7-deaza-8-azaguanine, 7-deaza-8-azaadenine, inosine, nebularine, nitropyrrole, nitroindole, 2-aminopurine, 2-amino-6-chloropurine, 2,6-diaminopurine, hypoxanthine, pseudouridine, 5-fluorocytosine, 5-chlorocytosine, 5-bromocytosine, 5-iodocytosine, pseudocytosine, pseudoisocytosine, 5-propynylcytosine, isocytosine, isoguanine, 7-deazaguanine, 2-thiopyrimidine, 6-thioguanine, 4-thiothymine, 4-thiouracil, O^6-methylguanine, N^6-methyladenine, O^4-methylthymine, 5,6-dihydrothymine, 5,6-dihydrouracil, 4-methylindole, and a pyrazolo[3,4-D]pyrimidine; and

R is selected from H, C_1-C_{18} alkyl, C_1-C_{18} substituted alkyl, C_2-C_{18} alkenyl, C_2-C_{18} substituted alkenyl, C_2-C_{18} alkynyl, C_2-C_{18} substituted alkynyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_2-C_{20} heterocycle, C_2-C_{20} substituted heterocycle, phosphonate, phosphophosphonate, diphosphophosphonate, phosphate, diphosphate, triphosphate, polyethyleneoxy or a physiologically functional derivative thereof; and

a pharmaceutically acceptable carrier.

B2. A composition of embodiment A1 wherein, in formula 1, R^1 and R^2 are independently selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_6-C_{20} arylalkyl, C_6-C_{20} substituted arylalkyl, acyloxymethyl esters $—CH_2OC(=O)R^9$ and acyloxymethyl carbonates $—CH_2OC(=O)OR^9$ where R^9 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl and C_6-C_{20} substituted aryl; and R^3, R^4, R^5, R^6 and R^7 are independently H or C_1-C_6 alkyl.

C3. A composition of embodiment A1 wherein, in formula 2, B is cytosine or a 5-halocytosine.

D4. A composition of embodiment A1 wherein, in formula 1, R^1 and R^2 are independently selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_6-C_{20} arylalkyl, C_6-C_{20} substituted arylalkyl, acyloxymethyl esters $—CH_2OC(=O)R^9$ and acyloxymethyl carbonates $—CH_2OC(=O)OR^9$ where R^9 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl and C_1-C_{20} substituted aryl; and R^3, R^4, R^5, R^6 and R^7 are independently H or C_1-C_6 alkyl; and, in formula 2, B is cytosine or a 5-halocytosine.

E5. A composition of embodiment D 4 wherein, in formula 1, R^1 and R^2 are independently selected from H, acyloxymethyl esters $—CH_2OC(=O)R^9$ and acyloxymethyl carbonates $—CH_2OC(=O)OR^9$ where R^9 is C_1-C_6 alkyl; and R^3, R^4, R^5, R^6 and R^7 are independently H or C_1-C_6 alkyl; and, in formula 2, B is cytosine or a 5-halocytosine and R is H.

F6. A composition of embodiment E5 wherein, in formula 1, R^1 and R^2 are independently selected from H and $—CH_2OC(=O)OCH(CH_3)_2$; R^3 is $—CH_3$; and R^4, R^5, R^6 and R^7 are H; and, in formula 2, B is 5-fluorocytosine and R is H.

G7. A pharmaceutical composition comprising a pharmaceutically effective amount of [2-(6-amino-purin-9-yl)-1-methyl-ethoxymethyl]-phosphonic acid diisopropoxycarbonyloxymethyl ester fumarate (tenofovir disoproxil fumarate) or a physiologically functional derivative thereof and a pharmaceutically effective amount of (2R, 5S,)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine) or a physiologically functional derivative thereof; and a pharmaceutically acceptable carrier.

H8. A pharmaceutical formulation of embodiment A1 to G7 further comprising a third active ingredient selected from the group consisting of a protease inhibitor, a nucleoside or nucleotide reverse transcriptase inhibitor, a non-nucleoside reverse transcriptase inhibitor, and an integrase inhibitor.

I9. A pharmaceutical formulation of embodiments A1 to H8 in unit dosage form.

J10. A method for the treatment or prevention of the symptoms or effects of an HIV infection in an infected animal which comprises administering to said animal a pharmaceutical composition of embodiments claims A1 to I9.

We claim:

1. A chemically stable fixed dose combination pharmaceutical dosage form comprising 300 mg tenofovir disoproxil fumarate and 200 mg emtricitabine; a binder selected from the group consisting of povidone, gelatin, hydroxypropyl methylcellulose, cellulose, microcrystalline cellulose, starch, and acacia; a disintegrant selected from sodium starch glycolate, crosslinked-povidone, cross-linked sodium carboxymethylcellulose, and alginic acid; and a lubricant selected from the group consisting of magnesium stearate, stearic acid, and talc;

wherein said pharmaceutical dosage form exhibits less than 10% degradation of the tenofovir disoproxil fumarate or emtricitabine after 6 months when packaged and stored with silica gel dessicant at 40° C./75% relative humidity.

2. The pharmaceutical dosage form of claim **1** wherein the dosage form is oral.

3. The pharmaceutical dosage form of claim 1 where there is less than 1% degradation of tenofovir disoproxil fumarate over a 24-hour period.

4. The pharmaceutical dosage form of claim 1 where there is less than 0.1% degradation of tenofovir disoproxil fumarate over a 24-hour period.

5. The pharmaceutical dosage form of claim 1 where there is less than 0.01% degradation of tenofovir disoproxil fumarate over a 24-hour period.

6. The pharmaceutical dosage form of claim 1 wherein less than 5% degradation of the tenofovir disoproxil fumarate or emtricitabine occurs after six months at 40° C./75% relative humidity when packaged and stored with desiccant.

7. The pharmaceutical dosage form of claim 1 comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, pregelatinized starch, croscarmellose sodium, lactose monohydrate, microcrystalline cellulose, and magnesium stearate.

8. The pharmaceutical dosage form of claim 7 comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, 50 mg pregelatinized starch, 60 mg croscarmellose sodium, 80 mg lactose monohydrate, 300 mg microcrystalline cellulose, and 10 mg magnesium stearate.

9. The pharmaceutical dosage form of claim 7 comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, 50 mg pregelatinized starch, 60 mg croscarmellose sodium, lactose monohydrate, 200 mg microcrystalline cellulose, and 10 mg magnesium stearate.

10. The pharmaceutical dosage form of claim 1 comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, pregelatinized starch, croscarmellose sodium, lactose monohydrate, microcrystalline cellulose, magnesium stearate, and colloidal silicon dioxide.

11. The pharmaceutical dosage form of claim 10 comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, 50 mg pregelatinized starch, 60 mg croscarmellose sodium, 175 mg lactose monohydrate, 200 mg microcrystalline cellulose, 10 mg magnesium stearate, and 5 mg colloidal silicon dioxide.

12. The pharmaceutical dosage form of claim 10 comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, hydroxypropyl methylcellulose, lactose, pregelatinized starch, and magnesium stearate.

13. The pharmaceutical dosage form of claim 10 comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, 112 mg hydroxypropyl methylcellulose, lactose, pregelatinized starch, and 7 mg magnesium stearate.

14. The pharmaceutical dosage form of claim 1 comprising less than 1% of impurities related to tenofovir disoproxil fumarate and emtricitabine.

15. A method for the treatment of the symptoms or effects of an HIV infection in an infected animal which comprises administering to said animal the pharmaceutical dosage form of claim 1.

16. A method for the treatment of the symptoms or effects of an HIV infection in an infected animal which comprises administering to said animal the pharmaceutical dosage form of claim 6.

17. A method for the treatment of the symptoms or effects of an HIV infection in an infected animal which comprises administering to said animal the pharmaceutical dosage form of claim 10.

18. The pharmaceutical dosage form of claim 1, wherein the starch is pregelatinized starch.

19. A chemically stable fixed dose combination pharmaceutical dosage form comprising 300 mg tenofovir disoproxil fumarate and 200 mg emtricitabine; a binder selected from the group consisting of povidone, gelatin, hydroxypropyl methylcellulose, cellulose, microcrystalline cellulose, pregelatinized starch, and acacia; a disintegrant selected from sodium starch glycolate, crosslinked-povidone, cross-linked sodium carboxymethylcellulose, maize starch, and alginic acid; and a lubricant selected from the group consisting of magnesium stearate, stearic acid, and talc;

wherein said pharmaceutical dosage form exhibits less than 10% degradation of the tenofovir disoproxil fumarate or emtricitabine after 6 months when packaged and stored with silica gel dessicant at 40° C./75% relative humidity.

20. A chemically stable fixed dose combination pharmaceutical dosage form comprising 300 mg tenofovir disoproxil fumarate and 200 mg emtricitabine; a binder selected from the group consisting of povidone, gelatin, hydroxypropyl methylcellulose, cellulose, microcrystalline cellulose, starch, and acacia; a disintegrant selected from sodium starch glycolate, crosslinked-povidone, cross-linked sodium carboxymethylcellulose, and alginic acid; and a lubricant selected from the group consisting of magnesium stearate, stearic acid, and talc;

wherein said pharmaceutical dosage form exhibits less than 1% degradation of the tenofovir disoproxil fumarate over a 24-hour period.

21. The pharmaceutical dosage form of claim 20, wherein there is less than 0.1% degradation of tenofovir disoproxil fumarate over a 24-hour period.

22. The pharmaceutical dosage form of claim 20, wherein there is less than 0.01% degradation of tenofovir disoproxil fumarate over a 24-hour period.

23. The pharmaceutical dosage form of claim 20, wherein the starch is pregelatinized starch.

24. A chemically stable fixed dose combination pharmaceutical dosage form comprising 300 mg tenofovir disoproxil fumarate and 200 mg emtricitabine; a binder selected from the group consisting of povidone, gelatin, hydroxypropyl methylcellulose, cellulose, microcrystalline cellulose, pregelatinized starch, and acacia; a disintegrant selected from sodium starch glycolate, crosslinked-povidone, cross-linked sodium carboxymethylcellulose, maize starch, and alginic acid; and a lubricant selected from the group consisting of magnesium stearate, stearic acid, and talc; wherein said pharmaceutical dosage form exhibits less than 1% degradation of the tenofovir disoproxil fumarate over a 24-hour period.

25. The pharmaceutical dosage form of claim 24, wherein there is less than 0.1% degradation of tenofovir disoproxil fumarate over a 24-hour period.

26. The pharmaceutical dosage form of claim 24, wherein there is less than 0.01% degradation of tenofovir disoproxil fumarate over a 24-hour period.

* * * * *

EXHIBIT D

US008716264B2

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

(10) **Patent No.: US 8,716,264 B2**
(45) **Date of Patent: *May 6, 2014**

(54) **COMPOSITIONS AND METHODS FOR COMBINATION ANTIVIRAL THERAPY**

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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.

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USPC **514/81**

(58) **Field of Classification Search**
USPC 514/45; 424/400, 408
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,524,846	A	8/1970	Moffatt et al.
3,622,677	A	11/1971	Short et al.
3,682,930	A	8/1972	Bourquin et al.
3,994,974	A	11/1976	Murakami et al.
4,003,878	A	1/1977	Skaar et al.
4,258,062	A	3/1981	Jonas et al.
4,355,032	A	10/1982	Verheyden et al.
4,384,005	A	5/1983	McSweeney
4,430,343	A	2/1984	Iemura et al.
4,476,248	A	10/1984	Gordon et al.
4,724,233	A	2/1988	De Clercq et al.
4,808,716	A	2/1989	Holy et al.
4,816,570	A	3/1989	Farquhar
4,879,288	A	11/1989	Warawa et al.
4,957,924	A	9/1990	Beauchamp
4,968,788	A	11/1990	Farquhar
5,047,407	A	9/1991	Belleau et al.
5,075,445	A	12/1991	Jarvest et al.
5,142,051	A	8/1992	Holy et al.
5,151,426	A	9/1992	Belleau et al.
5,155,268	A	10/1992	Hester
5,177,064	A	1/1993	Bodor
5,179,104	A	1/1993	Chu et al.
5,204,466	A	4/1993	Liotta et al.
5,208,221	A	5/1993	Kim et al.
5,210,085	A	5/1993	Liotta et al.
5,386,030	A	1/1995	Kim et al.
5,466,806	A	11/1995	Belleau et al.
5,476,938	A	12/1995	Vemishetti et al.
5,486,520	A	1/1996	Belleau et al.
5,506,347	A	4/1996	Erion et al.
5,512,596	A	4/1996	Kim et al.
5,514,798	A	5/1996	Bischofberger et al.
5,538,975	A	7/1996	Dionne et al.
5,587,480	A	12/1996	Belleau et al.
5,618,820	A	4/1997	Dionne et al.
5,618,964	A	4/1997	Cheng et al.
5,627,186	A	5/1997	Cameron et al.
5,663,159	A	9/1997	Starrett, Jr. et al.
5,696,254	A	12/1997	Mansour et al.
5,733,788	A	3/1998	Bischofberger
5,744,596	A	4/1998	Mansour et al.
5,756,706	A	5/1998	Mansour et al.
5,763,606	A	6/1998	Mansour et al.

(Continued)

FOREIGN PATENT DOCUMENTS

EP	0 182 024	A2	5/1986
EP	0 206 459	A2	12/1986

(Continued)

OTHER PUBLICATIONS

GILD—Gilead Sciences Conference Call to Discuss Triangle Pharmaceuticals Acquisition dated Dec. 4, 2002.*

Ristig et al. (Tenofovir Disoproxil Fumarate, TDF) Therapy for Chronic Hepatitis B in Human Immunodeficiency Virus/Hepatitis B Virus—Coinfected Individuals for Whom Interferon-alpha and Lamivudine Therapy Have Failed, JID 2002; 186 pp. 1844-1847.*

Pre-Grant Opposition Petition against Brazilian Patent Application PI 0406760-6 (Aug. 20, 2010).

(Continued)

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(57) **ABSTRACT**

The present invention relates to therapeutic combinations of [2-(6-amino-purin-9-yl)-1-methyl-ethoxymethyl]-phosphonic acid diisopropoxycarbonyloxymethyl ester (tenofovir disoproxil fumarate, Viread®) and (2R,5S,cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine, Emtriva™, (−)-cis FTC) and their physiologically functional derivatives. The combinations may be useful in the treatment of HIV infections, including infections with HIV mutants bearing resistance to nucleoside and/or non-nucleoside inhibitors. The present invention is also concerned with pharmaceutical compositions and formulations of said combinations of tenofovir disoproxil fumarate and emtricitabine, and their physiologically functional derivatives, as well as therapeutic methods of use of those compositions and formulations.

38 Claims, No Drawings

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,792,756	A	8/1998	Starrett, Jr. et al.
5,798,340	A	8/1998	Bischofberger et al.
5,814,639	A	9/1998	Liotta et al.
5,859,021	A	1/1999	Cameron et al.
5,905,082	A	5/1999	Roberts et al.
5,914,331	A	6/1999	Liotta et al.
5,922,695	A	7/1999	Arimilli et al.
5,935,946	A	8/1999	Munger, Jr. et al.
5,977,089	A	11/1999	Arimilli et al.
6,043,230	A	3/2000	Arimilli et al.
6,057,305	A	5/2000	Holy et al.
6,069,249	A	5/2000	Arimilli et al.
6,113,920	A	9/2000	Maye et al.
6,114,343	A	9/2000	Liotta et al.
6,121,315	A	9/2000	Nair et al.
6,194,391	B1	2/2001	Schinazi et al.
6,312,662	B1	11/2001	Erion et al.
RE38,333	E	11/2003	Arimilli et al.
7,094,413	B2	8/2006	Buelow et al.
2001/0012518	A1	8/2001	Makool-Morehead et al.
2001/0014352	A1	8/2001	Batra et al.
2003/0203969	A1	10/2003	Bevec et al.
2004/0180089	A1	9/2004	Plachetka et al.
2004/0224917	A1	11/2004	Dahl et al.
2004/0253218	A1	12/2004	Eisenbach-Schwartz et al.
2005/0197320	A1	9/2005	Chen et al.
2006/0128692	A1	6/2006	Chen et al.
2006/0246130	A1	11/2006	Dahl et al.
2007/0036861	A1	2/2007	Oury et al.
2007/0077295	A1	4/2007	Dahl et al.
2007/0099902	A1	5/2007	Dahl et al.
2009/0143314	A1	6/2009	Dahl et al.

FOREIGN PATENT DOCUMENTS

EP	0 269 947	A1	6/1988
EP	0 369 409	A1	5/1990
EP	0 481 214	A1	4/1992
EP	0 482 657	A2	4/1992
EP	0 632 048	A1	1/1995
EP	0 647 649	A1	4/1995
EP	0 694 547	A2	1/1996
EP	1 256 585	A1	11/2002
EP	1332757		8/2003
GB	942 152	A	11/1963
GB	1 523 865	A	9/1978
GB	2 111 043	A	6/1983
IN	698/KOLNP/2005		6/2004
WO	WO 88/05438		7/1988
WO	WO-91/19721		12/1991
WO	WO 92/01698		2/1992
WO	WO 92/09611	A1	6/1992
WO	WO 92/13869		8/1992
WO	WO-92/14743	*	9/1992
WO	WO 94/03466		2/1994
WO	WO 94/03467	A2	2/1994
WO	WO 95/07919		3/1995
WO	WO 95/07920	A1	3/1995
WO	WO 95/32957	A1	12/1995
WO	WO 96/18605	A1	6/1996
WO	WO 98/04569 AI		2/1998
WO	WO-99/25352	A1	5/1999
WO	WO 99/61026		12/1999
WO	WO-00/25797		11/2000
WO	WO 01/64221	A1	9/2001
WO	WO-02/08241	*	1/2002
WO	WO-02/62123	A2	8/2002
WO	WO-02/68058		9/2002
WO	WO-02/070518		9/2002
WO	WO 03/045327		6/2003
WO	WO 03/059327		7/2003
WO	WO-2004/064845	A1	8/2004
WO	WO 2005/021001		3/2005
WO	WO 2006/135933		12/2006
WO	WO-2006/135993	A2	12/2006
WO	WO 2007/068934	A2	6/2007

OTHER PUBLICATIONS

Office Action for Patent Application No. 2,512,475 issued by the Canadian Patent Office (Jan. 10, 2008).

First Office Action for Patent Application No. 200480002190.5 issued by the Patent Office of the People's Republic of China (Aug. 4, 2006) (translation).

Rejection Decision for Patent Application No. 200480002190.5 issued by the Patent Office of the People's Republic of China (Jan. 15, 2010) (translation).

Official Action for Patent Application No. 200501134/28 issued by the Eurasian Patent Office (Oct. 15, 2006) (translation).

Official Action for Patent Application No. 200501134/28 issued by the Eurasian Patent Office (Dec. 25, 2008) (translation).

Examiner's Remarks for Patent Application No. 200501134/28 issued by the Eurasian Patent Office (received Jul. 30, 2009) (translation).

Communication and Annex for Patent Publication EP1583542 (Application No. 04701819.7) issued by the European Patent Office (Oct. 24, 2005).

Communication and Annex for Patent Publication EP1583542 (Application No. 04701819.7) issued by the European Patent Office (Jul. 26, 2006).

Communication and Annex for Patent Publication EP1583542 (Application No. 04701819.7) issued by the European Patent Office (Jul. 17, 2007).

Decision to Grant Patent Publication EP1583542 (Application No. 04701819.7) issued by the European Patent Office and Druckexemplar (Nov. 5, 2007).

Teva Pharmaceutical Industries Ltd., Notice of Opposition of EP Patent EP1583542B1 (Application No. 04701819.7) (Mar. 13, 2009).

Generics [UK] Limited, Notice of Opposition of EP Patent EP1583542B1 (Application No. 04701819.7) (Mar. 18, 2009).

Communication of further notices of opposition pursuant to Rule 79(2) EPC for EP Patent EP1583542B1 (Application No. 04701819.7) and Request to File Observations (Apr. 23, 2009).

Reply of the Patent Proprietor to the Notices of Opposition of EP Patent EP1583542B1 (Application No. 04701819.7) (Jan. 4, 2010).

Letter Regarding the Opposition Procedure for EP Patent EP1583542B1 (Application No. 04701819.7) (Mar. 11, 2010).

Summons to Attend Oral Proceedings and Annex to the Communication for EP Patent EP1583542B1 (Application No. 04701819.7) (May 21, 2010).

Teva Pharmaceutical Industries Ltd., Written Submission in preparation for Oral Proceedings for EP Patent EP1583542B1 (Application No. 04701819.7) (Sep. 16, 2010).

Generics [UK] Limited, Written Submission in preparation for Oral Proceedings for EP Patent EP1583542B1 (Application No. 04701819.7) (Sep. 17, 2010).

Gilead Sciences Inc., Written Submission in preparation for Oral Proceedings for EP Patent EP1583542B1 (Application No. 04701819.7) (Sep. 17, 2010).

Information about the Results of Oral Proceedings for EP Patent EP1583542B1 (Application No. 04701819.7), Claims, Amended Claims and Minutes of the Oral Proceeding (Nov. 19, 2010).

Decision of the Opposition Division for EP Patent EP1583542B1 (Application No. 04701819.7).

Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc. Case No. 08-CV-10838 (Dec. 12, 2008).

Answer and Counterclaim against Gilead Sciences, Inc. filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Feb. 5, 2009).

Plaintiffs Reply to Teva's Counterclaim, filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Feb. 25, 2009).

(56) **References Cited**

OTHER PUBLICATIONS

First Amended Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Jul. 1, 2009).
Amended Answer and Counterclaim against Gilead Sciences, Inc. filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Jul. 20, 2009).
Plaintiffs Reply to Teva USA's Amended Counterclaim, filed by Gilead Sciences, Emory University, Case No. 08-CV-10838 (Aug. 10, 2009).
Second Amended Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Sep. 25, 2009).
Second Amended Answer and Counterclaim against Gilead Sciences, Inc. filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Filed Oct. 9, 2009).
Plaintiffs Reply to Teva USA's Second Amended Counterclaim, filed by Gilead Sciences, Emory University,Case No. 08-CV-10838 (Oct. 15, 2009).
Third Amended Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Jul. 6, 2010).
Third Amended Answer filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Filed Aug. 6, 2010).
Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Mar. 5, 2010).
Answer filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (May 10, 2010).
Declaration of Colleen Tracy in Support of a Motion for Leave to File Amended Complaint Case No. 10-CV-01796 (Apr. 11, 2011).
Memorandum of Law in Opposition of a Motion for Leave to File Amended Complaint Case No. 10-CV-01796 (Apr. 25, 2011).
Declaration of James Galbraith in Opposition of a Motion for Leave to File Amended Complaint Case No. 10-CV-01796 (Apr. 25, 2011).
Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc. and Emory University Case No. 10-CV-01798 (Mar. 5, 2010).
Answer filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case.
Plaintiff's Reply to Teva's Counterclaim, filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Feb. 25, 2009).
Third Amended Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Sep. 25, 2009.
Answer filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01798 (May 10, 2010).
Complaint for Patent Infrigement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company. Case No. 10-CV-01851 (Mar. 11, 2010).
Answer filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01851 (May 17, 2010).
Anderson, "Antiviral dynamics and sex differences of zidovudine and lamivudine triphosphate concentrations in HIV-infected individuals," AIDS 17:2159-2168 (2003).
Delehanty et al. Slides from the oral presentation for "A Randomized Study of Three Doses of FTC Versus 3TC in HIV-Infected Patients," 6th CROI (Jan. 31-Feb. 4, 1999) Chicago.
Delehanty et al., "A Phase I/II Randomized, Controlled Study of FTC Versus 3TC in HIV-Infected Patients," 6th CROI (Jan. 31-Feb. 4, 1999) Chicago, Session 5, Abstract 16.
Feng, J. et al, "Mechanistic studies show that 9-)-FTC-TP is a better inhibitor of HIV-1 reverse transcriptase than 3TC-TP," FASEB 13:1511-1517 (1999).
Harris et al., "Genotypic Analysis of HIV-1 Infected ART Naive Patients Receiving Emtricitabine (FTC) or Lamivudine (3TC) in a Double Blind Equivalence Trial," 5th International Workshop on Drug Resistance and Treatment Strategies, Jun. 4-8, No. 104 (2001).
Havlir et al., "In Vivo Antagonism with Zidovudine plus Stavudine Combination Therapy," J. Infect. Disease 182:321-325 (2000).
Hazen et al., "Relative Anti-HIV-1 Efficacy of Lamivudine and Emtricitabine In Vitro Is Dependent on Cell Type," J. AIDS 32:255-258 (2003).
Margot et al., "Development of HIV-1 Drug Resistance Through 144 Weeks in Antiretroviral-Naive Subjects on Emtricitabine, Tenofovir Disoproxil Fumarate, and Efavirenz Compared with Lamivudine/Zidovudine and Efavirenz in Study GS-01-934," JAIDS 52(2):209-221 (2009).
McColl et al., "Pooled Analysis of Recent Emtricitabine and Lamivudine Clinical Trials Reveals Differences in Rates of Development of the M184V/I Mutation," Poster No. PE7.3/17, 10th European AIDS Conference (EACS) Nov. 17-20, 2005, Dublin Ireland.
Mills et al., "ARTEMIS: Efficacy and Safety of Darunavir/ritonavir (DRV/r) 800/100mg Once-daily vs Lopinavir/ritonavir (LPV/r) in Treatment-naive, HIV-1-infected Patients at 96 wks," 48th Annual ICAAC/IDSA, 46th Annual Meeting, Washington, D.C. Oct. 25-28, 2008, Presentation No. H-1250c.
Molina et al., "A Lopinavir/Ritonavir-Based Once-Daily Regimen Results in Better Compliance and Is Non-inferior to a Twice-Daily Regimen Through 96 Weeks," AIDS Research and Human Retroviruses 23(12):1505-1514 (2007).
Sanne et al., "Two Randomized, Controlled, Equivalence Trials of Emtricitabine (FTC) to Lamivudine (3TC)," Poster 4432 presented at the XIV International AIDS Conference, Jul. 7-12, 2002, Barcelona, Spain.
Sanne et al., "Genotypic Analysis of HIV-1 Infected ART-Naive Patients Receiving Emtricitabine (FTC) or Lamivudine (3TC) in a Double Blind Equivalence Trial," Poster No. 4433, presented at the XIV International AIDS Conference Jul. 7-12, 2002, Barcelona, Spain.
Schinazi et al., "Selective Inhibition of Human Immunodeficiency Viruses by Racemates and Enantiomers of cis-5-Fluoro-1-[2-(Hydroxymethyl)-1,3-Oxathiolan-5-yl]Cytosine," Antimicrobial Agents and Chemotherapy 36(11):2423-2431(1992).
Schinazi et al., Letter to the Editor "Assessment of the Relative Potency of Emtricitabine and Lamivudine," J. AIDS 34(2)243-245 (2003).
Smith et al., "Randomized, double-blind, placebo-matched, multicenter trial of abavacir/lamivudine or tenofovir/emtricitabine with lopinavir/ritonavir for inital HIV treatment," AIDS 23:1547-1556 (2009).
Walmsley et al., "Gemini: A Noninferiority Study of Saquinavir/Ritonavir Versus Lopinavir/Ritonavir as Initial HIV-1 Therapy in Adults," J. Acquir. Immune Defic. Syndr. 50(4):367-374 (2009).
Wang et al. "Lack of Significant Pharmacokinetic Interactions between Emtricitabine and Other Nucleoside Antivirals in Healthy Volunteers," 41st ICAAC Abstracts, Chicago, IL, Sep. 22-25, 2001, Abstract A-505.
Wang et al. "Pharmacokinetic and pharmacodynamic characteristics of emtrictabine support its once daily dosing," Int. Conf. AIDS, Jul. 7-12 14:abstract TUPeB4546 (2002).
Search and Examination Report for Application No. AP/P/2005/003348 issued by the African Regional Intellectual Property Organization (Apr. 10, 2008).
Examiner's First Report on Patent Application No. 2004206821 issued by the Australian Patent Office (Aug. 28, 2007).
Examiner's Second Report on Patent Application No. 2004206821 issued by the Australian Patent Office (Aug. 20, 2008).
Examiner's First Report on Patent Application No. 2009200414 issued by the Australian Patent Office (Feb. 24, 2010).
Decision to Grant Patent Publication EP1583542 (Application No. 04701819.7) issued by the European Patent Office and Druckexemplar (May 23, 2008).

(56) **References Cited**

OTHER PUBLICATIONS

Decision of the Opposition Division for Ep Patent EP1583542B1 (Application No. 04701819.7), Claims, Grounds for the Decision and Provision of the minutes (Jan. 31 and Feb. 14, 2011).
Notice of Appeal of the Decision of the Opposition Division for EP Patent EP1583542B1 (Application No. 04701819.7) (Mar. 29, 2011).
Extended European Search Report for Patent Publication EP1923063 (Application No. 08152527.1) issued by the European Patent Office (Mar. 10, 2009).
Communication pursuant to Article 94(3) EPC for Patent Publication EP1923063 (Application No. 08152527.1) issued by the European Patent Office (Sep. 4, 2009).
Substantive Examination Report for Patent Application No. W0-200502145 issued by the Indonesian Patent Office (2010).
First Examination Report for Patent Application No. 3383/DELNP/2005 issued by the Indian Patent Office (Jul. 31, 2007).
Opponents Comments to the Reply Statement by the Applicant relating to Patent Application No. 3383/DELNP/2005 (Aug. 14, 2008).
Decision of Hearing of the Indian Patent Office for Patent Application No. 3383/DELNP/2005 (Mar. 25, 2009).
Office Action for Patent Application No. 2006-500939 issued by the Japanese Patent Office (Nov. 9, 2009) (translation).
Office Action for Patent Application No. 2006-500939 issued by the Japanese Patent Office (Mar. 25, 2010) (translation).
Office Action for Korean Patent Application No. 7013069/2005 issued by the Korean Intellectual Property Office (May 22, 2007) (translation).
Office Action for Patent Application No. 7007002/2008 issued by the Korean Intellectual Property Office (Jun. 11, 2008) (translation).
Decision of Rejection for Patent Application No. 7007002/2008 issued by the Korean Intellectual Property Office (Jan. 7, 2009) (translation).
Office Action for Patent Application No. 7009376/2009 issued by the Korean Intellectual Property Office (Oct. 9, 2009) (translation).
Examination Report for Patent Application No. 540728 issued by the Intellectual Property Office of New Zealand (Apr. 24, 2007).
Further Examination Report for Patent Application No. 540728 issued by the Intellectual Property Office of New Zealand (Feb. 11, 2008).
Further Examination Report for Patent Application No. 540728 issued by the Intellectual Property Office of New Zealand (Jun. 18, 2008).
Official Action for Patent Application No. 93100813 issued by the Taiwanese Intellectual Property Office (Jun. 22, 2005) (translation).
Rejection of Patent Application No. 93100813 issued by the Taiwanese Intellectual Property Office (Feb. 22, 2006) (translation).
Second Official Action for Patent Application No. a 2005 07947 issued by the Ukrainian Patent Office (2006) (translation).
Third Official Action for Patent Application No. a 2005 07947 issued by the Ukrainian Patent Office (2007) (translation).
First Official Action for Patent Application No. a 2008 00555 issued by the Ukrainian Patent Office (2011) (translation).
Opinion on Patent Application No. 1-2005-00812 issued by the Vietnamese Patent Office (Jul. 27, 2008).
Revised International Search Report for PCT/US2004/000832 (Aug. 5, 2004).
Written Opinion issued by the ISA for PCT/US2004/000832 (Jul. 12, 2004).
Byrn (editor), Solid State Chemistry of Drugs, 2cd Edition, p. 22, 1999.
Drugs and the Pharmaceutical Sciences, vol. 1999, p. 60 (Mark Gibson, ed), 2009.
Newman and Byrn, "Solid-state analysis of the active pharmaceutical ingredient in drug products" Drug Discovery Today, 8(19) 898-905 (2003).
Zhang et al. "Phase transformation considerations during process development and manufacture of solid oral dosage forms" Adv Drug Del Reviews 56(30), 371-390 (2004).
Office Action for Patent Application No. 2,611,520 issued by the Canadian Patent Office (Jun. 7, 2010).
Communication of Intent to Grant Patent Application No. 06773194.3 (EP1890681) and Druckexemplar issued by the European Patent Office (Jul. 15, 2008).
Decision to Grant Patent Application No. 06773194.3 (EP 1890681B1) issued by the European Patent Office (Dec. 11, 2008).
Teva Pharmaceuticals Industries Ltd., Notice of Opposition of EP 1890681B1 (Application No. 06773194.3) (Oct. 7, 2009).
Communication of notices of opposition pursuant to Rule 79(2) EPC for EP 1890681B1 (Application No. 06773194.3) and Request to File Observations (Nov. 12, 2009).
Reply of the Patent Proprietor to the Notice of Opposition of EP 1890681B1 (Application No. 06773194.3) (Jun. 22, 2010).
Summons to Attend Oral Proceedings and Annex to the Communication for the Opposition of EP 1890681B1 (Application No. 06773194.3) (Oct. 14, 2010).
Gilead Sciences Inc., Written Submission in preparation for Oral Proceedings for the Opposition of EP 1890681B1 (Application No. 06773194.3) (Feb. 4, 2011).
Gilead Sciences Inc., Written Submission in preparation for Oral Proceedings for the Opposition of EP 1890681B1 (Application No. 06773194.3) (Mar. 2 & 4, 2011).
Teva Pharmaceuticals Industries Ltd., Written Submission in preparation for Oral Proceedings for the Opposition of EP 1890681B1 (Application No. 06773194.3) (Mar. 17, 2011).
Information About the Results of the Oral Proceedings for EP 1890681B1 (Application No. 06773194.3) (Apr. 5, 2011).
Examination Report Patent Application No. 504045 issued by the Intellectual Property Office of New Zealand (Oct. 6, 2009).
International Search Report for PCT/US2006/023222 (Feb. 23, 2007).
Written Opinion of the ISA for PCT/US2006/023222 (Feb. 23, 2007).
Response to the Written Opinion of the ISA (May 10, 2007).
International Preliminary Report on Patentability for PCT/US2006/023222 (Oct. 8, 2007).
Alexander et al., "Investigation of (Oxodioxolenyl)methyl carbamates as nonchiral bioreversible prodrug moieties for chiral amines," J. Med. Chem. 39:480-486, 1996.
Arimilli et al., "Synthesis, in vitro biological evaluation and oral bioavailability of 9-[2-(phosphonomethoxy)propyl]adenine (PMPA) prodrugs," Antiviral Chem. & Chemo. 8(6):557-567, 1997.
Arimilli et al., "Orally bioavailable acylic nucleoside phosphonate prodrugs: Adefovir, Dipivoxil and Bis(POC)PMPA," vol. 3 (accepted for publication), Adv. Antiviral Drug Design, 1998.
Balzarini et al, "Differential antiherpesvirus and antiretrovirus effects of the (S) and (R) enantiomers of acyclic nucleoside phosphonates: potent and selective in vitro and in vivo antiretrovirus activities of (R)-9-(2-phosphonomethoxypropyl)-2,6-diaminopurine." Antimicrob Agents Chemother. Feb. 1993; 37(2): 332-338.
Benzaria et al., "New prodrugs of 9-(2-phosphonomethoxyethyl)adenine (PMEA): Synthesis and stability studies," Nucls. & Nuclt. 14(3-5):563-565, 1995.
Berge et al., "Pharmaceutical salts," J. Pharm. Sci. 66(1):1-19, 1977.
De Clercq et al., "(S)-9-(2,3-dihydroxypropyl)adenine: An aliphatic nucleoside analog with broad spectrum antiviral activity," Science 200:563-565, 1978.
Flaherty et al., "Synthesis and selective monoamine oxidase B-inhibiting properties of 1-Methyl-1,2,3,6-tetrahydropyrid-4-yl carbamate derivatives: potential prodrugs of (R)-and (S)-Nordeprenyl," J. Med. Chem. 39:4759-4761, 1996.
Hammer et al., "Ether, carbonate and urethane deoxynucleoside derivatives as prodrugs," Acta Chemica Scandinavia 50:609-622, 1996.
Ikeda et al., "Studies of prodrugs III. A convenient and practical preparation of Amphicillin prodrugs," Chem. Pharm. Bull. 32:4316-4322, 1984.
Iyer et al., "Synthesis of acyloxyalkyl acylphosphonates as potential prodrugs of the antiviral Trisodium phosphonoformate (Foscarnet sodium)," Tet. Lett. 30(51): 7141-7144, 1989.

(56) **References Cited**

OTHER PUBLICATIONS

Krise et al., "Prodrugs of phosphates, phosphonates, and phosphinates," Advanced Drug Delivery Reviews 19:287-310, 1996.
Landgrebe, J. A., "Crystallization and filtration," Theory and Practice in the Organic Laboratory, 3rd Edition, pp. 65-77, 1982.
Lindahl et al., "Synthesis of an acyloxymethyl prodrug of the Inositol phosphate alpha-Trinositol," J. Carbohydrate Chemistry 15(5):549-554, 1996.
Maillard et al., "Adenosine receptor prodrugs: Synthesis and biological activity of derivatives of potent A1-selective agonists," J. Pharm. Sci. 83(1):46-53, 1994.
Osol et al., Editor, Remington's Pharmaceutical Sciences, Sixteenth Edition, pp. 1554-1557, 1980.
Robinson et al., "Discovery of the Hemifumarate and (alpha-L-Alanyloxy)methylester as prodrugs of an antirheumatic oxindole: Prodrugs for enolic OH group," J. Med. Chem. 39:10-18, 1996.
Safadi et al., "Phosphoryloxymethyl carbamates and carbonates-Novel water soluble prodrugs for amines and hindered alcohols," Pharm. Res. 10(9):1350-1355, 1993.
Sakamoto et al., "Studies on prodrugs II. Preparation and characterization of (5-substituted 2-oxo-1, 3-dioxolen-4-yl)methyl esters of Ampicillin," Chem. Pharm. Bull. 32(6):2241-2248, 1983.
Samara et al., "Pharmacokinetic analysis of Diethylcarbonate prodrugs of Ibuprofen and Naproxen," Biopharmaceutics & Drug Disposition 16:201-210, 1995.
Shaw et al., "Metabolism and pharmacokinetics of novel oral prodrugs of 9-[(R)-2-(phosphonomethoxy)propyl]adenine (PMPA) in dogs," Pharm. Res. 14(12):1824-1829, 1997.
Srivastva et al., "Bioreversible phosphate protective groups: Synthesis and stability of model acyloxymethyl phosphates," Bioorg. Chem. 12:118-129, 1984.
Starret et al., "Synthesis and in vitro evaluation of a phosphonate prodrug:bis(pivaloyloxymethyl) 9-(2-phophonylmethoxyethyl)adenine," Antiviral Res. 19:267-273, 1992.
Starret et al., "Synthesis, oral bioavailability determination, and in vitro evaluation of prodrugs of the Antiviral agent 9-[2-(phosphonomethoxy)ethyl)adenine (PMEA)," J. Med. Chem. 37:1857-1864, 1994.
Sueoka et al., "Pharmacokinetics of Alkoxycarbonyloxy ester prodrugs of PMPA in dogs," Abstract, American Association of Pharmaceutical Science, Western Regional Meeting, Apr. 24-25, 1997.
Tsai et al., "Prevention of SIV infection in macaques by (R)-9-(2-phosphonylmethoxypropyl)adenine," Science 270:1197-1199, 1995.
Tsai et al., "Effects of (R)-9-(2-phophonylmethoxypropyl)adenine monotherapy on chronic SIV infection in macaques," Aids Res. & Hum. Retro. 13(8):707-712, 1997.
Ueda et al., "Vinyl compounds of nucleic acid bases I. Synthesis of N-vinyluracil, N-vinylthymine, and N-vinyladenine," Die Makromolekulare Chemie 120:13-20, 1968.
Examiner's First Report on Patent Application No. 85827/98 issued by the Australian Patent Office (Feb. 28, 2001).
Examiner's Second Report on Patent Application No. 85827/98 issued by the Australian Patent Office (Mar. 27, 2002).
Office Action for Patent Application No. 2,298,059 issued by the Canadian Intellectual Property Office (Apr. 25, 2007).
First Office Action for Patent Application No. 97197460.8 issued by the Chinese Patent Office (Jan. 16, 2004) (translation).
Second Office Action for Patent Application No. 97197460.8 issued by the Chinese Patent Office (Aug. 5, 2005) (translation).
Third Office Action for Patent Application No. 97197460.8 issued by the Chinese Patent Office (Jun. 9, 2006) (translation).
First Office Action for Patent Application No. 200410046290.X issued by the Chinese Patent Office (Jun. 17, 2005) (translation).
First Office Action for Patent Application No. 200510099916.8 issued by the Chinese Patent Office (Jun. 15, 2007) (translation).
Second Office Action for Patent Application No. 200510099916.8 issued by the Chinese Patent Office (Nov. 16, 2007) (translation).
Office Action for Patent Application No. 11-510067 issued by the Japanese Patent Office (Dec. 11, 2007) (translation) (translation).
Office Action for Patent Application No. 10-2000-7000636 issued by the Korean Intellectual Property Office (Aug. 19, 2005) (translation).
Decision of Rejection for Patent Application No. 7000636/2000 issued by the Korean Intellectual Property Office (May 10, 2006) (translation).
Office Action for Patent Application No. 87112168 issued by the Taiwanese Intellectual Property Office (Oct. 20, 2000) (translation).
Decision of Rejection for Patent Application No. 87112168 issued by the Taiwanese Intellectual Property Office (Apr. 6, 2001) (translation).
Office Action for Patent Application No. 87112168 issued by the Taiwanese Intellectual Property Office (May 6, 2002) (translation).
Office Action for Patent Application No. 87112168 issued by the Taiwanese Intellectual Property Office (Mar. 1, 2004) (translation).
Office Action for Patent Application No. 93112403 issued by the Taiwanese Intellectual Property Office (Apr. 27, 2005) (translation).
Office Action for U.S. Appl. No. 08/900,752 issued by the United States Patent and Trademark Office (Apr. 16, 1998).
International Search Report for PCT/US1998/015254 (Nov. 25, 1998).
Request for Ex Parte Reexamination of U.S. Patent No. 5,935,946 (submitted Apr. 30, 2007).
Order Granting Request for Ex Parte Reexamination of U.S. Patent No. 5,935,946 (Jul. 13, 2007).
Official Action for Ex Parte Reexamination of U.S. Patent No. 5,935,946 (Jan. 16, 2008).
Beauchamp et al., "Amino acid ester prodrugs of acyclovir," *Antivir. Chem. and Chemoth.* 3(3):157-64, 1992.
Colla et al., "Synthesis and antiviral activity of water-soluble esters of acyclovir [9-[(2-Hydroxyethoxy)methyl]guanine]," *J. Med. Chem.* 26:602-04, 1983.
Davidsen et al., "N-(Acyloxyalkyl)pyridinium salts as soluble prodrugs of a potent platelet activating factor antagonist," J. Med. Chem. 37(26):4423-4429, 1994.
Engel, R., "Phosphonates as analogues of natural phosphates," Chem. Rev. 77(3):349-367, 1977.
Farquhar et al., "Synthesis and antitumor evaluation of Bis[(pivaloxy)methyl] 2'-deoxy-5-fluorouridine 5'-monophosphate (FdUMP): a strategy to introduce nucleotides into cells," *J. Med. Chem.* 37(23):3902-03, 1994.
Folkmann et al., "Acyloxymethyl carbonochloridates. New intermediates in prodrug synthesis," Synthesis, pp. 1159-1166, 1990.
McIntee et al., "Probing the mechanism of action and decomposition of amino acid phosphomonoester amidates of antiviral nucleoside prodrugs," *J. Med. Chem.* 40(2):3323-31, 1997.
Naesens et al., "Antiretroviral activity and pharmacokinetics in mice of oral Bis(Pivaloyloxymethyl)-9-(2-phosphonylmethoxyethyl)adenine, the Bis(Pivaloyloxymethyl)ester prodrug of 9-(2-Phosphonylmethoxyethyl)adenine," Antimicro AG & Chemo. 40(1)22-28, 1996.
Notari, "Prodrug Design," *Pharmaceutical Therapy,* 14:25-53, 1981.
Serafinowska et al., "Synthesis and in vivo Evaluation of prodrug of 9-{2-(Phosphonomethoxy) ethoxy} adenine," J. Med. Chem. 38:1372-1379, 1995.
Srinivas et al., "Metabolism and in vitro antiretroviral activities of Bis(Pivaloyloxymethyl) prodrugs of acyclic nucleoside phosphonates," Antimicro AG & Chemo. 37(10):2247-2250, 1993.
Thornber "Isosterism and Molecular Modification in Drug design" Chem. Soc. Reviews 18:563-580, 1979.
Weller et al., "Orally active Fibrinogen receptor antagonists. 2. Amidoximes a prodrug of amidines," J. Med. Chem. 39:3139-3147, 1995.
Office Action for Patent Application No. 2,261,619 issued by the Canadian Patent Office (Dec. 22, 2004).
First Examination Report for Patent Application No. 602/DEL/2007 issued by the Indian Patent Office (Nov. 18, 2009).
Official Action for Patent Application No. 10-508318 issued by the Japanese Patent Office (Apr. 10, 2007) (translation).
Official Action for Patent Application No. 10-1999-7000806 issued by the Korean Intellectual Property Office (Apr. 28, 2005) (translation).
Decision of Rejection for Application No. 7000806/1999 issued by the Korean Intellectual Property Office (Jan. 20, 2006) (translation).

(56) **References Cited**

OTHER PUBLICATIONS

Official Action for Patent Application No. 333687 issued by the Intellectual Property Office of New Zealand (Mar. 2, 1999).
Decision of Rejection for Patent Application No. 86110757 issued by the Intellectual Property Office of Taiwan (Nov. 11, 1999) (translation).
Final Office Action for Patent Application No. 86110757 issued by the Intellectual Property Office of Taiwan (Sep. 5, 2000) (translation).
Final Office Action for Patent Application. No. 89123708 issued by the Intellectual Property Office of Taiwan (Apr. 12, 2001) (translation).
International Search Report for PCT/US1997/013244 (Oct. 20, 1997).
Request for Ex Parte Reexamination of U.S. Patent No. 5,922,695 (submitted Apr. 30, 2007).
Order Granting Request for Ex Parte Reexamination of U.S. Patent No. 5,922,695 (Jul. 13, 2007).
Official Action for Ex Parte Reexamination of U.S. Patent No. 5,922,695 (Dec. 11, 2007).
Request for Ex Parte Reexamination of U.S. Patent No. 5,977,089 (submitted Apr. 30, 2007).
Order Granting Request for Ex Parte Reexamination of U.S. Patent No. 5,977,089 (Jul. 13, 2007).
Official Action for Ex Parte Reexamination of U.S. Patent No. 5,977,089 (Jan. 16, 2008).
Request for Ex Parte Reexamination of U.S. Patent No. 6,043,230 (submitted Apr. 30, 2007).
Order Granting Request for Ex Parte Reexamination of U.S. Patent No. 6,043,230 (Jul. 13, 2007).
Official Action for Ex Parte Reexamination of U.S. Patent No. 6,043,230 (Dec. 11, 2007).
Anderson, P. (2003) "Antiviral dynamics and sex differences of zidovudine and lamivudine triphosphate concentrations in Hiv-infected individuals," *AIDS* 17:2159-2168.
Delehanty, J. et al "A Phase I/II Randomized, Controlled Study of FTC Versus 3TC in HIV-Infected Patients," 6th CROI (Jan. 31-Feb. 4, 1999) Chicago, Session 5, Abstract 16.
Delehanty, J. et al. Slides from the oral presentation for "A Randomized Study of Three Doses of FTC Versus 3TC in HIV-Infected Patients," 6th CROI (Jan. 3 31- Feb. 4, 1999) Chicago.
Feng, J. et al, (1999) "Mechanistic studies show that 9-)-FTC-TP is a better inhibitor of HIV-1 reverse transcriptase than 3TC-TP," *FASEB* 13:1511-17.
Harris et al. (2001) "Genotypic Analysis of HIV-1 Infected ART Naive Patients Receiving Emtricitabine (FTC) or Lamivudine (3TC) in a Double Blind Equivalence Trial," 5th International Workshop on Drug Resistance and Treatment Strategies, Jun. 4-8, No. 104.
Havlir, D. et al. (2000) "In Vivo Antagonism with Zidovudine plus Stavudine Combination Therapy," *The Journal of Infectious Diseases* 182:321-5.
Hazen, R. et al. (2003) "Relative Anti-HIV-1 Efficacy of Lamivudine and Emtricitabine In Vitro Is Dependent on Cell Type," *JAIDS* 32:255-258.
Margot, N. et al. (2009) "Development of HIV-1 Drug Resistance Through 144 Weeks in Antiretroviral-Naive Subjects on Emtricitabine, Tenofovir Disoproxil Fumarate, and Efavirenz Compared with Lamivudine/Zidovudine and Efavirenz in Study GS-01-934," *J Acquir Immune Defic Syndr* 52(2):209-221.
McColl, DJ. et al. "Pooled Analysis of Recent Emtricitabine and Lamivudine Clinical Trials Reveals Differences in Rates of Development of the M184V/I Mutation," Poster No. PE7.3/17, 10th European AIDS Conference (EACS) Nov. 17-20, 2005, Dublin Ireland.
Mills, A. et al. "ARTEMIS: Efficacy and Safety of Darunavir/ritonavir (DRV/r) 800/100mg Once-daily vs Lopinavir/ritonavir (LPV/r) in Treatment-naive, HIV-1-infected Patients at 96 wks," 48th Annual ICAAC/IDSA, 46th Annual Meeting, Washington, D.C. Oct. 25-28, 2008, Presentation No. H-1250c.
Molina, J. et al. "Castle: Atazanavir-Ritonavir vs Lopinavir-Ritonavir in Antiretroviral-Naive HIV-1 Infected Patients: 96 Week Efficacy & Safety," 48th Annual ICAAC/IDSA 46th Annual Meeting, Washington, D.C., Oct. 25-28, 2008, Presentation No. H-1250d.
Molina, J. et al. (2007) "A Lopinavir/Ritonavir-Based Once-Daily Regimen Results in Better Compliance and Is Non-inferior to a Twice-Daily Regimen Through 96 Weeks," *AIDS Research and Human Retroviruses* 23(12):1505-1514.
Sanne, I et al, "Two Randomized, Controlled, Equivalence Trials of Emtricitabine (FTC) to Lamivudine (3TC)," Poster 4432 presented at the XIV International AIDS Conference, Jul. 7-12, 2002, Barcelona, Spain.
Sanne, I, et al, "Genotypic Analysis of HIV-1 Infected ART-Naive Patients Receiving Emtricitabine (FTC) or Lamivudine (3TC) in a Double Blind Equivalence Trial," Pster No. 4433, resented at the XIV International AIDS Conference Jul. 7-12, 2002, Barcelona, Spain.
Schinazi, R. et al. (1992) "Selective Inhibition of Human Immunodeficiency Viruses by Racemates and Enantiomers of *cis*—5-Fluoro-1-[2-(Hydroxymethyl)-1,3-Oxathiolan-5-yl]Cytosine," *Antimircobial Agents and Chemotherapy* 36(11):2423-2431.
Schinazi, R. et al. (2003) Letter to the Editor "Assessment of the Relative Potency of Emtricitabine and Lamivudine," *JAIDS* 34(2)243-245.
Smith, K. et al. (2009) "Randomized, double-blind, placebo-matched, multicenter trial of abavacir/lamivudine or tenofovir/emtricitabine with lopinavir/ritonavir for -inital HIV treatment," *AIDS* 23:1547-1556.
Walmsley, S. et al. (2009) "Gemini: A Noninferiority Study of Saquinavir/Ritonavir Versus Lopinavir/Ritonavir as Initial HIV-1 Therapy in Adults," *J. Acquir Immune Defic Syndr* 50(4):367-374.
Wang (1998) "FTC: A Potent and Selective Anti-HIV and Anti-HBV Agent Demonstrating Desirable Pharmacokinetic (PK Characteristics," Abstracts of the IDSA, 36th Annual Meeting, Session 58, Poster 415, Hepatits A, B, and C in HIV-Infected Persons Friday, 4-6 pm.
Wang, L.H. et al. (2001) "Lack of Significant Pharmacokinetic Interactions between Emtricitabine and Other Nucleoside Antivirals in Healthy Volunteers," 41st ICAAC Abstracts, Chicago, IL, Sep. 22-25, 2001, Abstract A-505.
Wang, L. H. et al. (2002) "Pharmacokinetic and pharmacodynamic characteristics of emtrictabine support its once daily dosing," *Int.. Conf AIDS,* Jul. 7-12 14:abstract TUPeB4546.
"Atripla Fact Sheet", www.fda.gov, [Online], Jul. 12, 2006, pp. 1-2 retrieved from the internet www.fda.gov/cder/drug/inforpage/atripla/factsheet.htm [retrieved on Jan. 31, 2007.
Banker, Modern Pharmaceutics, Drugs and the Pharmaceutical Sciences, p. 340, Mercel Dekker, Inc. 1996.
European Medicines Agency: "Scientific discussion (Truvada)," EMEA, [on line], Feb. 2005, pp. 1-28, (retrieved from http://www.emea.eu.int/humandocs/PDFs/EPAR/truvada/2832505en6.pdf [retrieved on Jan. 31, 2007].
FDA: "Guidance for industry fixed dose combination and co-packaged drug products for treatment of HIV," www.fda.org. [on line], May 2004, pp. 1-17, (retrieved from http://www.fda.gov/oc/initiatives/hiv/hivguidance.html>[retrieved on Jan. 31, 2007].
Gilead: "Bristol-Myers Squibb and Gilead announce data supporting bioequivalence for single PIII fixed dose regimen of Sustiva®(efavirenz) and Truvada® (emtricitabine and tenofovir fumarate)" Gilead Press Release (online Jan. 9, 2006), pp. 1-5, retrieved from http://www.gilead.com/press.
Gilead: "Gilead provides update on development of fixed-dose regimen of Truvada (emtricitabine and tenofovir disoproxil fumarate) and Sustiva (efavirenz)," Gilead Press Release, [on line], Apr. 26, 2005, pp. 1-3, retrieved from http://www.gilead.com/press.
Gilead: "Gilead provides update on development of fixed-dose regimen of Truvada (emtricitabine and tenofovir disoproxil fumarate) and Sustiva (efavirenz)," Gilead Press Release, [on line], Aug. 9, 2005, pp. 1-3, retrieved from http://www.gilead.com/press.
"HIV Treatment Information," Project Inform, (on line), Jan. 2006, pp. 1-3, retrieved from http://www.projinf.org/bn/news_013006.html [retrieved on Jan. 31, 2007].
Ibbotson et al., Drugs 2003, 63(11), 1089-1096.
Kleinebudde et al. European journal of Pharmaceutics and Biopharmaceutics, 2004, 58, 317-326.
Lachman, et al. (1987) "The Theory and Practice of Industrial Pharmacy", Varghese Publishing House, Dadar Bombay, pp. 330-331.

(56) **References Cited**

OTHER PUBLICATIONS

Parikh, Handbook of Pharmaceutical Granulation Tech., NY, Marcel Dekker Inc., 1996.
Pharmaceutical Technology (2005), Big Pharma Companies Team Up to Develop Once-Daily Triple-Combination HIV Drug,vol. 29, No. 4.
Pujari et al., "Safety and long term effectiveness of generic fixed-dose formulations of nevirpine-based HAART amongst antiretroviral-naïve HIV-infected patients in India," World Health Organization, [on line], Dec. 16, 2003, pp. 99-116; (Retrieved from: http://libdoc.who.int/publications/2003/a86263.pdf.
Staszewski et al., NEJM, 1999, 341 5,1865-1873.
U.S. Department of Health and Human Services (2004) "Guidance for Industry Fixed Dose Combination and Co-Packaged Drug Products for Treatment of HIV—Draft Guidance" pp. 1-21.
Examiners First Report Patent Application No. 20026257795 issued by the Australian Patent Office (Sep. 29, 2009).
Official Action for Patent Application. No. 200800033/27 issued by the Eurasian Patent Office (2010) (translation).
Communication from the Examining Division of the EPO for Appln No. 06773195.3 (May 13, 2009).
Official Action for Patent Application No. 70010772008 issued by the Korean Intellectual Property Office (Sep. 13, 2010).
First Examination Report for Application No. 564102 Issued by the Intellectual Property Office of New Zealand (Oct. 6, 2009).
International Search Report for PCT/US2006/023223 (Feb. 23, 2007).
Written Opinion issued by the ISA for PCT/US2006/023223 (Feb. 23, 2007).
International Preliminary Report on Patentability for PCT/US2006/023223 (Oct. 8, 2007).
Ait-Khaled, et al., "Zidovudine appears to prevent selection of K65R and L74V mutations normally selected by abacavir mono- or combination therapies not containing zidovudine" *Antiviral Therapy,* 2002, 7:S107 (Abstract).
Borroto-Esoda, et al. "In Vitro evaluation of the anti-HIV activity and metabolic interactions of tenofovir and emtricitabine" *Antiviral therapy,* 2006, vol. 11, pp. 377-384.
Gallant, et al., "Early Non-Response to Tenofovir DF (TDF) + Abacavir (ABC) and Lamivudine (3TC) in a Randomized Trial Compared to Efavirenz (EFV) + ABC and 3TC: ESS30009 Unplanned Interim Analysis" *Abstr Intersci Conf Antimicrob Agents Chemother Intersci Conf Antimicrob Agents Chemother,* 2003, Abstract No. H-1722a.
Jamsek, et al. "Poor Virological Responses and Early Emergence of Resistance in Treatment Naïve, HIV-infected Patients Receiving a Once Daily Triple Nucleoside Regimen of Didanosine, Lamivudine, and Tenofovir DF" *11th Conf Retrovir Oppor Infect,* 2004, Abstract No. 51.
Lanier, et al. "Prediction of NRTI Optins by Linking Reverse Transcriptase Genotype to Phenotypic Breakpoints" *10th Conf Retrovir Oppor Infect,* 2003, Abstract No. 586.
Lu, et al., "Determination of Clinical Cut-Offs for Reduced Response to Tenofovir DF therapy in Antiretroviral-Experienced Patients" *Antiviral Therapy,* 2002, vol. 7(Suppl 1), 5104, Abstract No. 125.
Quail, et al., "Endogenous Reverse Transcriptase Assays Reveal Synergy between Combinations of the M184V and other Drug Resistance-conferring Mutations in Interactions with Nucleoside Analog Triphosphates" *J. Mol. Bio.,* 1998, vol. 227, pp. 237-247.
Fourth Amended Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. and Exhibits filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Oct. 3, 2011).
Forth Amended Answer filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Oct. 17, 2011).
Teva's Opening Claim Construction Brief filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Nov. 4, 2011).
Declaration of Michael J. Freno in Support of Teva's Opening Claim Construction Brief and Exhibits filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Nov. 4, 2011).
Plaintiffs' Opening Claim Construction Brief filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Nov. 4, 2011).
Declaration of Adam C. LaRock in Support of Plaintiffs' Claim Constructions and Exhibits filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Nov. 4, 2011).
Declaration of Paul A. Bartlett in Support of Plaintiffs' Claim Constructions and Exhibits filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Nov. 4, 2011).
Teva's Rebuttal Claim Construction Brief filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Dec. 16, 2011).
Declaration of Daniel P. Margolis in Support of Teva's Rebuttal Claim Construction Brief and Exhibits filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Dec. 16, 2011).
Plaintiffs' Rebuttal Claim Construction Brief filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Dec. 16, 2011).
Declaration of Adam C. LaRock in Support of Plaintiffs' Rebuttal Claim Constructions and Exhibits filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Dec. 16, 2011).
Order signed by Judge Richard J. Sullivan Case No. 08-CV-10838 (Dec. 19, 2011).
Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc. Case No. 09-CV-04463 (May 8, 2009).
Answer and Counterclaim filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 09-CV-04463 (Aug. 10, 2009).
Plaintiffs' Reply to Teva. USA's Counterclaim filed by Gilead Sciences, Inc., Emory University Case No. 09-CV-04463 (Aug. 31, 2009).
Order signed by Judge Richard J. Sullivan Case No. 08-CV-10838 (May 26, 2010).
First Amended Complaint for Patent Infringement filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Jun. 15, 2011).
Answer to Amended Complaint filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jun. 29, 2011).
Plaintiff's Opening Claim Construction Brief filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Dec. 2, 2011).
Teva's Opening Claim Construction Brief filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Dec. 2, 2011).
Declaration of Daniel P. Margolis in Support of Teva's Opening Claim Construction Brief and Exhibits filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Dec. 2, 2011).
Declaration of Natalie Lieber and Exhibits filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Dec. 2, 2011).
Declaration of Allan S. Myerson in Support of Plaintiffs' Claim Constructions and Exhibits filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Dec. 2, 2011).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01796 (Dec. 5, 2011).
Teva's Rebuttal Claim Construction Brief filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jan. 13, 2012).
Declaration of Daniel P. Margolis in Support of Teva's Rebuttal Claim Construction Brief and Exhibits filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jan. 13, 2012).
Plaintiffs' Rebuttal Claim Construction Brief filed by Gilead Sciences, Inc., Emory University Case No. 10-CV-01796 (Jan. 13, 2012).
Declaration of Natalie Lieber in Support of Plaintiffs' Rebuttal Claim Constructions and Exhibits filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Jan. 13, 2012).

(56) **References Cited**

OTHER PUBLICATIONS

Endorsed Letter to Judge Richard J. Sullivan Case No. 10-CV-01796 (Jan. 13, 2012).
Transcript of Proceedings held on Apr. 26, 2012 Case No. 10-CV-01796.
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01796 (Apr. 26, 2012).
Stipulation and Agreement Regarding U.S. Patent Nos. 5,922,965, 5,935,946, 5,977,089, and 6,043,230 Case No. 10-CV-01796 (Oct. 9, 2012).
Transcript of Proceedings held on Oct. 3, 3012, 2012 Case No. 10-CV-01796.
Plaintiff's Pretrial Memorandum filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Jan. 14, 2013).
Plaintiff's Proposed Findings of Fact and Conclusions of Law filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Jan. 14, 2013).
Defendants' Pretrial Memorandum filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jan. 14, 2013).
Defendants' Proposed Findings of Fact and Conclusions of Law filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jan. 14, 2013).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01796 (Jan. 18, 2013).
Defendants' Memorandum in Opposition to Plaintiffs Pretrial Memorandum filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jan. 28, 2013).
Plaintiff's Opposition to Defendants' Pretrial Memorandum by Gilead Sciences, Inc. Case No. 10-CV-01796 (Jan. 28, 2013).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01796 (Jan. 30, 2013).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01798 (May 26, 2010).
Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co's Opening Claim Construction Brief Case No. 10-CV-01851 (Dec. 2, 2011).
Affirmation of Andrew W. Williams in Support of Plaintiffs Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co's Opening Claim Construction Brief and Exhibits Case No. 10-CV-01851 (Dec. 2, 2011).
Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd.'s Opening Claim Construction Brief Case No. 10-CV-01851 (Dec. 2, 2011).
Declaration of Karen C. Shen in Support of Teva's Opening Claim Construction Brief and Exhibits Case No. 10-CV-01851 (Dec. 2, 2011).
Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd.'s Rebuttal Claim Construction Brief Case No. 10-CV-01851 (Jan. 13, 2012).
Declaration of Slaven Jesic in Support of Teva's Rebuttal Claim Construction Brief and Exhibits Case No. 10-CV-01851 (Jan. 13, 2012).
Declaration of Robin D. Rogers, Ph.D. In Support of Teva's Claim Constructions and Exhibits Case No. 10-CV-01851 (Jan. 13, 2012).
Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co's Rebuttal Claim Construction Brief Case No. 10-CV-01851 (Jan. 13, 2012).
Declaration of Andrew W. Williams in Support of Plaintiffs Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co's Rebuttal Claim Construction Brief and Exhibits Case No. 10-CV-01851 (Jan. 13, 2012).
"Annex I. Summary of Product Characteristics," for Epivir, 9 pages.
"Annex I. Summary of Product Characteristics," for Truvada film-coated tablets, 14 pages.
"graph," and "convolute of graphs,", cited as documents D54 and D55 in opposition proeedings of EP 04701819.7, Statement of Appeal Grounds filed Jun. 24, 2011.
Adis R&D Profile, "Emtricitabine/Tenofovir Disoproxil Fumarate," Drugs in R & D 5(3):160-161 (2004).
Analytical Profiles of Drug Substances, vol. 20, "Lactose Anhydrous," p. 373 Ed. Klaus Florey, Academic Press, Inc. (1990).
Ansel et al., "Pharmaceutical Dosage Forms and Rug Delivery Systems," 7th Edition, Lippincott Williams & Wilkins, pp. 209-213 (1999).
Arimilli et al., "Nucleotide Analogues," U.S. Appl. No. 60/022,708, 40 pages (filed Jul. 26, 1996).
Arzneiformenlehre. Ein Lehrbuch für Pharmazeuten, List et al., Eds., Wissenschaftliche Verlagsgesellschaft mbH, pp. 79 and 477 (1985).
BioWorld Today, "About BioWorld," 1 page, http://www.bioworld.com/servlet/com.accumedia.web.Dispatcher?next=aboutUs (2010).
Bristol Myers Squibb "Sustiva®," http://www.fda.gov/medwaTCH/SAFETY/2005/Sustiva_PI61005.pdf, pp. 3-40 (retrieved Jan. 31, 2007).
Brogan et al., "Cost-Effectiveness of Nucleoside Revers Transcriptase Inhibitor Pairs in Efavirenz-Based Regimens for Treatment-Naïve Adults with HIV Infection in the United States," Value in Health 14:657-664 (2011).
Castello and Mattocks, "Discoloration of Tablets Containing Amines and Lactose," J. Pharm. Sci. 106-108.
Communication concerning Correction of the EP Specification for Patent Publication EP 1583542 (Application No. 04701819.7) issued by the European Patent Office (Jul. 22, 2008).
Communication of Notice of Opposition of EP 1583542 B1 (Application No. 04701819.7)—first information to the patent proprietor (Mar. 26, 2009).
Communication of Notice of Opposition of EP 1583542 B1 (Application No. 04701819.7)—first information to the patent proprietor (Mar. 24, 2009).
Communication pursuant to Article 94(3) EPC for Patent Publication EP 1923063 (Application No. 08152527.1) issued by the European Patent Office (Mar. 26, 2012).
Correction of an Error in the Decision According to Rule 140 EPC for EP 1890681 B1 (Application No. 06773194.3) (Jul. 5, 2011).
Crowley, "Drug-Excipient Interactions," Pharm. Tech., 6 pages (2001).
Dahl et al., Amended Transmittal of U.S. Appl. No. 10/540,794, Compositions and methods for combination antiviral therapy, filed Mar. 20, 2006.
Decision to grant a European Patent for EP Appln No. 04701819.7 and Druckexemplar (May 23, 2008).
European Search Report, EP 2386294 (Application No. 11167101.2), 15 pages (Dec. 29, 2011).
Examiner's Remarks for Patent Application No. a 2008 00493 issued by the Ukranian Patent Office (2010).
Examiner's Second Report on Patent Application No. 2009200414 issued by the Australian Patent Office (Aug. 29, 2011).
Eyjolfsson, "Lisinopril-Lactose Incompatibility," Drug Devel. Indust. Pharm. 24(8):797-798 (1998).
First Examination Report for Patent Application No. 6665/DELNP/2008 issued by the Indian Patent Office (Jun. 30, 2011).
Gerhartz (editor) Ullmann's Encyclopedia of Industrial Chemistry vol. B2, Unit Operations I, 5th Edition, p. 3-7.
Gilead "Truvada®," http://www.fda.gov/medwatch/SAFETY/2005/Oct_PI/Truvada_PI.pdf, pp. 1-29 (retrieved Jan. 31, 2007).
Gilead Sciences Inc., Appeal Grounds against the Decision of the Opposition Division for EP 1890681 B1 (Application No. 06773194.3) (Oct. 17, 2011).
Gilead Sciences Inc., Notice of Appeal against the Decision of the Opposition Division for EP 1890681 B1 (Application No. 06773194.3) (Aug. 16, 2011).
Gilead Sciences Inc., Written Submission in preparation to/during Oral Proceedings for EP Patent EP 1583542 B1 (Application No. 04701819.7) (Sep. 17, 2010).
Gilead, Bristol-Myers Squibb "Atripla™," http://www.fda.gov/cder/foi/;abe;/2006/0219371b1.pdf, pp. 4-53 (retrieved Jan. 31, 2007).
Giron, "Applications of thermal analysis in the pharmaceutical industry," J. Pharm. Biomed. Anal. 4(6):755-770 (1986).
Glaxo Marketing Material, Epivir + Ziagen, 6 pages (2003).
Handbook of Pharmaceutical Excipients "Lactose,", 3rd ed. pp. 276-285 (2000).

(56) **References Cited**

OTHER PUBLICATIONS

Kusmierek et al., "Kinetics and Mechanisms of Hydrolytic Reactions of Methylated Cytidines under Acidic and Neutral Conditions," Acta Chem. Scand. 43:196-202 (1989).
Marcelin et al., "Resistance profiles of emtricitabine and lamivudine in tenofovir-containing regimens," J. Antimicrob. Chemother. 67:1475-1478 (2012).
Merck Index, 13th Edition, p. ONR-65 (2001).
Minutes of the Oral Proceedings before the Opposition Division for EP 1890681 B1 (Application No. 06773194.3) and Appendices (Apr. 5, 2011).
National Institutes of Health, "Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents," 9 pages (2011/2012).
Notice of Allegation and Detailed Statement in respect of Tenofovir Disoproxil Fumarate and Emtricitabine (Truvada®) and Canadian Patent Nos. 2,261,619 and 2,298,059, 56 pages (Nov. 22, 2011).
Notice of Allegation and Detailed Statement in respect of Truvada and Canadian Patent No. 2,512,475, 37 pages (Nov. 22, 2011).
Office Action for Korean Patent Application No. 7013069/2005 issued by the Korean Intellectual Property Office (Jan. 23, 2008).
Office Action, from U.S. Appl. No. 12/195,161 (mailed May 7, 2010).
Office Action, from U.S. Appl. No. 10/540,794 (mailed Sep. 21, 2006).
Office Action, from U.S. Appl. No. 10/540,794 (mailed Oct. 31, 2007).
Office Action, from U.S. Appl. No. 10/540,794 (mailed May 16, 2007).
Official Action and Preliminary Notice of Allowance from The Eurasian Patent Office for Application No. 200501134/28 (May 14, 2010).
Official Action for Application No. 095120445 issued by the Taiwanese Intellectual Property Office (Nov. 29, 2011) (translation).
Official Action for Application No. 2008-517083 issued by the Japanese Patent Office (Aug. 2, 2011) (translation).
Official Action for Application No. 2008-517083 issued by the Japanese Patent Office (Mar. 23, 2012) (translation).
Official Action for Patent Application No. 93100813 issued by the Taiwanese Intellectual Property Office (Mar. 16, 2011) (translation).
Official Action, from JP appl. No. 2010-175808 (mailed Nov. 6, 2012).
Official Communication for Patent Application No. 2100/DEL/2007 issued by the Indian Patent Office (Jan. 16, 2013).
Opposition filed in Indian Patent Appl. No. 9661/DELNP/2007 by Cipla Limited (Jun. 30, 2009).
Pharmaceutical Dosage Forms. Tablets. 2nd Ed., revised and expanded, Lieberman et al., eds., pp. 93 and 98 (1990).
Project Inform, "Perspective," pp. 1-28 (2003).
Reply to the statement of appeal grounds, EP 1583542 B1 (Application No. 04701819.7), 50 pages (Oct. 18, 2011).
Request for Correction of EP Appln No. 04701819.7 (Jul. 8, 2008).
Request for Correction of EP Appln No. 04701819.7 (Jun. 27, 2008).
Response to the Noting of Loss of Rights pursuant to Rule 112(1) EPC dated Sep. 3, 2012, Patent Publication EP 2386294 (Application No. 11167101.2) (Nov. 13, 2012).
Response to the noting of loss of rights pursuant to Rule 112(1)EPC dated Nov. 14, 2012, Patent Publication EP 1923063 (Application No. 08152527.1) (Jan. 24, 2013).
Response to the reply letter of Teva Pharmaceutical Industries Ltd. dated Oct. 18, 2011, EP 1583542 B1 (Application No. 04701819.7), 35 pages (Aug. 9, 2012).
Reversal of Rejection Decision for Application No. 200480002190.5 by the Patent Rexamination Board (Patent Office of the People's Republic of China) (Jun. 10, 2010).
Revocation of European Patent EP 1890681 B1 (Application No. 06773194.3) (Jun. 8, 2011).
Riaz and Ami, "Stability of Aminophylline," Pak. J. Pharm. Sci. 6(1):35-44 (1993).
Second Office Action for Application No. 200680026180.4 issued by the State Intellectual Property Office of the People's Republic of China (Oct. 20, 2011) (translation).
Second Office Action for Patent Application No. 200480002190.5 issued by the Patent Office of the People's Republic of China (May 11, 2011).
Smirnov et al., "A Comparative Study of the Kinetics of Cytarabine Hydrolytic Deamination in Aqueous Solutions," Pharm. Chem. J. 34(8):451-454 (2000).
Teva's Response to Patentee's Appeal, 5 pages, EP Pat. No. 1890681, EP Appl. No. 06773194.3 (mailed May 3, 2012).
Theory and Practice of Industrial Pharmacy, 3rd Edition, Lachman et al., Eds., Lea & Febiger, pp. 324-329 (1986).
Thiothi et al., "Investigation of the Kinetics of Degradation of Hexopyranosylated Cytosine Nucleosides Using Liquid Chromatography," Nucleosides, Nucleotides & Nucleic Acids 19(1&2):189-203 (2000).
USP 24 The United States Pharmacopeia (2000).
Wirth et al., "Maillard Reaction of Lactose and Fluoxetine Hydrochloride, a Secondary Amine," J. Pharm. Sci. 87(1):31-39 (1998).
Zalac et al., "Paracetamol-Propyphenazone Interaction and Formulation Difficulties Associated with Eutectic Formation in Combination Solid Dosage Forms," Chem. Pharm. Bull. 47(3):302-307 (1999).
Arribas et al. (2008) "Tenofovir Disoproxil Fumarate, Entricitabine and Efavirenz Compared with Zidovudine/Lamivudine and Efavirenz in Treatment -Naive Patients 144-Week Analysis," *J Acquire Immune Defic, Syndr.* 47(1).
Bartlett et al, "Overview of the Effectiveness of Triple Combination Therapy in Antiretroviral-Naive HIV-1 Infected Adults", 15:1369-1377, AIDS, 2001.
Benzaria et al, "Synthesis, in Vitro Antiviral Evaluation, and Stability Studies of Bis(S-acyl-2 thioethyl) Ester Derivatives of 9-[2-(Phosphonomethoxy)Ethyl]Adenine (PMEA) as Potential PMEA Prodrugs with Improved Oral Bioavailability", 39:4958-4965, J Med Chem, 1996.
Bioventure View, 17(25): pp. 6(3), Dec. 10, 2002.
Bioworld Today, 13(233), Dec. 5, 2002.
Bioworld Today, 13(239), Dec. 16, 2002.
Blackburn et al, "DNA and RNA Structure", pp. 15-81, Nucleic Acids in Chemistry and Biology, 1996.
Borroto-Esoda et al. (2006) "In Vitro evaluation of the anti-HIV activitiy and metaboloc interactions of tenofovir and emtricitabine," *Antiviral Therapy* 11:377-384.
BT Catalyst, 17(1): pp. 4(1), Jan. 1, 2003.
Bundgaard et al, "Design and Application of Prodrugs", pp. 113-191, Textbook of Drug Design and Development, 1991.
Chemical Market Reporter, 262(21): pp. 2(1), Dec. 9, 2002.
Compact Oxford English Dictionary, Extract on "Time-Release".
Conference Call Transcript—Gilead Sciences Conference call to Discuss Triangle Pharmaceuticals Acquistion. Event Date/Time Dec. 4.2002/ 9:00 AM ET (11 pages).
Dando et al. (2004) "Emtricitabine/Tenofovir Disoproxil Fumarite," *Drugs* 64(18):2075-2082.
De Lombaert, "N-Phosphonomethyl Dipeptides and their Phosphonate Prodrugs, a New Generation of Neutral Endopeptidase (NEP, EC 3.4.24.11) Inhibitors" 37:498-511, J Med Chem, 1994.
DeClercq, E. "New Development in Anti-HIV Chemotherapy", 8(13):1543-72, Current Medicinal Chemistry, 2001.
DeClercq, E. "New Developments in Anti-HIV Chemotherapy," Biochem Biophys Acta. 2002 18:1587(2-3):258-75.
DeClercq, E., "Highlights in the Development of New Antiviral Agents", 2(2): 163-175,Mini Rev Med Chem, 2002.
DeClercq, E., "New Developments in Anti-HIV Chemotherapy", 56(1-2): 3-12, Farmaco, 2001.
DeClercq, Erik "New-Anti-HIV Agents and Targets", 22(6):531-565, Medicinal Research Reviews, 2002.
DeClercq, Erik (2001) "Antiviral drugs: current state of the art," *Journal of Clinical Virology* 22:73-89.
Department of Health and Human Services, Nov. 3, 2008 "Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents" pp. 1, 33-49.
Encarta Dictionary, Extract on "Pill".

(56) **References Cited**

OTHER PUBLICATIONS

European Medicines Agency: Scientific Discussion (Truvada) EMEA Feb. 2005 (pp. 1-28).
Farquhar et al, "Biologically Reversible Phosphate-Protective Groups", 72:324-325, J Pharm Sci, 1983.
Fasman et al, pp, 385-394, Practical Handbook of Biochem and Molec Biol, 1989.
Fell et al, 20:657-659, J Pharm Pharmacol, 1968.
Feng et al. 2009 "The triple combination of tenofovir, emtricitabine and efavirenz show synergistic anti-HIV-1 activity in vitro: a mechanism of action study," *Retrovirology* 6:44, http://www.retrovirology.com/content/6/1/44.
Fiske et al. (1997) "Pharmcokinetics, safety and tolerability of single escalating doses of DMP 266, an HiIV non-nucleoside reverse transcriptase inhibitor, in healthy volunteers," *Pharmaceutical Research* (New York) 14(11) Suppl. S609 print.
Frampton et al. (2005) "Emtricitabine: A Review of Its Use in the Management of HIV Inspection," *Drugs* 65(10):1427-48.
Freeman et al, "3 Prodrug Design for Phosphate and Phosphonates", 34:112-147, Progress in Medicinal Chemistry, 1997.
Fridland, Arnold, "Tenofovir", 2(3):295-301, Current Opinion in Anti-Infective Investigational Drugs, 2000.
FTC 101 Virology analysis, TPI Document No. 14022.
Fung, Horatio B., "Tenofovir Disoproxil Fumarate: A Nucleotide Reverse Transcriptase Inhibitor for the Treatment of HIV Infection", 24(10):1515-1548, Clinical Therapeutics, 2002.
Gilead Sciences, Inc., Data Comparing Viread (R) and Emtriva (R) to Combivir (R) as Part of Combination HIV Therapy Published in New England Journal of Medicine, pp. 1-5, Press Release, 2006.
Gilead Sciences, Inc., "Gilead Sciences to Acquire Triangle Pharmaceuticals for $464 Million Gilead to Launch Coviracil in 2003 Will Develop Co-Formulation of Viread and Coviracil", Press Release 2002.
Gilead Sciences, Inc., "U.S. FDA Approves Gilead Sciences' Emtriva a One-Capsule, Once-Daily Medication for the Treatment of HIV", pp. 3-7, Press Release 2003.
Glaxo Marketing Material 2003, Epivir + Ziagen (6 pages).
Hostetler et al, "Greatly Enhanced Inhibition of Human Immunodeficiency Virus Type 1 Replication in CEM and HT4-6C Cells by 3'-Deoxythymidine Diphosphate Dimyristoylglycerol, a Lipid Prodrug of 3'-Deoxythymidine", 36(9):2025-2029, Antimicro AG & Chemo, 1992.
Hostetler et al, "Synthesis and Antiretroviral Activity of Phospholipid Analogs of Azidothymidine and Other Antiviral Nucleosides", 265(11):6112-6117, J Biol Chem, 1990.
International Preliminary Report on Patentability for PCT/US2004/000832, international filing date Jan. 13, 2004.
International Search Report and Written Opinion mailed Jul. 12, 2004 for PCT/US2004/000832, Intl Filing Date Jan. 13, 2004.
Ishida K., "Self-Association and Unique DNA Binding Properties of the Anti-Cancer Agent TAS-103, a Dual Inhibitor of Topoisomerases I and II", 1587(2-3):155-163, Biochem Biophys Acta, 2002.
Jones et al, "Minireview: Nucleotide Prodru.s", 27:1-17, Antiviral Res, 1995.
Kearney et al, Abstracts of the Interscience Conference on Antimicrobial Agents and Chemotherapy, 41(81): Abstract A-505, 2001.
Khamnei et al, "Neighboring Group Catalysis in the Design of Nucleotide Prodrugs", 39:4109-4115, J Med Chem, 1996.
King et al. (2002) "Potency of Nonnucleoside Reverse Transcriptase Inhibitors (NNRTIs) Used in Combination with Other Human Immunodeficiency Virus NNRTIs, NRTIs, or Protease Inhibitors," *Antimicrobial Agents and Chemotherapy* 46(6):1640-1646.
Kucera et al, "Novel Membrane-Interactive Ether Lipid Analogs That Inhibit Infectious HIV-1 Production and Induce Defective Virus Formation", 6:491-501, AIDS Res & Hum Retro, 1990.
Lieberman et al, 1:177-178, Pharmaceutical Dosage Forms, 1989.
Lindahl, Tomas, "Instability and Decay of the Primary Structure of DNA", 362:709-715, Nature, 1993.
Liu, "Thymidylate Synthase as a Translational Regulator of Cellular Gene Expression", 1587(2-3):174-182, Biochem Biophys Acta, 2002.
Loveday, C., "Nucleoside Reverse Transcriptase Inhibitor Resistance", 26:510-S24, J AIDS, 2001.
Margot et al. (2002) "Genotypic and phenotypic analyses of HIV-1 in antiretroviral-experienced patients treated with tenofovir DF," *AIDS* 16:1227-1235.
Margot et al. (2006) "In Vitro Human Immnuodeficiency Virus Type 1 Resistance Selections with Combinations of Tenofovir and Emtricitabine or Abacavir and Lamivudine," *Antimicrobial Agents and Chemotherapy* 50(12):4087-4095.
Margot et al. (2006) "Resistance development over 144 weeks in treatment -naive patients receiving tenofovir disoproxil fumarate or stavudine with lamivudine and efavirenz in Study 903," *HIV Medicine* 7:442-450.
Masho et al. (2007) "Review of Tenofovir -Emtricitabine," *Ther. Clin. Risk Manag.* 3(6):1097-1104.
Miller et al. Sixth International Congress on Drug Therapy in HIV Infection, Nov. 17-21, 2002 (1 page).
Molina et al. (2000) "Once-Daily Combination Therapy with Emtricitabine, Didanosine, and Efavirenz in Human Immunodeficiency Virus-Infected Patients," *The Journal of Infectious Diseases* 182:599-602.
Monthly Index of Medical Specialities, pp. 194-198, 2002.
Mulato et al, "Anti-HIV Activity of Adefovir (PMEA) and PMPA in Combination With Antiretroviral Compounds: In vitro Analyses", 36(2):91-97 Antiviral Res, 1997.
Murry, et al, Reversion of the M184V Mutation in Simian Immunodeficiency Virus Reverse Transcriptase is Selected by Tenofovir, Even in the Presence of Lamivudine, 77(2):1120-1130, J Virol, 2003.
Pallella et al, 338:853-860, J Med Chem, 1998.
Pharma Marketletter, "Gilead Buys Triangle in $464M Deal", Dec. 9, 2002.
Pharmaceutical Dosage Forms and Drug Delivery Systems, 6th Ed. (1995) pp. 171-174.
Physician Insert for Truvada 2007 Gilead Sciences, Inc, pp. 1-30.
PI Perspective, "New Uses for Tenofovir; More Questions about d4T", 35:15-16, Jan. 2003.
Piantadosi et al, "Synthesis and Evaluation of Novel Ether Lipid Nucleoside Conjugates for Anti-HIV-1 Activity", 34:1408-1414, J Med Chem, 1991.
Pozniak et all (2006) "Tenofovir Disoproxil Fumarate, Emtricitabine, and Efavirenz Versus Fixed-Dose Zidovudine/Lamivudine and Efavirenz in Antiretroviral -Naive Patients," *J Acquir Immun Def Syndr.* 43(5):535-540.
Puech et al, "Intracellular Delivery of Nucleoside Monophosphates Through a Reductase-Mediated Activation Process", 22:155-174, Antiviral Res, 1993.
Rescriptor Patient Information Leaflet, 2001.
Richman, Douglas D., "Antiretroviral Activity of Emtricitabine, a Potent Nucleoside Reverse Transcriptase Inhibitor", 6:83-88, Antiviral Therapy, 2001.
Richman, Douglas, "HIV Chemotherapy", 410:995-1001, Nature, 2001.
Ristig, Maria B., "Tenofovir Disoproxil Fumarate Therapy for Chronic Hepatitis B in Human Immunodeficiency Virus/Hepatitis B Virus—Coinfected Individuals for Whom Interferon-α and Lamivudine Therapy Have Failed", 186:1844-1847, Journ of Infect Disease, 2002.
Rousseau et al. (2001) "Prototype trial design for rapid dose selection of antireftroviral drugs: an example using emtricitabine (Coviracil)," *Journal of Antimicrobial Chemotherapy* 48:507-513.
Schinazi et al. (1993) "Characterization of Human Immunodeficiency Viruses Resistant to Oxathiolane-Cytosine Nucleosides," *Antimicrobial Agents and Chemotherapy* 37(4):875-881.
Scrip, "Gilead Acquires Triangle for $464 Million", Dec. 6, 2002.
Siddiqui et al, "Design and Synthesis of Lipophilic Phosphoramidate D4T-MP Prodrugs Expressing High Potency Against HIV in Cell Culture: Structural Determinants for in Vitro Activity and QSAR", 42(20):4122-4128, J Med Chem, 1999.

(56) **References Cited**

OTHER PUBLICATIONS

Tamari, Marisa, "A Decade in HIV Treatment: What is the State of the Art and How Did We Arrive", 5(1):4-12, Clinical Excellence for Nurse Practitioners, 2001.

The Body, 2002 "Five New Drugs Enter the Homestretch", Dec. 2002.

Tisdale et al. (1993) "Rapid in vitro selection of human immunodeficiency virus type 1 resistant to 3'-thiacytidine inhibitors due to a mutation in the YMDD region of reverse transcriptase," Proc. Natl. Acad. Sci. USA 90:5653-5656.

Truvada Patient Information Leaflet, 2008.

Viread (Tenofovir Disoproxil Fumarate Tablets) Summary of Product Characteristics, EMEA, SmPC, Feb. 5, 2002.

Viread 2001 Label pp. 1-44.

Viread Patient Information Leaflet, 2002.

Wainberg et al. (1999) "In vitro selection and characterization of HIV-1 with reduced susceptability to PMPA," *Antiviral Therapy* 4:87-94.

WWW.PROJECTINFORM.ORG, "Anti-HIV Drug Updates-Three Drugs on the Near Horizon", 35:4-7, PI Perspective, 2003.

Yeni et al, "Antiretroviral Treatment for Adult HIV Infection in 2002", 288(2):222-235, JAMA, 2002.

Yuan et al, "Degradation Kinetics of Oxycarbonyloxyrnethyl Prodrugs of Phosphonates in Solution", 18(2):234-237, Pharm Res, 2001.

Teva Pharmaceutical Industries, Ltd.(Opponent), Opposition Brief against Israel Patent No. 169243, dated Jul. 26, 2009.

Generics (UK) Limited (Opponent), Oppositiion Brief against European Patent No. EP 1 583 542, dated Mar. 18, 2009.

Teva Pharamaceuticals Industries, Inc.(Opponent), Opposition Brief against European Patent No. 1 583 542 dated Mar. 13, 2009.

Cipla Ltd (Opponent), Pre-Grant Opposition against Indian patent application 3383/DELNP/2005A, dated Jul. 29, 2005.

Decision of Controller in Indian Patent Application No. 3383/DELNP/2005; CIPLA Opposition dated Mar. 25, 2009.

* cited by examiner

COMPOSITIONS AND METHODS FOR COMBINATION ANTIVIRAL THERAPY

This non-provisional application is a continuation of U.S. patent application Ser. No. 10/540,794, filed Mar. 20, 2006, now abandoned which is a national stage entry of PCT/US04/00832, filed Jan. 13, 2004 which claims the benefit of Provisional Application Nos. 60/440,246 and 60/440,308, both filed Jan. 14, 2003, which is incorporated herein by reference.

FIELD OF THE INVENTION

The invention relates generally to combinations of compounds with antiviral activity and more specifically with anti-HIV properties. In particular, it relates to chemically stable combinations of structurally diverse anti-viral agents.

BACKGROUND OF THE INVENTION

Human immunodeficiency virus (HIV) infection and related diseases are a major public health problem worldwide. Human immunodeficiency virus type 1 (HIV-1) encodes at least three enzymes which are required for viral replication: reverse transcriptase (RT), protease (Prt), and integrase (Int). Although drugs targeting reverse transcriptase and protease are in wide use and have shown effectiveness, particularly when employed in combination, toxicity and development of resistant strains have limited their usefulness (Palella, et al N. *Engl. J. Med.* (1998) 338:853-860; Richman, D. D. *Nature* (2001) 410:995-1001). Human immunodeficiency virus type 1 (HIV-1) protease (Prt) is essential for viral replication and is an effective target for approved antiviral drugs. The HIV Pit cleaves the viral Gag and Gag-Pol polyproteins to produce viral structural proteins (p17, p24, p7 and p6) and the three viral enzymes. Combination therapy with RT inhibitors has proven to be highly effective in suppressing viral replication to unquantifiable levels for a sustained period of time. Also, combination therapy with RT and Prt inhibitors (PI) have shown synergistic effects in suppressing HIV replication. Unfortunately, a high percentage, typically 30 to 50% of patients currently fail combination therapy due to the development of drug resistance, non-compliance with complicated dosing regimens, pharmacokinetic interactions, toxicity, and lack of potency. Therefore, there is a need for new HIV-1 inhibitors that are active against mutant HIV strains, have distinct resistance profiles, fewer side effects, less complicated dosing schedules, and are orally active. In particular, there is a need for a less onerous dosage regimen, such as once per day oral dosing, optimally with as few pills as possible.

The use of combinations of compounds can yield an equivalent antiviral effect with reduced toxicity, or an increase in drug efficacy. Lower overall drug doses can reduce the frequency of occurrence of drug-resistant variants of HIV. Many different methods have been used to examine the effects of combinations of compounds acting together in different assay systems (Furman WO 02/068058). Lower doses predict better patient compliance when pill burden decreases, dosing schedules are simplified and, optionally, if synergy between compounds occurs (Loveday, C. "Nucleoside reverse transcriptase inhibitor resistance" (2001) *JAIDS Journal of Acquired Immune Deficiency Syndromes* 26:S10-S24). AZT (Zidovudine™, 3',-azido, 3'-deoxythymidine) demonstrates synergistic antiviral activity in vitro in combination with agents that act at HIV-1 replicative steps other than reverse transcription, including recombinant soluble CD4 castanospermine and recombinant interferon-α. However, it must be noted that combinations of compounds can give rise to increased cytotoxicity. For example, AZT and recombinant interferon-α have an increased cytotoxic effect on normal human bone marrow progenitor cells.

Chemical stability of combinations of antiviral agents is an important aspect of co-formulation success and the present invention provides examples of such combinations.

There is a need for new combinations of orally-active drugs for the treatment of patients infected with certain viruses, e.g. HIV, that provide enhanced therapeutic safety and efficacy, impart lower resistance, and predict higher patient compliance.

SUMMARY OF THE INVENTION

The present invention provides combinations of antiviral compounds, in particular compositions and methods for inhibition of HIV. In an exemplary aspect, the invention includes a composition including tenofovir disoproxil fumarate and emtricitabine which has anti-HIV activity. The composition of tenofovir DF and emtricitabine is both chemically stable and either synergistic and/or reduces the side effects of one or both of tenofovir DF and emtricitabine. Increased patient compliance is likely in view of the lower pill burden and simplified dosing schedule.

The present invention relates to therapeutic combinations of [2-(6-amino-purin-9-yl)-1-methyl-ethoxymethyl]-phosphonic acid diisopropoxycarbonyloxymethyl ester fumarate (tenofovir disoproxil fumarate, tenofovir DF, TDF, Viread®) and (2R,5S,cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine, Emtriva™, (−)-cis FTC) and their use in the treatment of HIV infections including infections with HIV mutants bearing resistance to nucleoside and/or non-nucleoside inhibitors. The present invention is also concerned with pharmaceutical compositions and formulations of said combinations of tenofovir disoproxil fumarate and emtricitabine. Another aspect of the invention is a pharmaceutical formulation comprising a physiologically functional derivative of tenofovir disoproxil fumarate or a physiologically functional derivative of emtricitabine.

Therapeutic combinations and pharmaceutical compositions and formulations of the invention include the combination of PMEA or PMPA (tenofovir) compounds with emtricitabine or (2R,5S,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (3TC, lamivudine, Epivir™), and their use in the treatment of HIV infections.

One aspect of the invention is a method for the treatment or prevention of the symptoms or effects of an HIV infection in an infected animal which comprises administering to, i.e. treating, said animal with a therapeutically effective amount of a combination comprising [2-(6-amino-purin-9-yl)-1-methyl-ethoxymethyl]-phosphonic acid diisopropoxycarbonyloxymethyl ester fumarate (tenofovir DF, TDF) or a physiologically functional derivative thereof and (2R,5S,cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine) or a physiologically functional derivative thereof.

Another aspect of the invention is a unit dosage form of a therapeutic combination comprising tenofovir disoproxil fumarate and emtricitabine, or physiological functional derivatives thereof. The unit dosage form may be formulated for administration by oral or other routes and is unexpectedly chemically stable in view of the properties of the structurally diverse components.

Another aspect of the invention is directed to chemically stable combination antiviral compositions comprising tenofovir disoproxil fumarate and emtricitabine. In a further

aspect of the invention, the chemically stable combinations of tenofovir disoproxil fumarate and emtricitabine further comprise a third antiviral agent. In this three-component mixture, the unique chemical stability of tenofovir disoproxil fumarate and emtricitabine is taken advantage of in order to enable the combination with the third antiviral agent. Particularly useful third agents include, by way of example and not limitation, those of Table A. Preferably, the third component is an agent approved for antiviral use in humans, more preferably, it is an NNRTI or a protease inhibitor (PI), more preferably yet, it is an NNRTI. In a particularly preferred embodiment, the invention is directed to a combination of the chemically stable mixture of tenofovir disoproxil fumarate and emtricitabine together with efavirenz.

Another aspect of the invention is a patient pack comprising at least one, typically two, and optionally, three active ingredients and other antiviral agents selected from tenofovir disoproxil fumarate and emtricitabine, and an information insert containing directions on the use of tenofovir disoproxil fumarate and emtricitabine together in combination.

Another aspect of the invention is a process for preparing the combinations hereinbefore described, which comprises bringing into association tenofovir DF and emtricitabine of the combination in a medicament to provide an antiviral effect. In a further aspect of the present invention, there is provided the use of a combination of the present invention in the manufacture of a medicament for the treatment of any of the aforementioned viral infections or conditions.

DETAILED DESCRIPTION OF THE INVENTION

While the invention will be described in conjunction with the enumerated claims, it will be understood that they are not intended to limit the invention to those claims. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the scope of the present invention as defined by the claims.

DEFINITIONS

Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings:

When tradenames are used herein, applicants intend to independently include the tradename product and the active pharmaceutical ingredient(s) of the tradename product.

The term "chemical stability" means that the two primary antiviral agents in combination are substantially stable to chemical degradation. Preferably, they are sufficiently stable in physical combination to permit commercially useful shelf life of the combination product. Typically, "chemically stable" means that a first component of the mixture does not act to degrade a second component when the two are brought into physical combination to form a pharmaceutical dosage form. More typically, "chemically stable" means that the acidity of a first component does not catalyzes or otherwise accelerate the acid decomposition of a second component. By way of example and not limitation, in one aspect of the invention, "chemically stable" means that tenofovir disoproxil fumarate is not substantially degraded by the acidity of emtricitabine. "Substantially" in this context means at least about less than 10%, preferably less than 1%, more preferably less than 0.1%, more preferably yet, less than 0.01% acid degradation of tenofovir disoproxil fumarate over a 24-hour period when the products are in a pharmaceutical dosage form.

The terms "synergy" and "synergistic" mean that the effect achieved with the compounds used together is greater than the sum of the effects that results from using the compounds separately, i.e. greater than what would be predicted based on the two active ingredients administered separately. A synergistic effect may be attained when the compounds are: (1) co-formulated and administered or delivered simultaneously in a combined formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation therapy, a synergistic effect may be attained when the compounds are administered or delivered sequentially, e.g. in separate tablets, pills or capsules, or by different injections in separate syringes. In general, during alternation therapy, an effective dosage of each active ingredient is administered sequentially, i.e. serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together. A synergistic antiviral effect denotes an antiviral effect which is greater than the predicted purely additive effects of the individual compounds of the combination.

The term "physiologically functional derivative" means a pharmaceutically active compound with equivalent or near equivalent physiological functionality to tenofovir DF or emtricitabine when administered in combination with another pharmaceutically active compound in a combination of the invention. As used herein, the term "physiologically functional derivative" includes any: physiologically acceptable salt, ether, ester, prodrug, solvate, stereoisomer including enantiomer, diastereomer or stereoisomerically enriched or racemic mixture, and any other compound which upon administration to the recipient is capable of providing (directly or indirectly) such a compound or an antivirally active metabolite or residue thereof.

"Bioavailability" is the degree to which the pharmaceutically active agent becomes available to the target tissue after the agent's introduction into the body. Enhancement of the bioavailability of a pharmaceutically active agent can provide a more efficient and effective treatment for patients because, for a given dose, more of the pharmaceutically active agent will be available at the targeted tissue sites.

The compounds of the combinations of the invention may be referred to as "active ingredients" or "pharmaceutically active agents."

The term "prodrug" as used herein refers to any compound that when administered to a biological system generates the drug substance, i.e. active ingredient, as a result of spontaneous chemical reaction(s), enzyme catalyzed chemical reaction(s), and/or metabolic chemical reaction(s).

"Prodrug moiety" means a labile functional group which separates from the active inhibitory compound during metabolism, systemically, inside a cell, by hydrolysis, enzymatic cleavage, or by some other process (Bundgaard, Hans, "Design and Application of Prodrugs" in *Textbook of Drug Design and Development* (1991), P. Krogsgaard-Larsen and H. Bundgaard, Eds. Harwood Academic Publishers, pp. 113-191). Prodrug moieties can serve to enhance solubility, absorption and lipophilicity to optimize drug delivery, bioavailability and efficacy. A "prodrug" is thus a covalently modified analog of a therapeutically-active compound.

"Alkyl" means a saturated or unsaturated, branched, straight-chain, branched, or cyclic hydrocarbon radical derived by the removal of one hydrogen atom from a single carbon atom of a parent alkane, alkene, or alkyne. Typical alkyl groups consist of 1-18 saturated and/or unsaturated carbons, such as normal, secondary, tertiary or cyclic carbon atoms. Examples include, but are not limited to: methyl, Me (—CH_3), ethyl, Et (—CH_2CH_3), acetylenic (—C≡CH), ethylene, vinyl (—CH=CH_2), 1-propyl, n-Pr, n-propyl (—$CH_2CH_2CH_3$), 2-propyl, i-Pr, i-propyl (—$CH(CH_3)_2$),

allyl (—$CR_2CH{=}CH_2$), propargyl (—$CH_2C{\equiv}CH$), cyclopropyl (—C_3H_5), 1-butyl, n-Bu, n-butyl (—$CH_2CH_2CH_2CH_3$), 2-methyl-1-propyl, i-Bu, 1-butyl (—$CH_2CH(CH_3)_2$), 2-butyl, s-Bu, s-butyl (—$CH(CH_3)CH^2CH_3$), 2-methyl-2-propyl, t-Bu, t-butyl (—$C(CH_3)_3$), 1-pentyl, n-pentyl, (—$CH_2CH_2CH_2CH_2CH_3$), 2-pentyl (—$CH(CH_3)CH_2CH_2CH_3$), 3-pentyl (—$CH(CH_2CH_3)_2$), 2-methyl-2-butyl (—$C(CH_3)_2CH_2CH_3$), cyclopentyl (—C_5H_9), 3-methyl-2-butyl (—$CH(CH_3)CH(CH_3)_2$), 3-methyl-1-butyl (—$CH_2CH_2CH(CH_3)_2$), 2-methyl-1-butyl (—$CH_2CH(CH_3)CH_2CH_3$), 1-hexyl (—$CH_2CH_2CH_2CH_2CH_2CH_3$), 5-hexenyl (—$CH_2CH_2CH_2CH_2CH{=}CH_2$) 1-hexyl (—$CH(CH_3)CH_2CH_2CH_2CH_3$), 3-hexyl (—$CH(CH_2CH_3)(CH_2CH_2CH_3)$), cyclohexyl (—$C_6H_{11}$), 2-methyl-2-pentyl (—$C(CH_3)_2CH_2CH_2CH_3$), 3-methyl-2-pentyl (—$CH(CH_3)CH(CH_3)CH_2CH_3$), 4-methyl-2-pentyl (—$CH(CH_3)CH_2CH(CH_3)_2$), 3-methyl-3-pentyl (—$C(CH_3)(CH_2CH_3)_2$), 2-methyl-3-pentyl (—$CH(CH_2CH_3)CH(CH_3)_2$), 2,3-dimethyl-2-butyl (—$C(CH_3)_2CH(CH_3)_2$), and 3,3-dimethyl-2-butyl (—$CH(CH_3)C(CH_3)_3$.

"Aryl" means a monovalent aromatic hydrocarbon radical of 6-20 carbon atoms derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system. Typical aryl groups include, but are not limited to, radicals derived from benzene, substituted benzene, naphthalene, anthracene, biphenyl, and the like.

"Arylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or Sp^3 carbon atom, is replaced with an aryl radical. Typical arylalkyl groups include, but are not limited to, benzyl, 2-phenylethan-1-yl, 2-phenylethen-1-yl, naphthylmethyl, 2-naphthylethan-1-yl, 2-naphthylethen-1-yl, naphthobenzyl, 2-naphthophenylethan-1-yl and the like. The arylalkyl group 6 to 20 carbon atoms e.g., the alkyl moiety, including alkanyl, alkenyl or alkynyl groups, of the arylalkyl group is 1 to 6 carbon atoms and the aryl moiety is 5 to 14 carbon atoms.

"Substituted alkyl", "substituted aryl", and "substituted arylalkyl" mean alkyl, aryl, and arylalkyl respectively, in which one or more hydrogen atoms are each independently replaced with a substituent. Typical substituents include, but are not limited to, —X, —R, —O^-, —OR, —SR, —S^-, —NR_2, —NR_3, ═NR, —CX_3, —CN, —OCN, —SCN, —N═C═O, —NCS, —NO, —NO_2, ═N_2, —N_3, NC(═O)R, —C(═O)R, —C(═O)NRR—$S(═O)_2O^-$, —$S(═O)_2$OH, —$S(═O)_2$R, —OS(═O)OR, —$S(═O)_2$NR, —S(═O)R, —$OP(═O)O_2RR$ —$P(═O)O_2RR$ —$P(═O)(O^-)_2$, —$P(═O)(OH)_2$, —C(═O)R, —C(═O)X, —C(S)R, —C(O)OR, —$C(O)O^-$, —C(S)OR, —C(O)SR, —C(S)SR, —C(O)NRR, —C(S)NRR, —C(NR)NRR, where each X is independently a halogen: F, Cl, Br, or I; and each R is independently —H, alkyl, aryl, heterocycle, or prodrug moiety.

"Heteroaryl" and "Heterocycle" refer to a ring system in which one or more ring atoms is a heteroatom, e.g. nitrogen, oxygen, and sulfur. Heterocycles are described in: Katritzky, Alan R., Rees, C. W., and Scriven, E. *Comprehensive Heterocyclic Chemistry* (1996) Pergamon Press; Paquette, Leo A.; *Principles of Modern Heterocyclic Chemistry* W. A. Benjamin, New York, (1968), particularly Chapters 1, 3, 4, 6, 7, and 9; "The Chemistry of Heterocyclic Compounds, A series of Monographs" (John Wiley & Sons, New York, 1950 to present), in particular Volumes 13, 14, 16, 19, and 28. Exemplary heterocycles include but are not limited to substituents, i.e. radicals, derived from pyrrole, indole, furan, benzofuran, thiophene, benzothiophene, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-quinolyl, 3-quinolyl, 4-quinolyl, 2-imidazole, 4-imidazole, 3-pyrazole, 4-pyrazole, pyridazine, pyrimidine, pyrazine, purine, cinnoline, pthalazine, quinazoline, quinoxaline, 3-(1,2,4-N)-triazolyl, 5-(1,2,4-N)-triazolyl, 5-tetrazolyl, 4-(1-O,3-N)-oxazole, 5-(1-O,3-N)-oxazole, 4-(1-S,3-N)-thiazole, 5-(1-S,3-N)-thiazole, 2-benzoxazole, 2-benzothiazole, 4-(1,2,3-N)-benzotriazole, and benzimidazole.

Stereochemical definitions and conventions used herein generally follow S. P. Parker, Ed., *McGraw-Hill Dictionary of Chemical Terms* (1984) McGraw-Hill Book Company, New York; and Eliel, E. and Wilen, S., *Stereochemistry of Organic Compounds* (1994) John Wiley & Sons, Inc., New York. Many organic compounds exist in optically active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L or R and S are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (−) are employed to designate the sign of rotation of plane-polarized light by the compound, with (−) or l meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, these compounds, called stereoisomers, are identical except that they are mirror images of one another. A specific stereoisomer is also referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture or a racemate. The terms "racemic mixture" and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.

The term "chiral" refers to molecules which have the property of non-superimposability of the mirror image partner, while the term "achiral" refers to molecules which are superimposable on their mirror image partner.

The term "stereoisomers" refers to compounds which have identical chemical constitution, but differ with regard to the arrangement of the atoms or groups in space.

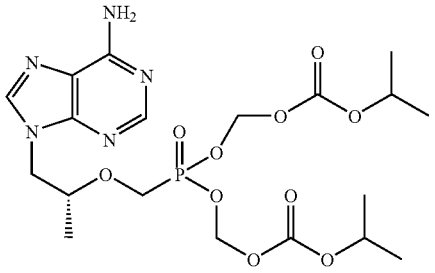
"Diastereomer" refers to a stereoisomer with two or more centers of chirality and whose molecules are not mirror images of one another. Diastereomers have different physical properties, e.g. melting points, boiling points, spectral properties, and reactivities. Mixtures of diastereomers may separate under high resolution analytical procedures such as electrophoresis and chromatography.

"Enantiomers" refer to two stereoisomers of a compound which are non-superimposable mirror images of one another.

Active Ingredients of the Combinations

The present invention provides novel combinations of two or more active ingredients being employed together. In some embodiments, a synergistic antiviral effect is achieved. In other embodiments, a chemically stable combination is obtained. The combinations include at least one active ingredient selected from (1) tenofovir disoproxil fumarate and physiologically functional derivatives, and at least one active ingredient selected from (2) emtricitabine and physiologically functional derivatives. The term "synergistic antiviral effect" is used herein to denote an antiviral effect which is greater than the predicted purely additive effects of the individual components (a) and (b) of the combination.

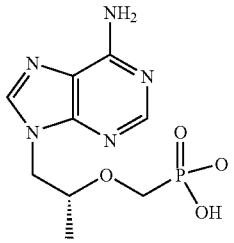
Tenofovir disoproxil fumarate (also known as Viread®, Tenofovir DF, Tenofovir disoproxil, TDF, Bis-POC-PMPA (U.S. Pat. Nos. 5,935,946, 5,922,695, 5,977,089, 6,043,230, 6,069,249) is a prodrug of tenofovir, and has the structure:



and including fumarate salt ($HO_2CCH_2CH_2CO_2^-$).

The chemical names for Tenofovir disoproxil include: [2-(6-amino-purin-9-yl)-1-methyl-ethoxymethyl]-phosphonic acid diisopropoxycarbonyloxymethyl ester; 9-[(R)-2-[[bis [[(isopropoxycarbonyl)oxy]methoxy]phosphinyl]methoxy] propyl]adenine; and 2,4,6,8-tetraoxa-5-phosphanonanedioic acid, 5-[[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy] methyl]-, bis(1-methylethyl)ester, 5-oxide. The CAS Registry numbers include: 201341-05-1; 202138-50-9; 206184-49-8. It should be noted that the ethoxymethyl unit of tenofovir has a chiral center. The R (rectus, right handed configuration) enantiomer is shown. However, the invention also includes the S isomer. The invention includes all enantiomers, diastereomers, racemates, and enriched stereoisomer mixtures of tenofovir (PMPA) and physiologically functional derivatives thereof.

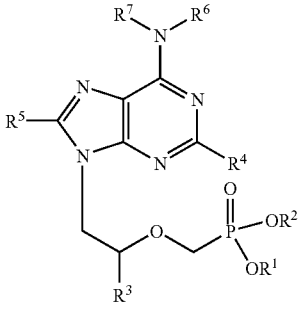
PMPA or tenofovir (U.S. Pat. Nos. 4,808,716, 5,733,788, 6,057,305) has the structure:



The chemical names of PMPA, tenofovir include: (R)-9-(2-phosphonylmethoxypropyl)adenine; and phosphonic acid, [[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy] methyl]. The CAS Registry number is 147127-20-6.

Tenofovir disoproxil fumarate (DF) is a nucleotide reverse transcriptase inhibitor approved in the United States in 2001 for the treatment of HIV-1 infection in combination with other antiretroviral agents. Tenofovir disoproxil fumarate or Viread® (Gilead Science, Inc.) is the fumarate salt of tenofovir disoproxil. Viread® may be named as: 9-[; R)-2-[[bis [[(isopropoxycarbonyl)oxy]methoxy]phosphinyl]methoxy] propyl]adenine fumarate (1:1); or 2,4,6,8-tetraoxa-5-phosphanonanedioic acid, 5-[[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy]methyl]-, bis(1-methylethyl) ester, 5-oxide, (2E)-2-butenedioate (1:1). The CAS Registry number is 202138-50-9.

Physiologically functional derivatives of tenofovir disoproxil fumarate include PMEA (adefovir, 9-((R)-2-(phosphonomethoxy)ethyl)adenine) and PMPA compounds. Exemplary combinations include a PMEA or PMPA compound in combination with emtricitabine or 3TC. PMEA and PMPA compounds have the structures:



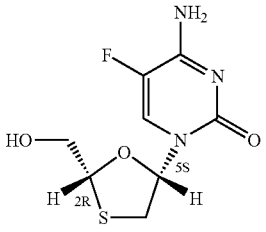
where PMEA (R^3 is H) and PMPA (R^3 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, or CH_2OR^8 where R^8 is C_1-C_6 alkyl, C_1-C_6 hydroxyalkyl or C_1-C_6 haloalkyl. R^6 and R^7 are independently H or C_1-C_6 alkyl. R^4 and R^5 are independently H, NH_2, NHR or NR_2 where R is C_1-C_6 alkyl. R^1 and R^2 are independently H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_6-C_{20} arylalkyl, C_6-C_{20} substituted arylalkyl, acyloxymethyl esters —$CH_2C(=O)R^9$ (e.g. POM) or acyloxymethyl carbonates —$CH_2OC(=O)OR^9$ (e.g. POC) where R^9 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl or C_6-C_{20} substituted aryl. For example, R_1 and R_2 may be pivaloyloxymethoxy, POM, —$CH_2C(=O)C(CH_3)_3$; $CH_2OC(=O)OC(CH_3)_3$; or POC, —$CH_2C(=O)OCH(CH_3)_2$. Also for example, tenofovir has the structure where R^3 is CH_3, and R^1, R^2, R^4, R^5, R^6 and R^7 are H. Dialkyl phosphonates may be prepared according to the methods of: Quast et al (1974) *Synthesis* 490; Stowell et al (1990) *Tetrahedron Lett.* 3261; U.S. Pat. No. 5,663,159.

The PMPA compound may be enantiomerically-enriched or purified (single stereoisomer) where the carbon atom bearing R^3 may be the R or S enantiomer. The PMPA compound may be a racemate, i.e. a mixture of R and S stereoisomers.

Adefovir (9-(2-phosphonomethoxyethyl)adenine where R_1-R_7═H) is an exemplary PMEA compound (U.S. Pat. Nos. 4,808,716, 4,724,233). As the bis-pivalate prodrug, Adefovir dipivoxil, also known as bis-POM PMEA, (R_3-R_7═H, R_1 and R_2=—$CH_2CO(=O)C(CH_3)_3$, pivoxil, POM, pivaloyloxymethoxy), is effective against HIV and Hepatitis B infections (U.S. Pat. Nos. 5,663,159, 6,451,340). Adefovir dipivoxil has demonstrated minor to moderate synergistic inhibition of HIV replication in combination with other compounds with anti-HIV activity including PMPA, d4T, ddC, nelfinavir, ritonavir, and saquinavir (Mulato et al (1997) *Antiviral Research* 36:91-97).

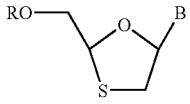
The invention includes all enantiomers, diastereomers, racemates, and enriched stereoisomer mixtures of PMEA and PMPA, and physiologically functional derivatives thereof.

Emtricitabine ((−)-cis-FTC, Emtriva™), a single enantiomer of FTC, is a potent nucleoside reverse transcriptase inhibitor approved for the treatment of HIV (U.S. Pat. Nos. 5,047,407, 5,179,104, 5,204,466, 5,210,085, 5,486,520, 5,538,975, 5,587,480, 5,618,820, 5,763,606, 5,814,639, 5,914,331, 6,114,343, 6,180,639, 6,215,004; WO 02/070518). The single enantiomer emtricitabine has the structure:



The chemical names for emtricitabine include: (−)-cis-FTC; β-L-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane; (2R,5S)-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine; and 4-amino-5-fluoro-1-(2-hydroxymethyl-[1,3]-(2R,5S)-oxathiolan-5-yl)-1H-pyrimidin-2-one. The CAS Registry numbers include: 143491-57-0; 143491-54-7. It should be noted that FTC contains two chiral centers, at the 2 and 5 positions of the oxathiolane ring, and therefore can exist in the form of two pairs of optical isomers (i.e. enantiomers) and mixtures thereof including racemic mixtures. Thus, FTC may be either a cis or a trans isomer or mixtures thereof. Mixtures of cis and trans isomers are diastereomers with different physical properties. Each cis and trans isomer can exist as one of two enantiomers or mixtures thereof including racemic mixtures. The invention includes all enantiomers, diastereomers, racemates, and enriched stereoisomer mixtures of emtricitabine and physiologically functional derivatives thereof. For example, the invention includes physiological functional derivatives such as the 1:1 racemic mixture of the enantiomers (2R,5S,cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine) and its mirror image (2S,5R, cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one, or mixtures of the two enantiomers in any relative amount. The invention also includes mixtures of cis and trans forms of FTC.

Physiologically functional derivatives of emtricitabine include 1,3 oxathiolane nucleosides having the structure:

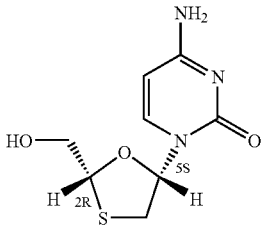


In the 1,3 oxathiolane nucleoside structure above, B is a nucleobase including any nitrogen-containing heterocyclic moiety capable of forming Watson-Crick hydrogen bonds in pairing with a complementary nucleobase or nucleobase analog, e.g. a purine, a 7-deazapurine, or a pyrimidine. Examples of B include the naturally occurring nucleobases: adenine, guanine, cytosine, uracil, thymine, and minor constituents and analogs of the naturally occurring nucleobases, e.g. 7-deazaadenine, 7-deazaguanine, 7-deaza-8-azaguanine, 7-deaza-8-azaadenine, inosine, nebularine, nitropyrrole, nitroindole, 2-aminopurine, 2-amino-6-chloropurine, 2,6-diaminopurine, hypoxanthine, pseudouridine, 5-fluorocytosine, 5-chlorocytosine, 5-bromocytosine, 5-iodocytosine, pseudocytosine, pseudoisocytosine, 5-propynylcytosine, isocytosine, isoguanine, 7-deazaguanine, 2-thiopyrimidine, 6-thioguanine, 4-thiothymine, 4-thiouracil, O^6-methylguanine, N^6-methyladenine, O^4-methylthymine, 5,6-dihydrothymine, 5,6-dihydrouracil, 4-methylindole, pyrazolo[3,4-D]pyrimidines (U.S. Pat. Nos. 6,143,877 and 6,127,121; WO 01/38584), and ethenoadenine (Fasman (1989) in *Practical Handbook of Biochemistry and Molecular Biology*, pp. 385-394, CRC Press, Boca Raton, Fla.).

Nucleobases B may be attached in the configurations of naturally-occurring nucleic acids to the 1,3 oxathiolane moiety through a covalent bond between the N-9 of purines, e.g. adenin-9-yl and guanin-9-yl, or N-1 of pyrimidines, e.g. thymin-1-yl and cytosin-1-yl (Blackburn, G. and Gait, M. Eds. "DNA and RNA structure" in *Nucleic Acids in Chemistry and Biology*, 2nd Edition, (1996) Oxford University Press, pp. 15-81).

Also in the 1,3 oxathiolane nucleoside structure above, R is H, C_1-C_{18} alkyl, C_1-C_{18} substituted alkyl, C_2-C_{18} alkenyl, C_2-C_{18} substituted alkenyl, C_2-C_{18} alkynyl, C_2-C_{18} substituted alkynyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_2-C_{20} heterocycle, C_2-C_{20} substituted heterocycle, phosphonate, phosphophosphonate, diphosphophosphonate, phosphate, diphosphate, triphosphate, polyethyleneoxy, or a prodrug moiety.

Physiologically functional derivatives of emtricitabine also include 3TC (lamivudine, Epivir®), a reverse transcriptase inhibitor approved in the United States for the treatment of HIV-1 infection in combination with AZT as Combivir® (GlaxoSmithKline). U.S. Pat. Nos. 5,859,021; 5,905,082; 6,177,435; 5,627,186; 6,417,191. Lamivudine (U.S. Pat. Nos. 5,587,480, 5,696,254, 5,618,820, 5,756,706, 5,744,596, 568,164, 5,466,806, 5,151,426) has the structure:



For example and for some therapeutic uses, 3TC may be a physiologically functional derivative of emtricitabine in combination with tenofovir DF or a physiologically functional derivative of tenofovir DF.

It will be appreciated that tenofovir DF and emtricitabine, and their physiologically functional derivatives may exist in keto or enol tautomeric forms and the use of any tautomeric form thereof is within the scope of this invention. Tenofovir DF and emtricitabine will normally be utilized in the combinations of the invention substantially free of the corresponding enantiomer, that is to say no more than about 5% w/w of the corresponding enantiomer will be present.

Prodrugs

The invention includes all prodrugs of tenofovir and emtricitabine. An exemplary prodrug of tenofovir is tenofovir disoproxil fumarate (TDF, Viread®). A large number of structurally-diverse prodrugs have been described for phosphonic acids (Freeman and Ross in *Progress in Medicinal Chemistry* 34: 112-147 (1997). A commonly used prodrug class is the acyloxyalkyl ester, which was first used as a prodrug strategy for carboxylic acids and then applied to phosphates and phosphonates by Farquhar et al (1983) *J. Pharm. Sci.* 72: 324; also U.S. Pat. Nos. 4,816,570, 4,968,788, 5,663,159 and 5,792,756. Subsequently, the acyloxyalkyl ester was used to deliver phosphonic acids across cell membranes and to enhance oral bioavailability. A close variant of the acyloxyalkyl ester strategy, the alkoxycarbonyloxyalkyl ester, may also enhance oral bioavailability as a prodrug moiety in the compounds of the combinations of the invention. Aryl esters of phosphorus

groups, especially phenyl esters, are reported to enhance oral bioavailability (DeLambert et al (1994) *J. Med. Chem.* 37: 498). Phenyl esters containing a carboxylic ester ortho to the phosphate have also been described (Khamnei and Torrence, (1996) *J. Med. Chem.* 39:4109-4115). Benzyl esters are reported to generate the parent phosphonic acid. In some cases, substituents at the ortho- or para-position may accelerate the hydrolysis. Benzyl analogs with an acylated phenol or an alkylated phenol may generate the phenolic compound through the action of enzymes, e.g. esterases, oxidases, etc., which in turn undergoes cleavage at the benzylic C—O bond to generate the phosphoric acid and the quinone methide intermediate. Examples of this class of prodrugs are described by Mitchell et al (1992) *J. Chem. Soc. Perkin Trans. I*2345; Brook et al WO 91/19721. Still other benzylic prodrugs have been described containing a carboxylic ester-containing group attached to the benzylic methylene (Glazier et al WO 91/19721). Thio-containing prodrugs are reported to be useful for the intracellular delivery of phosphonate drugs. These proesters contain an ethylthio group in which the thiol group is either esterified with an acyl group or combined with another thiol group to form a disulfide. Deesterifecation or reduction of the disulfide generates the free thio intermediate which subsequently breaks down to the phosphoric acid and episulfide (Puech et al (1993) *Antiviral Res.,* 22: 155-174; Benzaria et al (1996) *J. Med. Chem.* 39: 4958). Cyclic phosphonate esters have also been described as prodrugs of phosphorus-containing compounds.

Prodrug esters in accordance with the invention are independently selected from the following groups: (1) mono-, di-, and tri-phosphate esters of tenofovir or emtricitabine or any other compound which upon administration to a human subject is capable of providing (directly or indirectly) said mono-, di, or triphosphate ester; (2) carboxylic acid esters (3) sulphonate esters, such as alkyl- or aralkylsulphonyl (for example, methanesulphonyl); (4) amino acid esters (for example, alanine, L-valyl or L-isoleucyl); (5) phosphonate; and (6) phosphonamidate esters.

Ester groups (1)-(6) may be substituted with; straight or branched chain C_1-C_{18} alkyl (for example, methyl, n-propyl, t-butyl, or n-butyl); C_3-C_{12} cycloalkyl; alkoxyalkyl (for example, methoxymethyl); arylalkyl (for example, benzyl); aryloxyalkyl (for example, phenoxymethyl); C_5-C_{20} aryl (for example, phenyl optionally substituted by, for example, halogen, C_1-C_4 alkyl, C_1-C_4 alkoxy, or amino; acyloxymethyl esters —$CH_2C(=O)R^9$ (e.g. POM) or acyloxymethyl carbonates —$CH_2C(=O)OR^9$ (e.g. POC) where R^9 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl or C_6-C_{20} substituted aryl. For example, ester groups may be: —$CH_2OC(O)C(CH_3)_3$, —$CH_2OC(O)OC(CH_3)_3$ or —$CH_2C(=O)OCH(CH_3)_2$.

An exemplary aryl moiety present in such esters comprises a phenyl or substituted phenyl group. Many phosphate prodrug moieties are described in U.S. Pat. No. 6,312,662; Jones et al (1995) *Antiviral Research* 27:1-17; Kucera et al (1990) *AIDS Res. Hum. Retro Viruses* 6:491-501; Piantadosi et al (1991) *J. Med. Chem.* 34:1408-14; Hosteller et al (1992) *Antimicrob. Agents Chemother.* 36:2025-29; Hostetler et al (1990) *J. Biol. Chem.* 265:611127; and Siddiqui et al (1999) *J. Med. Chem.* 42:4122-28.

Pharmaceutically acceptable prodrugs refer to a compound that is metabolized in the host, for example hydrolyzed or oxidized, by either enzymatic action or by general acid or base solvolysis, to form an active ingredient. Typical examples of prodrugs of the active ingredients of the combinations of the invention have biologically labile protecting groups on a functional moiety of the active compound. Prodrugs include compounds that can be oxidized, reduced, aminated, deaminated, esterified, deesterified, alkylated, dealkylated, acylated, deacylated, phosphorylated, dephosphorylated, or other functional group change or conversion involving forming or breaking chemical bonds on the prodrug.

Chemical Stability of a Pharmaceutical Formulation

The chemical stability of the active ingredients in a pharmaceutical formulation is of concern to minimize the generation of impurities and ensure adequate shelf-life. The active ingredients, tenofovir disoproxil fumarate and emtricitabine, in the pharmaceutical formulations of the invention have relatively low pKa values, indicative of the potential to cause acidic hydrolysis of the active ingredients. Emtricitabine, with a pKa of 2.65 (Emtriva™ Product Insert, Gilead Sciences, Inc. 2003, available at gilead.com) is subject to hydrolytic deamination of the 5-fluoro cytosine nucleobase to form the 5-fluoro uridine nucleobase. Tenofovir disoproxil fumarate, with a pKa of 3.75 (Yuan L. et al "Degradation Kinetics of Oxycarbonyloxymethyl Prodrugs of Phosphonates in Solution", *Pharmaceutical Research* (2001) Vol. 18, No. 2, 234-237), is subject also to hydrolytic deamination of the exocyclic amine of the adenine nucleobase, and to hydrolysis of one or both of the POC ester groups (U.S. Pat. No. 5,922, 695). It is desirable to formulate a therapeutic combination of tenofovir disoproxil fumarate and emtricitabine, and the physiological functional derivatives thereof, with a minimum of impurities and adequate stability.

The combinations of the present invention provide combination pharmaceutical dosage forms which are chemically stable to acid degradation of: (1) a first component (such as tenofovir disoproxil fumarate, and physiological functional derivatives; (2) a second component (such as emtricitabine, and physiological functional derivatives; and (3) optionally a third component having antiviral activity. The third component includes anti-HIV agents and include: protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), and integrase inhibitors. Exemplary third active ingredients to be administered in combination with first and second components are shown in Table A. First and second components are as defined in the above section entitled: *ACTIVE INGREDIENTS OF THE COMBINATIONS.*

Salts

Any reference to any of the compounds in the compositions of the invention also includes any physiologically acceptable salt thereof. Examples of physiologically acceptable salts of tenofovir DF, emtricitabine and their physiologically functional derivatives include salts derived from an appropriate base, such as an alkali metal (for example, sodium), an alkaline earth (for example, magnesium), ammonium and NX_4^+ (wherein X is C_1-C_4 alkyl), or an organic acid such as fumaric acid, acetic acid, succinic acid. Physiologically acceptable salts of an hydrogen atom or an amino group include salts of organic carboxylic acids such as acetic, benzoic, lactic, fumaric, tartaric, maleic, malonic, malic, isethionic, lactobionic and succinic acids; organic sulfonic acids, such as methanesulfonic, ethanesulfonic, benzenesulfonic and p-toluenesulfonic acids; and inorganic acids, such as hydrochloric, sulfuric, phosphoric and sulfamic acids. Physiologically acceptable salts of a compound of an hydroxy group include the anion of said compound in combination with a suitable cation such as Na^+ and NX_4^+ (wherein X is independently selected from H or a C_1-C_4 alkyl group).

For therapeutic use, salts of active ingredients of the combinations of the invention will be physiologically acceptable, i.e. they will be salts derived from a physiologically accept-

able acid or base. However, salts of acids or bases which are not physiologically acceptable may also find use, for example, in the preparation or purification of a physiologically acceptable compound. All salts, whether or not derived from a physiologically acceptable acid or base, are within the scope of the present invention.

Administration of the Formulations

While it is possible for the active ingredients of the combination to be administered alone and separately as monotherapies, it is preferable to administer them as a pharmaceutical co-formulation. A two-part or three-part combination may be administered simultaneously or sequentially. When administered sequentially, the combination may be administered in one, two, or three administrations.

Preferably, two-part or three-part combinations are administered in a single pharmaceutical dosage form. More preferably, a two-part combination is administered as a single oral dosage form and a three-part combination is administered as two identical oral dosage forms. Examples include a single tablet of tenofovir disoproxil fumarate and emtricitabine, or two tablets of tenofovir disoproxil fumarate, emtricitabine, and efavirenz.

It will be appreciated that the compounds of the combination may be administered: (1) simultaneously by combination of the compounds in a co-formulation or (2) by alternation, i.e. delivering the compounds serially, sequentially, in parallel or simultaneously in separate pharmaceutical formulations. In alternation therapy, the delay in administering the second, and optionally a third active ingredient, should not be such as to lose the benefit of a synergistic therapeutic effect of the combination of the active ingredients. By either method of administration (1) or (2), ideally the combination should be administered to achieve peak plasma concentrations of each of the active ingredients. A one pill once-per-day regimen by administration of a combination co-formulation may be feasible for some HIV-positive patients. Effective peak plasma concentrations of the active ingredients of the combination will be in the range of approximately 0.001 to 100 μM. Optimal peak plasma concentrations may be achieved by a formulation and dosing regimen prescribed for a particular patient. It will also be understood that tenofovir DF and emtricitabine, or the physiologically functional derivatives of either thereof, whether presented simultaneously or sequentially, may be administered individually, in multiples, or in any combination thereof. In general, during alternation therapy (2), an effective dosage of each compound is administered serially, where in co-formulation therapy (1), effective dosages of two or more compounds are administered together.

Formulation of the Combinations

When the individual components of the combination are administered separately they are generally each presented as a pharmaceutical formulation. The references hereinafter to formulations refer unless otherwise stated to formulations containing either the combination or a component compound thereof. It will be understood that the administration of the combination of the invention by means of a single patient pack, or patient packs of each formulation, within a package insert diverting the patient to the correct use of the invention is a desirable additional feature of this invention. The invention also includes a double pack comprising in association for separate administration, formulations of tenofovir disoproxil fumarate and emtricitabine, or a physiologically functional derivative of either or both thereof.

The combination therapies of the invention include: (1) a combination of tenofovir DF and emtricitabine or (2) a combination containing a physiologically functional derivative of either or both thereof.

The combination may be formulated in a unit dosage formulation comprising a fixed amount of each active pharmaceutical ingredient for a periodic, e.g. daily, dose or subdose of the active ingredients.

Pharmaceutical formulations according to the present invention comprise a combination according to the invention together with one or more pharmaceutically acceptable carriers or excipients and optionally other therapeutic agents. Pharmaceutical formulations containing the active ingredient may be in any form suitable for the intended method of administration. When used for oral use for example, tablets, troches, lozenges, aqueous or oil suspensions, dispersible powders or granules, emulsions, hard or soft capsules, syrups or elixirs may be prepared (Remington's Pharmaceutical Sciences (Mack Publishing Co., Easton, Pa.). Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents including antioxidants, sweetening agents, flavoring agents, coloring agents and preserving agents, in order to provide a palatable preparation. Tablets containing the active ingredient in admixture with non-toxic pharmaceutically acceptable excipient which are suitable for manufacture of tablets are acceptable. These excipients may be, for example, inert diluents, such as calcium or sodium carbonate, lactose, lactose monohydrate, croscarmellose sodium, povidone, calcium or sodium phosphate; granulating and disintegrating agents, such as maize starch, or alginic acid; binding agents, such as cellulose, microcrystalline cellulose, starch, gelatin or acacia; and lubricating agents, such as magnesium stearate, stearic acid or talc. Tablets may be uncoated or may be coated by known techniques including microencapsulation to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate alone or with a wax may be employed.

Formulations for oral use may be also presented as hard gelatin capsules where the active ingredient is mixed with an inert solid diluent, for example pregelatinized starch, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, such as peanut oil, liquid paraffin or olive oil.

Aqueous suspensions of the invention contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients include a suspending agent, such as sodium carboxymethylcellulose, methylcellulose, hydroxypropyl methylcelluose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as a naturally occurring phosphatide (e.g., lecithin), a condensation product of an alkylene oxide with a fatty acid (e.g., polyoxyethylene stearate), a condensation product of ethylene oxide with a long chain aliphatic alcohol (e.g., heptadecaethyleneoxycetanol), a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol anhydride (e.g., polyoxyethylene sorbitan monooleate). The aqueous suspension may also contain one or more preservatives such as ethyl or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents, such as sucrose, sucralose or saccharin.

Oil suspensions may be formulated by suspending the active ingredient in a vegetable oil, such as arachis oil, olive

oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oral suspensions may contain a thickening agent, such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents, such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid, BHT, etc.

Dispersible powders and granules of the invention suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, a suspending agent, and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those disclosed above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions or liposome formulations. The oily phase may be a vegetable oil, such as olive oil or arachis oil, a mineral oil, such as liquid paraffin, or a mixture of these. Suitable emulsifying agents include naturally-occurring gums, such as gum acacia and gum tragacanth, naturally occurring phosphatides, such as soybean lecithin, esters or partial esters derived from fatty acids and hexitol anhydrides, such as sorbitan monooleate, and condensation products of these partial esters with ethylene oxide, such as polyoxyethylene sorbitan monooleate. The emulsion may also contain sweetening and flavoring agents. Syrups and elixirs may be formulated with sweetening agents, such as glycerol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, a flavoring or a coloring agent.

The pharmaceutical compositions of the invention may be in the form of a sterile injectable preparation, such as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, such as a solution in 1,3-butane-diol or prepared as a lyophilized powder. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile fixed oils may conventionally be employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid may likewise be used in the preparation of injectables.

The pharmaceutical compositions of the invention may be injected parenterally, for example, intravenously, intraperitoneally, intrathecally, intraventricularly, intrasternally, intracranially, intramuscularly or subcutaneously, or they may be administered by infusion techniques. They are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art.

The pharmaceutical compositions of the invention may also be administered intranasally or by inhalation and are conveniently delivered in the form of a dry powder inhaler or an aerosol spray presentation from a pressurized container or a nebuliser with the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFC 134a), carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurized container or nebuliser may contain a solution or suspension of the composition, e.g. using a mixture of ethanol and the propellant as the solvent, which may additional contain a lubricant, e.g. sorbitan trioleate. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated to contain a powder mix of a compound of the formula (I) and a suitable powder base such as lactose or starch. Aerosol or dry powder formulations are preferably arranged so that each metered dose or "puff" contains from 20 μg to 20 mg of a composition for delivery to the patient. The overall daily dose with an aerosol will be in the range of from 20 μg to 20 mg which may be administered in a single dose or, more usually, in divided doses throughout the day.

The amount of active ingredient that may be combined with the carrier material to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a time-release formulation intended for oral administration to humans may contain approximately 1 to 1000 mg of active material compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95% of the total compositions (weight:weight). The pharmaceutical composition can be prepared to provide easily measurable amounts for administration. For example, an aqueous solution intended for intravenous infusion may contain from about 3 to 500 μg of the active ingredient per milliliter of solution in order that infusion of a suitable volume at a rate of about 30 mL/hr can occur. As noted above, formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be administered as a bolus, electuary or paste.

The combinations of the invention may conveniently be presented as a pharmaceutical formulation in a unitary dosage form. A convenient unitary dosage formulation contains the active ingredients in any amount from 1 mg to 1 g each, for example but not limited to, 10 mg to 300 mg. The synergistic effects of tenofovir DF in combination with emtricitabine may be realized over a wide ratio, for example 1:50 to 50:1 (tenofovir DF:emtricitabine). In one embodiment the ratio may range from about 1:10 to 10:1. In another embodiment, the weight/weight ratio of tenofovir to emtricitabine in a co-formulated combination dosage form, such as a pill, tablet, caplet or capsule will be about 1, i.e. an approximately equal amount of tenofovir DF and emtricitabine. In other exemplary co-formulations, there may be more or less tenofovir than FTC. For example, 300 mg tenofovir DF and 200 mg emtricitabine can be co-formulated in a ratio of 1.5:1 (tenofovir DF: emtricitabine). In one embodiment, each compound will be employed in the combination in an amount at which it exhibits antiviral activity when used alone. Exemplary Formulations A, B, C, D, E, and F (Examples) have ratios of 12:1 to 1:1 (tenofovir F: emtricitabine). Exemplary Formulations A, B, C, D, E, and F use amounts of tenofovir DF and emtricitabine ranging from 25 mg to 300 mg. Other ratios and amounts of the compounds of said combinations are contemplated within the scope of the invention.

A unitary dosage form may further comprise tenofovir DF and emtricitabine, or physiologically functional derivatives of either thereof, and a pharmaceutically acceptable carrier.

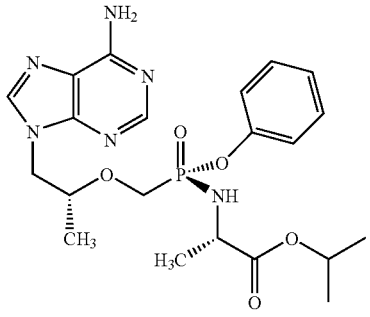
It will be appreciated by those skilled in the art that the amount of active ingredients in the combinations of the invention required for use in treatment will vary according to a variety of factors, including the nature of the condition being treated and the age and condition of the patient, and will ultimately be at the discretion of the attending physician or health care practitioner. The factors to be considered include the route of administration and nature of the formulation, the animal's body weight, age and general condition and the nature and severity of the disease to be treated. For example, in a Phase I/II monotherapy study of emtricitabine, patients received doses ranging from 25 mg to 200 mg twice-a-day for two weeks. At each dose regimen greater or equal to 200 mg, a 98-percent (1.75 log 10) or greater viral suppression was observed. A once-a-day dose of 200 mg of emtricitabine reduced the viral load by an average of 99 percent (1.92 log 10). Viread® (tenofovir DF) has been approved by the FDA for the treatment and prophylaxis of HIV infection as a 300 mg oral tablet. Emtriva™ (emtricitabine) has been approved by the FDA for the treatment of HIV as a 200 mg oral tablet.

It is also possible to combine any two of the active ingredients in a unitary dosage form for simultaneous or sequential administration with a third active ingredient. The three-part combination may be administered simultaneously or sequentially. When administered sequentially, the combination may be administered in two or three administrations. Third active ingredients have anti-HIV activity and include protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), and integrase inhibitors. Exemplary third active ingredients to be administered in combination with tenofovir DF, emtricitabine, and their physiological functional derivatives, are shown in Table A.

TABLE A

5,6 dihydro-5-azacytidine
5-aza 2'deoxycytidine
5-azacytidine
5-yl-carbocyclic 2'-deoxyguanosine (BMS200,475)
9 (arabinofuranosyl)guanine; 9-(2' deoxyribofuranosyl)guanine
9-(2'-deoxy 2'fluororibofuranosyl)-2,6-diaminopurine
9-(2'-deoxy 2'fluororibofuranosyl)guanine
9-(2'-deoxyribofuranosyl)-2,6 diaminopurine
9-(arabinofuranosyl)-2,6 diaminopurine
Abacavir, Ziagen ®
Acyclovir, ACV; 9-(2-hydroxyethoxylmethyl)guanine
Adefovir dipivoxil, Hepsera ®
amdoxivir, DAPD
Amprenavir, Agenerase ®
araA; 9-β-D-arabinofuranosyladenine (Vidarabine)
atazanivir sulfate (Reyataz ®)
AZT; 3'-azido-2',3'-dideoxythymdine, Zidovudine, (Retrovir ®)
BHCG; (.+−.)-(1a,2b,3a)-9-[2,3-bis(hydroxymethyl)cyclobutyl]guanine
BMS200,475; 5-yl-carbocyclic 2'-deoxyguanosine
Buciclovir; (R) 9-(3,4-dihydroxybutyl)guanine
BvaraU; 1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil (Sorivudine)
Calanolide A
Capravirine
CDG; carbocyclic 2'-deoxyguanosine
Cidofovir, HPMPC; (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine
Clevudine, L-FMAU; 2'-Fluoro-5-methyl-β-L-arabino-furanosyluracil
Combivir ® (lamivudine/zidovudine)
Cytallene; [1-(4'-hydroxy-1',2'-butadienyl)cytosine]
d4C; 3'-deoxy-2',3'-didehydrocytidine
DAPD; (−)-β-D-2,6-diaminopurine dioxolane
ddA; 2',3'-dideoxyadenosine
ddAPR; 2,6-diaminopurine-2',3'-dideoxyriboside
ddC; 2',3'-dideoxycytidine (Zalcitabine)
ddI; 2',3'-dideoxyinosine, didanosine, (Videx ®, Videx ® EC)
Delavirdine, Rescriptor ®
Didanosine, ddI, Videx ®; 2',3'-dideoxyinosine
DXG; dioxolane guanosine
E-5-(2-bromovinyl)-2'-deoxyuridine
Efavirenz, Sustiva ®
Enfuvirtide, Fuzeon ®
F-ara-A; fluoroarabinosyladenosine (Fludarabine)
FDOC; (−)-β-D-5-fluoro-1-[2-(hydroxymethyl)-1,3-dioxolane]cytosine
FEAU; 2'-deoxy-2'-fluoro-1-β-D-arabinofuranosyl-5-ethyluracil
FIAC; 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine
FIAU; 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouridine
FLG; 2',3'-dideoxy-3'-fluoroguanosine
FLT; 3'-deoxy-3'-fluorothymidine
Fludarabine; F-ara-A; fluoroarabinosyladenosine
FMAU; 2'-Fluoro-5-methyl-β-L-arabino-furanosyluracil
FMdC
Foscarnet; phosphonoformic acid, PFA
FPMPA; 9-(3-fluoro-2-phosphonylmethoxypropyl)adenine
Gancyclovir, GCV; 9-(1,3-dihydroxy-2-propoxymethyl)guanine
GS-7340; 9-[R-2-[[(S)-[[(S)-1-(isopropoxycarbonyl)ethyl]amino]-phenoxyphosphinyl]methoxy]propyl]adenine
HPMPA; (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine
HPMPC; (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (Cidofovir)
Hydroxyurea, Droxia ®
Indinavir, Crixivan ®
Kaletra ® (lopinavir/ritonavir)
Lamivudine, 3TC, Epivir ™; (2R,5S,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one
L-d4C; L-3'-deoxy-2',3'-didehydrocytidine
L-ddC; L-2',3'-dideoxycytidine
L-Fd4C; L-3'-deoxy-2',3'-didehydro-5-fluorocytidine
L-FddC; L-2',3'-dideoxy-5-fluorocytidine
Lopinavir
Nelfinavir, Viracept ®
Nevirapine, Viramune ®
Oxetanocin A; 9-(2-deoxy-2-hydroxymethyl-β-D-erythro-oxetanosyl)adenine
Oxetanocin G; 9-(2-deoxy-2-hydroxymethyl-β-D-erythro-oxetanosyl)guanine
Penciclovir
PMEDAP; 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine
PMPA, tenofovir; (R)-9-(2-phosphonylmethoxypropyl)adenine
PPA; phosphonoacetic acid
Ribavirin; 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide
Ritonavir, Norvir ®
Saquinavir, Invirase ®, Fortovase ®
Sorivudine, BvaraU; 1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil
Stavudine, d4T, Zerit ®; 2',3'-didehydro-3'-deoxythymidine
Trifluorothymidine, TFT; Trifluorothymidine
Trizivir ® (abacavir sulfate/lamivudine/zidovudine)
Vidarabine, araA; 9-β-D-arabinofuranosyladenine
Zalcitabine, Hivid ®, ddC; 2',3'-dideoxycytidine
Zidovudine, AZT, Retrovir ®; 3'-azido-2',3'-dideoxythymdine
Zonavir; 5-propynyl-1-arabinosyluracil

Another aspect of the present invention is a three-part combination comprising tenofovir DF, FTC, and 9-[(R)-2-[[(S)-[[(S)-1-(isopropoxycarbonyl)ethyl]amino]phenoxy-phosphinyl]methoxy]propyl]adenine, also designated herein as GS-7340, which has the structure:



GS-7340 is a prodrug of tenofovir and the subject of commonly owned, pending application, U.S. Ser. No. 09/909,560, filed Jul. 20, 2001 and Becker et al WO 02/08241.

For example, a ternary unitary dosage may contain 1 mg to 1000 mg of tenofovir disoproxil fumarate, 1 mg to 1000 mg of emtricitabine, and 1 mg to 1000 mg of the third active ingredient. As a further feature of the present invention, a unitary dosage form may further comprise tenofovir DF, emtricitabine, the third active ingredient, or physiologically functional derivatives of the three active ingredients thereof, and a pharmaceutically acceptable carrier.

Combinations of the present invention enable patients greater freedom from multiple dosage medication regimens and ease the needed diligence required in remembering and complying with complex daily dosing times and schedules. By combining tenofovir disoproxil fumarate and emtricitabine into a single dosage form, the desired daily regimen may be presented in a single dose or as two or more sub-doses per day. The combination of co-formulated tenofovir DF and emtricitabine may be administered as a single pill, once per day.

A further aspect of the invention is a patient pack comprising at least one active ingredient: tenofovir disoproxil fumarate, emtricitabine, or a physiologically functional derivative of either of the combination and an information package or product insert containing directions on the use of the combination of the invention.

Segregation of active ingredients in pharmaceutical powders and granulations is a widely recognized problem that can result in inconsistent dispersions of the active ingredients in final dosage forms. Some of the main factors contributing to segregation are particle size, shape and density. Segregation is particularly troublesome when attempting to formulate a single homogenous tablet containing multiple active ingredients having different densities and different particle sizes. Glidants are substances that have traditionally been used to improve the flow characteristics of granulations and powders by reducing interparticulate friction. See Lieberman, *Lachman, & Schwartz, Pharmaceutical Dosage Forms: Tablets, Volume* 1, p. 177-178 (1989), incorporated herein by reference. Glidants are typically added to pharmaceutical compositions immediately prior to tablet compression to facilitate the flow of granular material into the die cavities of tablet presses. Glidants include: colloidal silicon dioxide, asbestos free talc, sodium aluminosilicate, calcium silicate, powdered cellulose, microcrystalline cellulose, corn starch, sodium benzoate, calcium carbonate, magnesium carbonate, metallic stearates, calcium stearate, magnesium stearate, zinc stearate, stearowet C, starch, starch 1500, magnesium lauryl sulfate, and magnesium oxide. Exemplary Tablet Formulation A has colloidal silicon dioxide (Examples). Glidants can be used to increase and aid blend composition homogeneity in formulations of anti-HIV drugs (U.S. Pat. No. 6,113,920). The novel compositions of the present invention may contain glidants to effect and maintain homogeneity of active ingredients during handling prior to tablet compression.

The present invention provides pharmaceutical formulations combining the active ingredients tenofovir DF and emtricitabine, or physiologically functional derivatives thereof in a sufficiently homogenized form, and a method for using this pharmaceutical formulation. An object of the present invention is to utilize glidants to reduce the segregation of active ingredients in pharmaceutical compositions during pre-compression material handling. Another object of the present invention is to provide a pharmaceutical formulation combining the active ingredients tenofovir DF and emtricitabine, or physiologically functional derivatives thereof, with a pharmaceutically acceptable glidant, resulting in a mixture characterized by a pharmaceutically acceptable measure of homogeneity.

Formulations include those suitable for oral, rectal, nasal, topical (including transdermal, buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods represent a further feature of the present invention and include the step of bringing into association the active ingredients with the carrier, which constitutes one or more accessory ingredients, and maintaining chemical stability. In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, caplets, cachets or tablets each containing a predetermined amount of the active ingredients; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. povidone, gelatin, hydroxypropyl methylcellulose), lubricant, inert diluent, preservative, disintegrant (e.g. sodium starch glycollate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose) surface-active or dispersing agent. Molded tablets may be made by molding a mixture of the powdered compound moistened with an inert liquid diluent in a suitable machine. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredients therein using, for example, cellulose ether derivatives (e.g., hydroxypropyl methylcellulose) or methacrylate derivatives in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredients in a flavored base, usually sucrose and acacia or tragacanth; pastiles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier. Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for

example, cocoa butter or a salicylates. Topical administration may also be by means of a transdermal iontophoretic device.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for penile administration for prophylactic or therapeutic use may be presented in condoms, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Pharmaceutical formulations suitable for rectal administration wherein the carrier is a solid are most preferably presented as unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art. The suppositories may be conveniently formed by admixture of the active combination with the softened or melted carrier(s) followed by chilling and shaping in moulds.

Formulations suitable for parenteral administration include aqueous and nonaqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and nonaqueous sterile suspensions which may include suspending agents and thickening agents; and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Exemplary unit dosage formulations are those containing a daily dose or daily subdose of the active ingredients, as hereinbefore recited, or an appropriate fraction thereof. It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include such further agents as sweeteners, thickeners and flavoring agents.

The compounds of the combination of the present invention may be obtained in a conventional manner, known to those skilled in the art. Tenofovir disoproxil fumarate can be prepared, for example, as described in U.S. Pat. No. 5,977,089. Methods for the preparation of FTC are described in WO 92/14743, incorporated herein by reference.

Composition Use

Compositions of the present invention are administered to a human or other mammal in a safe and effective amount as described herein. These safe and effective amounts will vary according to the type and size of mammal being treated and the desired results of the treatment. Any of the various methods known by persons skilled in the art for packaging tablets, caplets, or other solid dosage forms suitable for oral administration, that will not degrade the components of the present invention, are suitable for use in packaging. The combinations may be packaged in glass and plastic bottles. Tablets, caplets, or other solid dosage forms suitable for oral administration may be packaged and contained in various packaging materials optionally including a dessicant e.g. silica gel. Packaging may be in the form of unit dose blister packaging. For example, a package may contain one blister tray of tenofovir DF and another blister tray of emtricitabine pills, tablets, caplets, or capsule. A patient would take one dose, e.g. a pill, from one tray and one from the other. Alternatively, the package may contain a blister tray of the co-formulated combination of tenofovir DF and emtricitabine in a single pill, tablet, caplet or capsule. As in other combinations and packaging thereof, the combinations of the invention include physiological functional derivatives of tenofovir DF and FTC.

The packaging material may also have labeling and information related to the pharmaceutical composition printed thereon. Additionally, an article of manufacture may contain a brochure, report, notice, pamphlet, or leaflet containing product information. This form of pharmaceutical information is referred to in the pharmaceutical industry as a "package insert." A package insert may be attached to or included with a pharmaceutical article of manufacture. The package insert and any article of manufacture labeling provides information relating to the pharmaceutical composition. The information and labeling provides various forms of information utilized by health-care professionals and patients, describing the composition, its dosage and various other parameters required by regulatory agencies such as the United States Food and Drug Agency.

Assays of the Combinations

The combinations of the inventions may be tested for in vitro activity against HIV and sensitivity, and for cytotoxicity in laboratory adapted cell lines, e.g. MT2 and in peripheral blood mononuclear cells (PBMC) according to standard assays developed for testing anti-HIV compounds, such as WO 02/068058 and U.S. Pat. No. 6,475,491. Combination assays may be performed at varying concentrations of the compounds of the combinations to determine EC_{50} by serial dilutions.

EXEMPLARY FORMULATIONS

The following examples further describe and demonstrate particular embodiments within the scope of the present invention. Techniques and formulations generally are found in *Remington's Pharmaceutical Sciences* (Mack Publishing Co., Easton, Pa.). The examples are given solely for illustration and are not to be construed as limitations as many variations are possible without departing from spirit and scope of the Invention. The following examples are intended for illustration only and are not intended to limit the scope of the invention in any way. "Active ingredient" denotes tenofovir disoproxil fumarate, emtricitabine, or a physiologically functional derivative of either thereof.

Tablet Formulation

The following exemplary formulations A, B, C, D, E, and F are prepared by wet granulation of the ingredients with an aqueous solution, addition of extragranular components and then followed by addition of magnesium stearate and compression.

	mg/tablet
Formulation A:	
Tenofovir Disoproxil Fumarate	300
emtricitabine	200
Microcrystalline Cellulose	200
Lactose Monohydrate	175
Croscarmellose Sodium	60
Pregelatinized Starch	50
Colloidal silicon dioxide	5
Magnesium Stearate	10
total:	1000
Formulation B:	
Tenofovir Disoproxil fumarate	300
emtricitabine	100
Microcrystalline Cellulose	200
Lactose Monohydrate	180
Sodium Starch Glycollate	60
Pregelatinized Starch	50
Magnesium Stearate	10
total:	900
Formulation C:	
Tenofovir Disoproxil fumarate	200
emtricitabine	200
Microcrystalline Cellulose	200
Lactose Monohydrate	180
Sodium Starch Glycollate	60
Pregelatinized Starch	50
Magnesium Stearate	10
total:	900
Formulation D:	
Tenofovir Disoproxil fumarate	300
emtricitabine	25
Microcrystalline Cellulose	200
Lactose Monohydrate	180
Sodium Starch Glycollate	60
Pregelatinized Starch	50
Magnesium Stearate	10
total:	825
Formulation E:	
Tenofovir Disoproxil fumarate	200
emtricitabine	25
Microcrystalline Cellulose	200
Lactose Monohydrate	180
Sodium Starch Glycollate	60
Pregelatinized Starch	50
Magnesium Stearate	10
total:	725
Formulation F:	
Tenofovir Disoproxil fumarate	100
emtricitabine	100
Microcrystalline Cellulose	200
Lactose Monohydrate	180
Sodium Starch Glycollate	60
Pregelatinized Starch	50
Magnesium Stearate	10
total:	700

Formulation G (Controlled Release Formulation):

This formulation is prepared by wet granulation of the ingredients with an aqueous solution, followed by the addition of magnesium stearate and compression.

	mg/tablet
Tenofovir Disoproxil fumarate	300
emtricitabine	200
Hydroxypropyl Methylcellulose	112
Lactose B.P.	53
Pregelatinized Starch B.P.	28
Magnesium Stearate	
total:	700

Drug release takes place over a period of about 6-8 hours and is complete after 12 hours.

Capsule Fomulations

Formulation H:

A capsule formulation is prepared by admixing the ingredients and filling into a two-part hard gelatin or hydroxypropyl methylcellulose capsule.

	mg/capsule
Active Ingredient	500
Microcrystalline Cellulose	143
Sodium Starch Glycollate	25
Magnesium Stearate	2
total:	670

Formulation I (Controlled Release Capsule):

The following controlled release capsule formulation is prepared by extruding ingredients a, b, and c using an extruder, followed by spheronization of the extrudate and drying. The dried pellets are then coated with release-controlling membrane (d) and filled into a two-piece, hard gelatin or hydroxypropyl methylcellulose capsule.

	mg/capsule
(a) Active Ingredient	500
(b) Microcrystalline Cellulose	125
(c) Lactose B.P.	125
(d) Ethyl Cellulose	13
total:	763

Formulation J (Oral Suspension):

The active ingredients are admixed with the ingredients and filling them as dry powder.

Purified water is added and mixed well before use.

Active Ingredient	500 mg
Confectioner's Sugar	2000 mg
Simethicone	300 mg
Methylparaben	30 mg
Propylparaben	10 mg
Flavor, Peach	500 mg
Purified Water q.s. to	5.00 ml

Formulation K (Suppository):

One-fifth of the Witepsol H15 is melted in a steam-jacketed pan at 45° C. maximum. The active ingredients are sifted through a 200 micron sieve and added to the molten base with mixing, using a Silverson fitted with a cutting head, until a smooth dispersion is achieved. Maintaining the mixture at

45° C., the remaining Witepsol H15 is added to the suspension and stirred to ensure a homogenous mix. The entire suspension is passed through a 250 micron stainless steel screen and, with continuous stirring, is allowed to cool to 40° C. At a temperature of 38° C. to 40° C., 2.02 g of the mixture is filled into suitable, 2 ml plastic molds. The suppositories are allowed to cool to room temperature.

	mg/Suppository
Active Ingredient	500
Hard Fat, B.P. (Witepsol H15-Dynamit Nobel)	1770
total	2270

Fixed Dose Combination Tablet

A fixed dose combination tablet of tenofovir disoproxil fumarate (TDF) 300 mg/emtricitabine 200 mg was formulated using a wet granulation/fluid-bed drying process using conventional methods. See: U.S. Pat. No. 5,935,946; L. Young (editor). Tableting Specification Manual 5th ed., American Pharmaceutical Association, Washington, D.C., (2001); L. Lachman, H. Lieberman (editors). Pharmaceutical Dosage Forms: Tablets (Vol 2), Marcel Dekker Inc., New York, 185-202 (1981); J. T. Fell and J. M. Newton, J. Pharm. Pharmacol. 20, 657-659 (1968); US Pharmacopeia 24-National Formulary 19, "Tablet Friability", Chapter <1216>, Page 2148 (2000).

The effects of granulation water level (ranging from 40% to 50% w/w) and wet massing time were studied on the physicochemical properties of the final powder blend and its performance with respect to blend uniformity and compressibility (tablet compactibility). In addition, content uniformity, assay, stability and dissolution performance was evaluated for the TDF/emtricitabine fixed dose combination tablets.

Formulation Equipment

Equipment included a high shear mixer equipped with a pressure tank and spray nozzle tip to add the granulating water, a fluid-bed dryer, a mill, a tumble blender, a rotary tablet press, and a tablet deduster.

Formulation Process

The dried, milled powder was blended with the extragranular microcrystalline cellulose and croscarmellose sodium and then blended with magnesium stearate. Powder samples were removed after mixing with the magnesium stearate. The blend samples were evaluated for, bulk density, mesh analysis and compressibility. The powder blend mixed with the magnesium stearate was compressed into tablets on a press setup.

Materials

The following Table 1 lists the quantitative composition of the TDF/emtricitabine tablet formulation.

TABLE 1

Ingredient	% w/w	Unit Formula for tablet cores (mg/tablet)	Quantity per 12 kg Batch (kg)
Tenofovir Disoproxil Fumarate[a]	30.0	300.0	3.60
Emtricitabine[a]	20.0	200.0	2.40
Pregelatinized Starch, NF/EP	5.0	50.0	0.60
Croscarmellose Sodium, NF/EP	6.0	60.0	0.72
Lactose Monohydrate, NF/EP[a]	8.0	80.0	0.96
Microcrystalline Cellulose, NF/EP[c]	30.0	300.0	3.60
Magnesium Stearate, NF/EP	1.0	10.0	0.12
Purified Water, USP/EP	[b]	[b]	[b]
Totals	100.0	1000.0	12.00

[a]Actual weight is adjusted based on the Drug Content Factor (DCF) of tenofovir disoproxil fumarate and emtricitabine.
[b]Water removed during drying.

Characterization Equipment

Moisture content was measured by loss on drying using a heat lamp/balance system. The powder blend was sampled with a sampling thief fitted with chambers to determine powder blend uniformity. Duplicate samples were removed from each of several locations in the blender. Blend uniformity analysis was performed on one sample from each location.

Particle size analysis of the final powder blend was determined by sifting a multi-gram sample through a screen using a sonic sifter. The quantity of final powder blend retained on each sieve and the fines collector was determined by calculating the difference in weight between the sieves and fines collector before and after the test. The geometric mean diameter particle size was calculated by logarithmic weighting of the sieved distribution.

Bulk density was determined by filling a graduated cylinder with the final powder blend and measuring the weight differential between the empty and filled graduate cylinder per unit volume.

Tablets were characterized for friability using a friabilator, a hardness tester, a thickness micrometer equipped with a printer, and a weighing balance.

Compression characteristics were determined using a rotary tablet press equipped with a flat-faced, beveled edged punch to a target weight of 400 mg. The powder blends were compressed using target upper punch pressures ranging from approximately 100 to 250 MPa. The apparent normalized ejection force was determined and normalized for tablet thickness and diameter.

Tablet hardness was determined using a hardness tester. Tablet thickness was determined using a micrometer, and tablet weights were determined using a top loading balance.

Wet Granulation

The powders were blended in a granulator and then granulated using water. The impeller and chopper speeds were kept constant in the blender at a low setting during the granulation and wet massing operations. After water addition, the impeller and chopper were stopped and the granulator bowl was opened to observe the granulation consistency and texture. The lid was closed and the wet massing phase was performed. Acceptable granules had 40% w/w and 60% w/w water, respectively.

Wet Milling

To facilitate a uniform drying process, each wet granulation was deagglomerated with a mill fitted with a screen and an impeller. The milled wet granules were charged into a fluid-bed dryer immediately following wet milling.

Fluid-Bed Drying

Milled wet granules were dried using an inlet air setpoint temperature of about 70° C. and airflow of approximately 100 cfm. The target LOD was about 1.0% with a range of not more than (NMT) 1.5%. The total fluid-bed drying time ranged from 53 to 75 minutes. Final LOD ranged from 0.4% to 0.7% for all of the batches dried. The final exhaust temperatures for all the batches ranged from 47° C. to 50° C.

Dry Milling

All dried granules were milled through a perforated screen. The mill was equipped with a square impeller and operated. The lots were milled and manually transferred to the V-blender.

Blending

Each lot was blended using the V-blender. In one set of three formulations, starting with 12 kg materials, final powder blend yield available for compression after blending ranged from 10.5 kg (87.5%) to 11.1 kg (92.5%). The final powder blend bulk density ranged from 0.48 to 0.58 g/cc and the geometric mean diameter particle size ranged from 112 to 221 μm. Percent water and wet massing time affect final powder blend particle size and bulk density.

The powder blending for both tenofovir DF and emtricitabine gave a mean (n=10) strength value for tenofovir DF ranged from 100.6% to 102.8% of target strength for the lots and the relative standard deviation (RSD) was from 0.5% to 1.7%. The mean (n=10) strength value for emtricitabine ranged from 101.3% to 104.1% of target strength for the lots with the relative standard deviation (RSD) ranged from 0.6% to 1.7%. The final powder blend moisture level ranged from 0.8% to 1.1% LOD.

Tablet Compression

The final blends were compressed using a rotary tablet press and the tablets were film-coated.

Three 300 gm formulations (Table 2) were granulated in a granulator equipped with a 1-L bowl. The quantities of intragranular components were based on a 300 g total batch size. The formulations in lots 1 and 2 differed in the amount of microcrystalline cellulose 30% vs. 20% w/w, respectively. Lots 2 and 3 were identical except for the type of binder. Lot 2 contained 5% w/w of pregelatinized starch and lot 3 contained 5% w/w povidone as binder.

TABLE 2

Ingredient	Lot 1 % w/w	Lot 2 % w/w	Lot 3 % w/w
Tenofovir Disoproxil Fumarate	30.0	30.0	30.0
Emtricitabine	20.0	20.0	20.0
Pregelatinized Starch, NF/EP	5.0	5.0	N/A
Povidone, USP/NF (C-30)	N/A	N/A	5.0
Croscarmellose Sodium, NF/EP	6.0	6.0	6.0
Lactose Monohydrate, NF/EP	8.0	18.0	18.0
Microcrystalline Cellulose, NF/EP[a]	30.0	20.0	20.0
Magnesium Stearate, NF/EP	1.0	1.0	1.0
Purified Water, USP/EP	[a]	[a]	[a]
Total	100.0	100.0	100.0

[a]Water removed during drying.

After water addition, the impeller and chopper were stopped and the granulator bowl was opened to observe the granulation consistency and texture. To achieve similar granulation consistency, lots 1, 2, and 3 were granulated with 45%, 40%, and 30% w/w water, respectively. The lid was closed and the wet massing phase was performed. All lots had a 30 sec wet massing resulting in acceptable granulations. The wet granulations from all batches were hand screened through a sieve to deagglomerate. The resulting granulations were tray dried in a convection oven set at 60° C. for approximately 20 hours to an LOD <1.0%. The dried granulations from all batches were hand screened through a sieve. In order to fit the granulation into the small scale (300 mL) V-blender, the final blend batch size was adjusted to 100 g. A portion, 81 g of the resulting blend from Lot 1 was blended with 15 g microcrystalline cellulose, 3 g croscarmellose sodium and 1 g magnesium stearate. 86 g of the resulting granulation from Lot 2 and Lot 3 were each blended with 10 g microcrystalline cellulose, 3 g croscarmellose sodium and 1 g magnesium stearate.

Purity analysis was conducted by reverse-phase HPLC (high performance liquid chromatography). Impurities related to tenofovir disoproxil fumarate and emtricitabine were characterized and measured in the bulk API (active pharmaceutical ingredient) before formulation in the three lots of Table 2, and again after formulation in the resulting tablets. The impurities include by-products from hydrolysis of the exocyclic amino groups of tenofovir disoproxil fumarate and emtricitabine, and the hydrolysis of the disoproxil (POC) esters of tenofovir disoproxil fumarate. In each lot, the sum total of impurities related to tenofovir disoproxil fumarate and emtricitabine was less than 1% after formulation and tablet manufacture.

The physicochemical properties of tenofovir disoproxil fumarate and emtricitabine tablets were evaluated by visual appearance, water content, label strength, impurity and degradation product contents, and tablet dissolution. Stability studies were conducted on drug product packaged in container-closure systems that are identical to the intended clinical and commercial container-closure system. There was no sign of discoloration or tablet cracking during the course of the stability study. Film-coated tenofovir disoproxil fumarate and emtricitabine tablets exhibited satisfactory stability at 40° C./75% RH (relative humidity) for up to six months when packaged and stored with silica gel desiccant. No significant loss (defined as >5% degradation) in % label strength of tenofovir DF or emtricitabine was observed after six months at 40° C./75% RH, when packaged and stored with desiccant. The increase in the total degradation products was 1.5% for tenofovir DF and 0.6-0.7% for emtricitabine after six months at 40° C./75% RH when packaged and stored with 3 grains of desiccant.

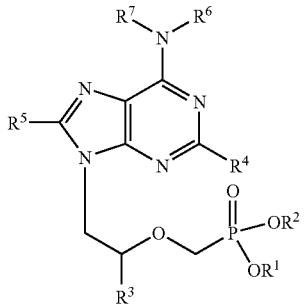
All publications and patent applications cited herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although certain embodiments are described in detail above, those having ordinary skill in the art will clearly understand that many modifications are possible in the claims without departing from the teachings thereof. All such modifications are intended to be encompassed within the claims of the invention.

EMBODIMENTS OF THE INVENTION

A1. A pharmaceutical composition comprising an effective amount of a compound of the formula:

(1)



wherein R^1 and R^2 are independently selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_6-C_{20} arylalkyl, C_6-C_{20} substituted arylalkyl, acyloxymethyl esters —$CH_2C(=O)R^9$ and acyloxymethyl carbonates —$CH_2C(=O)OR^9$ where R^9 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl and C_6-C_{20} substituted aryl;

R^3 is selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, or CH_2OR^8 where R^8 is C_1-C_6 alkyl, C_1-C_6 hydroxyalkyl and C_1-C_6 haloalkyl;

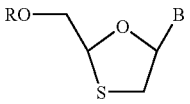
R^4 and R^5 are independently selected from H, NH_2, NHR and NR_2 where R is C_1-C_6 alkyl; and

R^6 and R^7 are independently selected from H and C_1-C_6 alkyl;

or a physiologically functional derivative thereof;

in combination with an effective amount of a compound of the formula

(2)



wherein B is selected from adenine, guanine, cytosine, uracil, thymine, 7-deazaadenine, 7-deazaguanine, 7-deaza-8-azaguanine, 7-deaza-8-azaadenine, inosine, nebularine, nitropyrrole, nitroindole, 2-aminopurine, 2-amino-6-chloropurine, 2,6-diaminopurine, hypoxanthine, pseudouridine, 5-fluorocytosine, 5-chlorocytosine, 5-bromocytosine, 5-iodocytosine, pseudocytosine, pseudoisocytosine, 5-propynylcytosine, isocytosine, isoguanine, 7-deazaguanine, 2-thiopyrimidine, 6-thioguanine, 4-thiothymine, 4-thiouracil, O^6-methylguanine, N^6-methyladenine, O^4-methylthymine, 5,6-dihydrothymine, 5,6-dihydrouracil, 4-methylindole, and a pyrazolo[3,4-D]pyrimidine; and

R is selected from H, C_1-C_{18} alkyl, C_1-C_{18} substituted alkyl, C_2-C_{18} alkenyl, C_2-C_{18} substituted alkenyl, C_1-C_{18} alkynyl, C_2-C_{18} substituted alkynyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_2-C_{20} heterocycle, C_2-C_{20} substituted heterocycle, phosphonate, phosphophosphonate, diphosphophosphonate, phosphate, diphosphate, triphosphate, polyethyleneoxy or a physiologically functional derivative thereof; and

a pharmaceutically acceptable carrier.

B2. A composition of embodiment A1 wherein, in formula 1, R^1 and R^2 are independently selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_6-C_{20} arylalkyl, C_6-C_{20} substituted arylalkyl, acyloxymethyl esters —$CH_2C(=O)R^9$ and acyloxymethyl carbonates —$CH_2C(=O)OR^9$ where R^9 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl; and R^3R^4, R^5, R^6 and R^7 are independently H or C_1-C_6 alkyl.

C3. A composition of embodiment A1 wherein, in formula 2, B is cytosine or a 5-halocytosine.

D4. A composition of embodiment A1 wherein, in formula 1, R^1 and R^2 are independently selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_6-C_{20} arylalkyl, C_6-C_{20} substituted arylalkyl, acyloxymethyl esters —$CH_2C(=O)R^9$ and acyloxymethyl carbonates $CH_2C(=O)OR^9$ where R^9 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl and C_6-C_{20} substituted aryl; and R^3 R^4, R^5, R^6 and R^7 are independently H or C_1-C_6 alkyl; and, in formula 2, B is cytosine or a 5-halocytosine.

E5. A composition of embodiment D 4 wherein, in formula 1, R^1 and R^2 are independently selected from H, acyloxymethyl esters —$CH_2C(=O)R^9$ and acyloxymethyl carbonates $CH_2C(=O)OR^9$ where R^9 is C_1-C_6 alkyl; and R^3, R^4, R^5, R^6 and R^7 are independently H or C_1-C_6 alkyl; and, in formula 2, B is cytosine or a 5-halocytosine and R is H.

F6. A composition of embodiment E5 wherein, in formula 1, R^1 and R^2 are independently selected from H and —$CH_2C(=O)OCH(CH_3)_2$; R^3 is —CH_3; and R^4, R^5, R^6 and R^7 are H; and, in formula 2, B is 5-fluorocytosine and R is H.

G7. A pharmaceutical composition comprising a pharmaceutically effective amount of [2-(6-amino-purin-9-yl)-1-methyl-ethoxymethyl]-phosphonic acid diisopropoxycarbonyloxymethyl ester fumarate (tenofovir disoproxil fumarate) or a physiologically functional derivative thereof and a pharmaceutically effective amount of (2R,5S)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine) or a physiologically functional derivative thereof; and a pharmaceutically acceptable carrier.

H8. A pharmaceutical formulation of embodiment A1 to G7 further comprising a third active ingredient selected from the group consisting of a protease inhibitor, a nucleoside or nucleotide reverse transcriptase inhibitor, a non-nucleoside reverse transcriptase inhibitor, and an integrase inhibitor.

I9. A pharmaceutical formulation of embodiments A1 to H8 in unit dosage form.

J10. A method for the treatment or prevention of the symptoms or effects of an HIV infection in an infected animal which comprises administering to said animal a pharmaceutical composition of embodiments claims A1 to 19.

We claim:

1. A chemically stable fixed-dose combination comprising 300 mg of tenofovir disoproxil fumarate and 200 mg of emtricitabine wherein the combination exhibits less than 10% degradation of the tenofovir disoproxil fumarate and emtricitabine after six months at 40° C./75% relative humidity when packaged and stored with silica gel desiccant at 40° C./70% relative humidity.

2. The chemically stable combination of claim **1** in the form of a pharmaceutical dosage form.

3. The chemically stable combination of claim **2** wherein the dosage form is oral.

4. The pharmaceutical dosage form of claim **2** wherein the tenofovir disoproxil fumarate is not substantially degraded.

5. The pharmaceutical dosage form of claim **4** where there is less than 10% degradation of tenofovir disoproxil fumarate over a 24-hour period.

6. The pharmaceutical dosage form of claim **4** where there is less than 1% degradation of tenofovir disoproxil fumarate over a 24-hour period.

7. The pharmaceutical dosage form of claim **4** where there is less than 0.1% degradation of tenofovir disoproxil fumarate over a 24-hour period.

8. The pharmaceutical dosage form of claim **4** where there is less than 0.01% degradation of tenofovir disoproxil fumarate over a 24-hour period.

9. The pharmaceutical dosage form of claim **2** wherein less than 5% degradation of the tenofovir disoproxil fumarate and emtricitabine occurs after six months.

10. A chemically stable fixed-dose combination comprising 300 mg of tenofovir disoproxil fumarate and 200 mg of emtricitabine wherein the combination exhibits less than 10% degradation of tenofovir disoproxil fumarate over a 24-hour period.

11. The chemically stable combination of claim **10**, in the form of a pharmaceutical dosage form.

12. The chemically stable combination of claim **11**, wherein the dosage form is oral.

13. The pharmaceutical dosage form of claim **11**, wherein there is less than 1% degradation of tenofovir disoproxil fumarate.

14. The pharmaceutical dosage form of claim **11**, wherein there is less than 0.1% degradation of tenofovir disoproxil fumarate.

15. The pharmaceutical dosage form of claim **11**, wherein there is less than 0.01% degradation of tenofovir disoproxil fumarate.

16. The pharmaceutical dosage form of claim **11**, wherein the combination exhibits less than 10% degradation of the tenofovir disoproxil fumarate and emtricitabine after six months at 40° C./75% relative humidity when packaged and stored with silica gel desiccant at 40° C./70% relative humidity.

17. The pharmaceutical dosage form of claim **11** wherein the combination exhibits less than 5% degradation of the tenofovir disoproxil fumarate and emtricitabine after six months at 40° C./75% relative humidity when packaged and stored with silica gel desiccant at 40° C./70% relative humidity.

18. The pharmaceutical dosage form of claim **2** or **11** comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, pregelatinized starch, croscarmellose sodium, lactose monohydrate, microcrystalline cellulose, and magnesium stearate.

19. The pharmaceutical dosage form of claim **18** comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, 50 mg pregelatinized starch, 60 mg croscarmellose sodium, 80 mg lactose monohydrate, 300 mg microcrystalline cellulose, and 10 mg magnesium stearate.

20. The pharmaceutical dosage form of claim **18** comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, 50 mg pregelatinized starch, 60 mg croscarmellose sodium, 180 mg lactose monohydrate, 200 mg microcrystalline cellulose, and 10 mg magnesium stearate.

21. The pharmaceutical dosage form of claim **2** or **11** comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, pregelatinized starch, croscarmellose sodium, lactose monohydrate, microcrystalline cellulose, magnesium stearate, and colloidal silicon dioxide.

22. The pharmaceutical dosage form of claim **21** comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, 50 mg pregelatinized starch, 60 mg croscarmellose sodium, 175 mg lactose monohydrate, 200 mg microcrystalline cellulose, 10 mg magnesium stearate, and 5 mg colloidal silicon dioxide.

23. The pharmaceutical dosage form of claim **21** comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, hydroxypropyl methylcellulose, lactose B.P., pregelatinized starch B.P, and magnesium stearate.

24. The pharmaceutical dosage form of claim **21** comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, 112 g hydroxypropyl methylcellulose, 53 mg lactose B.P., 28 mg pregelatinized starch B.P, and 7 mg magnesium stearate.

25. The pharmaceutical dosage form of claim **2** or **11** comprising less than 1% of impurities related to tenofovir disoproxil fumarate and emtricitabine.

26. The pharmaceutical dosage form of claim **2** or **11**, further comprising a third anti-viral agent.

27. The pharmaceutical dosage form of claim **26**, wherein the third antiviral agent is selected from the group consisting of protease inhibitors, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and integrase inhibitors.

28. The pharmaceutical dosage form of claim **27**, wherein the third antiviral agent is a protease inhibitor.

29. The pharmaceutical dosage form of claim **27**, wherein the third antiviral agent is a nucleoside reverse transcriptase inhibitor.

30. The pharmaceutical dosage form of claim **27**, wherein the third antiviral agent is a non-nucleoside reverse transcriptase inhibitor.

31. The pharmaceutical dosage form of claim **27**, wherein the third antiviral agent is an integrase inhibitor.

32. The pharmaceutical dosage form of claim **30**, wherein the third antiviral agent is efavirenz.

33. A method for the treatment of the symptoms or effects of an HIV infection in an infected animal which comprises administering to said animal the pharmaceutical dosage form of claim **2** or **11**.

34. A method for the treatment of the symptoms or effects of an HIV infection in an infected animal which comprises administering to said animal the pharmaceutical dosage form of claim **9** or **13**.

35. A method for the treatment of the symptoms or effects of an HIV infection in an infected animal which comprises administering to said animal the pharmaceutical dosage form of claim **21**.

36. A method for the treatment of the symptoms or effects of an HIV infection in an infected animal which comprises administering to said animal the pharmaceutical dosage form of claim **26**.

37. A method for the treatment of the symptoms or effects of an HIV infection in an infected animal which comprises administering to said animal the pharmaceutical dosage form of claim **27**.

38. A method for the treatment of the symptoms or effects of an HIV infection in an infected animal which comprises administering to said animal the pharmaceutical dosage form of claim **32**.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 8,716,264 B2
APPLICATION NO. : 12/204174
DATED : May 6, 2014
INVENTOR(S) : Dahl 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

Claim 1, Column 30, lines 51-52:

Delete "at 40° C./70% relative humidity."

Claim 16, Column 31, lines 29-30:

Delete "at 40° C./70% relative humidity."

Claim 17, Column 31, lines 35-36:

Delete "at 40° C./70% relative humidity."

Signed and Sealed this
Tenth Day of February, 2015

Michelle K. Lee

Michelle K. Lee
Deputy Director of the United States Patent and Trademark Office

EXHIBIT E

US009457036B2

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

(10) **Patent No.: US 9,457,036 B2**
(45) **Date of Patent: *Oct. 4, 2016**

(54) **COMPOSITIONS AND METHODS FOR COMBINATION ANTIVIRAL THERAPY**

(71) Applicant: **Gilead Sciences, Inc.**, Foster City, CA (US)

(72) Inventors: **Terrence C. Dahl**, Sunnyvale, CA (US); **Mark M. Menning**, San Francisco, CA (US); **Reza Oliyai**, Foster City, CA (US)

(73) Assignee: **Gilead Sciences, Inc.**, Foster City, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 59 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **14/523,758**

(22) Filed: **Oct. 24, 2014**

(65) **Prior Publication Data**

US 2015/0111855 A1 Apr. 23, 2015

Related U.S. Application Data

(63) Continuation of application No. 14/227,653, filed on Mar. 27, 2014, which is a continuation of application No. 12/204,174, filed on Sep. 4, 2008, now Pat. No. 8,716,264, which is a continuation of application No. 10/540,794, filed as application No. PCT/US2004/000832 on Jan. 13, 2004, now abandoned.

(60) Provisional application No. 60/440,246, filed on Jan. 14, 2003, provisional application No. 60/440,308, filed on Jan. 14, 2003.

(51) **Int. Cl.**

A01N 43/54	(2006.01)
A61K 31/505	(2006.01)
A61K 31/675	(2006.01)
A61K 31/513	(2006.01)
A61K 31/7076	(2006.01)
A61K 45/06	(2006.01)
A61K 31/683	(2006.01)
A61K 9/20	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 31/675*** (2013.01); ***A61K 9/2009*** (2013.01); ***A61K 9/2013*** (2013.01); ***A61K 9/2018*** (2013.01); ***A61K 9/2054*** (2013.01); ***A61K 9/2059*** (2013.01); ***A61K 31/513*** (2013.01); ***A61K 31/683*** (2013.01); ***A61K 31/7076*** (2013.01); ***A61K 45/06*** (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,524,846	A	8/1970	Moffatt et al.
3,622,677	A	11/1971	Short et al.
3,682,930	A	8/1972	Bourquin et al.
3,994,974	A	11/1976	Murakami et al.
4,003,878	A	1/1977	Skaar et al.
4,258,062	A	3/1981	Jonas et al.
4,355,032	A	10/1982	Verheyden et al.
4,384,005	A	5/1983	McSweeney
4,430,343	A	2/1984	Iemura et al.
4,476,248	A	10/1984	Gordon et al.
4,724,233	A	2/1988	De Clercq et al.
4,808,716	A	2/1989	Holy et al.
4,816,570	A	3/1989	Farquhar
4,879,288	A	11/1989	Warawa et al.
4,935,507	A	6/1990	Takaya et al.
4,957,924	A	9/1990	Beauchamp
4,968,788	A	11/1990	Farquhar
5,047,407	A	9/1991	Belleau et al.
5,075,445	A	12/1991	Jarvest et al.
5,142,051	A	8/1992	Holy et al.
5,151,426	A	9/1992	Belleau et al.
5,155,268	A	10/1992	Hester

(Continued)

FOREIGN PATENT DOCUMENTS

EP	0 182 024 A2	5/1986
EP	0 206 459 A2	12/1986

(Continued)

OTHER PUBLICATIONS

Gild—Gilead Sciences Conference Call to Discuss Triangle Pharmaceuticals Acquisition dated Dec. 4, 2002, Ristig et al. (Tenofovir Disoproxil Fumarate, TDF) Therapy for Chronic Hepatitis B in Human Immunodeficiency Virus/Hepatitis B Virus—Coinfected Individuals for Whom Interferon-alpha and Lamivudine Therapy Have Failed, JID 2002; 186 pp. 1844-1847.*

Memorandum Opinion and Order Construing Patent Claims, signed by Judge Irene M. Keeley, Case No. 1:14-cv-00099-IMK (Dated: May 12, 2015), 44 pages.

(Continued)

Primary Examiner — Alton Pryor

(74) *Attorney, Agent, or Firm* — Cooley LLP

(57) **ABSTRACT**

The present invention relates to therapeutic combinations of [2-(6-amino-purin-9-yl)-1-methyl-ethoxymethyl]-phosphonic acid diisopropoxycarbonyloxymethyl ester (tenofovir disoproxil fumarate, Viread®) and (2R,5S,cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine, Emtriva™, (−)-cis FTC) and their physiologically functional derivatives. The combinations may be useful in the treatment of HIV infections, including infections with HIV mutants bearing resistance to nucleoside and/or non-nucleoside inhibitors. The present invention is also concerned with pharmaceutical compositions and formulations of said combinations of tenofovir disoproxil fumarate and emtricitabine, and their physiologically functional derivatives, as well as therapeutic methods of use of those compositions and formulations.

16 Claims, No Drawings

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,177,064	A	1/1993	Bodor	
5,179,104	A	1/1993	Chu et al.	
5,204,466	A	4/1993	Liotta et al.	
5,208,221	A	5/1993	Kim et al.	
5,210,085	A	5/1993	Liotta et al.	
5,386,030	A	1/1995	Kim et al.	
5,432,172	A	7/1995	Kashman et al.	
5,453,503	A	9/1995	Aikins et al.	
5,466,806	A	11/1995	Belleau et al.	
5,476,938	A	12/1995	Vemishetti et al.	
5,486,520	A	1/1996	Belleau et al.	
5,506,347	A	4/1996	Erion et al.	
5,512,596	A	4/1996	Kim et al.	
5,514,557	A	5/1996	Moghaddam	
5,514,798	A	5/1996	Bischofberger et al.	
5,519,021	A	5/1996	Payne et al.	
5,538,975	A	7/1996	Dionne	
5,587,480	A	12/1996	Belleau et al.	
5,618,820	A	4/1997	Dionne	
5,618,964	A	4/1997	Cheng et al.	
5,627,186	A	5/1997	Cameron et al.	
5,663,159	A	9/1997	Starrett, Jr. et al.	
5,696,254	A	12/1997	Mansour et al.	
5,733,788	A	3/1998	Bischofberger	
5,744,596	A	4/1998	Mansour et al.	
5,756,706	A	5/1998	Mansour et al.	
5,763,606	A	6/1998	Mansour et al.	
5,792,756	A	8/1998	Starrett, Jr. et al.	
5,798,340	A	8/1998	Bischofberger et al.	
5,814,639	A	9/1998	Liotta et al.	
5,859,021	A	1/1999	Cameron et al.	
5,905,082	A	5/1999	Roberts et al.	
5,914,331	A	6/1999	Liotta et al.	
5,922,695	A	7/1999	Arimilli et al.	
5,935,946	A	8/1999	Munger, Jr. et al.	
5,977,089	A	11/1999	Arimilli et al.	
6,043,230	A	3/2000	Arimilli et al.	
6,057,305	A	5/2000	Holy et al.	
6,069,249	A	5/2000	Arimilli et al.	
6,069,252	A	5/2000	Liotta et al.	
6,113,920	A	9/2000	Maye et al.	
6,114,343	A	9/2000	Liotta et al.	
6,121,315	A	9/2000	Nair et al.	
6,180,639	B1	1/2001	Coates et al.	
6,194,391	B1	2/2001	Schinazi et al.	
6,312,662	B1	11/2001	Erion et al.	
6,417,191	B1	7/2002	Barry et al.	
6,639,071	B2	10/2003	Thompson et al.	
RE38,333	E	11/2003	Arimilli et al.	
6,642,245	B1	11/2003	Liotta et al.	
6,703,396	B1	3/2004	Liotta et al.	
6,812,233	B1	11/2004	Liotta	
6,939,964	B2	9/2005	Thompson et al.	
7,094,413	B2	8/2006	Buelow et al.	
7,795,217	B2	9/2010	Adra	
7,851,165	B2	12/2010	Fuhrmann et al.	
8,067,473	B2	11/2011	Alchanati et al.	
8,592,397	B2	11/2013	Dahl et al.	
8,598,185	B2 *	12/2013	Dahl et al.	514/263.32
8,716,264	B2 *	5/2014	Dahl et al.	514/81
8,871,271	B2	10/2014	Dahl et al.	
2001/0012518	A1	8/2001	Makooi-Morehead et al.	
2001/0014352	A1	8/2001	Batra et al.	
2003/0203969	A1	10/2003	Bevec et al.	
2004/0180089	A1	9/2004	Plachetka et al.	
2004/0224917	A1	11/2004	Dahl et al.	
2004/0253218	A1	12/2004	Eisenbach-Schwartz et al.	
2005/0197320	A1	9/2005	Chen et al.	
2006/0128692	A1	6/2006	Chen et al.	
2006/0246130	A1	11/2006	Dahl et al.	
2007/0036861	A1	2/2007	Oury et al.	
2007/0077295	A1	4/2007	Dahl et al.	
2007/0099902	A1	5/2007	Dahl et al.	
2009/0036408	A1	2/2009	Dahl et al.	
2009/0143314	A1	6/2009	Dahl et al.	
2014/0037732	A1	2/2014	Dahl et al.	
2014/0213556	A1	7/2014	Dahl et al.	
2014/0370102	A1	12/2014	Dahl et al.	

FOREIGN PATENT DOCUMENTS

EP	0 269 947	A1	6/1988
EP	0 369 409	A1	5/1990
EP	0 382 526	A2	8/1990
EP	0 481 214	A1	4/1992
EP	0 482 657	A2	4/1992
EP	0 595 635		5/1994
EP	0 632 048	A1	1/1995
EP	0 647 649	A1	4/1995
EP	0 694 547	A2	1/1996
EP	1 256 585	A1	11/2002
EP	1 332 757	A1	8/2003
GB	942 152	A	11/1963
GB	1 523 865	A	9/1978
GB	2 111 043	A	6/1983
WO	WO 88/05438		7/1988
WO	WO 91/19721	A1	12/1991
WO	WO 92/01698		2/1992
WO	WO 92/09611	A1	6/1992
WO	WO 92/13869		8/1992
WO	WO 92/14743	*	9/1992
WO	WO 92/14743	A2	9/1992
WO	WO 93/03027	A1	2/1993
WO	WO 94/03466		2/1994
WO	WO 94/03467	A2	2/1994
WO	WO 95/07919		3/1995
WO	WO 95/07920	A1	3/1995
WO	WO 95/32957	A1	12/1995
WO	WO 96/18605	A1	6/1996
WO	WO 98/04569	A1	2/1998
WO	WO 99/25352	A1	5/1999
WO	WO 99/61026		12/1999
WO	WO 00/16755		3/2000
WO	WO 00/25797	A1	5/2000
WO	WO 00/64427	A2	11/2000
WO	WO 01/64221	A1	9/2001
WO	WO 02/08241	A2 *	1/2002
WO	WO 02/062123	A2	8/2002
WO	WO 02/068058	A2	9/2002
WO	WO 02/070518	A1	9/2002
WO	WO 03/045327	A2	6/2003
WO	WO 03/059327		7/2003
WO	WO 2004/052296	A2	6/2004
WO	WO 2004/064845	A1	8/2004
WO	WO 2005/021001	A1	3/2005
WO	WO 2006/135933	A2	12/2006
WO	WO 2006/135993	A2	12/2006
WO	WO 2007/068934	A2	6/2007
WO	WO 2012/068535		5/2012

OTHER PUBLICATIONS

Plaintiffs' Motion to Compel Defendants to Produce Documents, Case No. 1:14-cv-00099-IMK (Filed: May 28, 2015), 9 pages.

Plaintiffs' Response to Defendants' Pretrial Memrorandum filed by Bristol-Meyers, Squibb Company, Merck, Sharp &Dohme Corp., Case No. 1:10-cv-01851-RJS, (Filed May 28, 2013), 19 pages.

Affidavit of Paul A. Bartlett, Ph.D. filed by Emory University, Defendants' Memorandum of Law in Oppositon to Plaintiffs' Opening Pretrial Brief ffiled by Teva Pharmaceutical Industries, Ltd., Teva Pharmaceuticals USA, Inc., Case No. 1:10-cv-01851-RJS, (Filed May 28, 2013), 18 pages.

Stipulation and Order of Dismissal, Case No. 1:10-cv-01851-RJS (Dated Aug. 16, 2013), 2 pages.

Affidavit of Calvin J. Cohen, M.D. filed by Emory University, Gilead Sciences, Inc., Case No. Case: 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 41 pages. Gilead Sciences, Inc.,Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 39 pages.

Affidavit of Carlo-Federico Perno, M.D., Ph.D. filed by Emory University, Gilead Sciences, Inc.,Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 50 pages.

(56) **References Cited**

OTHER PUBLICATIONS

Affidavit of Dennis C. Liotta, Ph.D. filed by Emory University, Gilead Sciences, Inc.,Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 31 pages.
Affidavit of Judi Weissinger, Ph.D. filed by Emory University, Gilead Sciences, Inc.,Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 13 pages.
Affidavit of Stephen G. Davies, Ph.D. filed by Emory University, Gilead Sciences, Inc.,Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 42 pages.
Affidavit of Daniel C. Smith, Ph.D. filed by Emory University, Gilead Sciences, Inc.,Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 31.
Affidavit of James Meyers filed by Emory University, Gilead Sciences, Inc.,Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 15.
Plaintiffs' Pretrial Memorandum filed by Emory University, Gilead Sciences, Inc.,Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 60 pages.
Plaintiffs' Proposed Findings of Fact and Conclusions of Law, filed by Emory University, Gilead Sciences, Inc.,Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 85 pages.
Declaration of Harry Boghigian filed by Teva Pharmaceutical Industries Limited, Teva Pharmaceuticals USA, Inc., Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 17 pages.
Declaration of Stanley Roberts, Ph.D., filed by Teva Pharmaceutical Industries Limited, Teva Pharmaceuticals USA, Inc., Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 78 pages.
Defendants' Pretiral Memorandum filed by Teva Pharmaceutical Industries Limited, Teva Pharmaceuticals USA, Inc., Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 47 pages.
Defendants' Proposed Findings of Fact and Conclusions of Law filed by Teva Pharmaceutical Industries Limited, Teva Pharmaceuticals USA, Inc., Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 68 pages.
Affidavit of Dennis C. Liotta,Ph.D. filed by Emory University, Gilead Sciences, Inc. Case No. 1:08-cv-10838-RJS (Filed Sep. 19, 2013), 31 pages.
Affidavit of Stephen G. Davies, Ph.D. filed by Emory University, Gilead Sciences, Inc. Case No. 1:08-cv-10838-RJS (Filed Sep. 19, 2013), 42.
Plaintiffs' Pretrial Memorandum filed by Emory University, Gilead Sciences, Inc. Case No. 1:08-cv-10838-RJS (Filed Sep. 19, 2013), 60 pages.
Plaintiffs' Proposed Findings of Fact and Conculsions of Law filed by Emory University, Gilead Sciences, Inc. Case No. 1:08-cv-10838-RJS (Filed Sep. 19, 2013), 85 pages.
Defendants' Memorandum of Law in Opposition to Plantiffs' Pretrial Memorandum filed by Teva Pharmaceutical Industries Limited, Teva Pharmaceuticals USA, Inc., Case No. 1:08-cv-10838-RJS (Filed Sep. 23, 2013), 31 pages.
Plaintiffs' Opposition to Teva's Pretrial Memorandum, filed by Emory University, Gilead Sciences, Inc. Case No. 1:08-cv-10838-RJS (Filed Sep. 23, 2013), 41 pages.
Amendend Declaration of Stanley Roberts, Ph.D. filed by Teva Pharmaceutical Industries Limited, Teva Pharmaceuticals USA, Inc., Case No. 1:08-cv-10838-RJS (Filed Oct. 4, 2013), 77 pages.
Transcript of Proceedings regarding Trial held on Oct. 8, 2013 before Judge Richard J. Sullivan, Case No. 1:08-cv-10838-RJS (Dated Oct. 21, 2013), 222 pages.
Transcript of Proceedings regarding Trial held on Oct. 9, 2013 before Judge Richard J. Sullivan, Case No. 1:08-cv-10838-RJS (Dated Oct. 21, 2013), 220 pages.
Transcript of Proceedings regarding Trial held on Oct. 10, 2013 before Judge Richard J. Sullivan, Case No. 1:08-cv-10838-RJS (Dated Oct. 21, 2013), 38 pages.
Transcript of Proceedings regarding Trial held on Oct. 28, 2013 before Judge Richard J. Sullivan, Case No. 1:08-cv-10838-RJS (Dated Nov. 7, 2013), 67 pages.
Stipulated Statement of Corrections to Trial Transcript, Oct. 8-10, 28, 2013 filed by Emory University, Gilead Sciences, Inc. Case No. 1:08-cv-10838-RJS (Filed Dec. 4, 2013), 42 pages.
Plaintiffs' Post-Trial Brief filed by Emory University, Gilead Sciences, Inc. Case No. 1:08-cv-10838-RJS (Filed Dec. 4, 2013), 43 pages.
Defendants' Post-Trial Memorandum filed by Teva Pharmaceutical Industries Limited, Teva Pharmaceuticals USA, Inc., Case No. 1:08-cv-10838-RJS (Filed Dec. 4, 2013), 43 pages.
Defendants' Revised Proposed Findings of Fact and Conclusions of Law filed by Teva Pharmaceutical Industries Limited, Teva Pharmaceuticals USA, Inc., Case No. 1:08-cv-10838-RJS (Filed Dec. 4, 2013), 102 pages.
Plaintiffs' Proposed Findings of Fact and Conclusions of Law filed by Emory University, Gilead Sciences, Inc. Case No. 1:08-cv-10838-RJS (Dec. 5, 2013), 111 Pages.
Plaintiffs' Notice of PTX, A, B, and C (exhibits) in Support of Post-trial Memorandum regarding Post Trial Memorandum filed by Emory University, Gilead Sciences, Inc. Case No. 1:08-cv-10838-RJS, (Filed Dec. 5, 2013), 81 pages.
Plaintiffs' Opposition to Defendants' Post-Trial Memorandum filed by Emory University, Gilead Sciences, Inc. Case No. 1:08-cv-10838-RJS (Filed Dec. 18, 2013), 22 pages.
Defendants' Post-Trial Reply Memorandum filed by Teva Pharmaceutical Industries Limited, Teva Pharmaceuticals USA, Inc., Case No. 1:08-cv-10838-RJS (Filed Dec. 18, 2013), 20 pages.
Stipulation of Dismissal, Case No. 1:08-cv-10838-RJS (Dated Jan. 29, 2014), 3 pages.
Order, Case No. 1:08-cv-10838-RJS (Dated Feb. 13, 2014), 1 page.
Order on Stipulation for Dismissal, Case No. 1:08-cv-10838_RJS (Dated Apr. 30, 2014), 2 pages.
Staszewski, S., J. Gallant, A. Pozniak, J. M. A. H. Suleiman, E. DeJesus, E. Koenig, S. Coleman et al. "Efficacy and safety of tenofovir disoproxil fumarate (TDF) versus stavudine (d4T) when used in combination with lamivudine (3TC) and efavirenz (EFV) in HIV-1 infected patients naive to antiretroviral therapy (ART): 48-week interim results." Oral 17, 14th International AIDS Conference, Jul. 7-12, 2002 (poster).
Staszewski, S., J. Gallant, A. Pozniak, J. M. A. H. Suleiman, E. DeJesus, E. Koenig, S. Coleman et al. "Efficacy and safety of tenofovir disoproxil fumarate (TDF) versus stavudine (d4T) when used in combination with lamivudine (3TC) and efavirenz (EFV) in HIV-1 infected patients naive to antiretroviral therapy (ART): 48-week interim results of Study GS-99-903" Oral 17, 14th International AIDS Conference, Jul. 7-12, 2002 (presentation), 23 pages.
Declaration of Dr. Jonathan A V Coates in the matter Australian patent application 2011253996 in the name of Gilead Sciences, Inc. and in the matter of Opposition by Alphapharm, Jun. 2, 2015.
Declaration of Andrew David Carr in the matter Australian patent application 2011253996 in the name of Gilead Sciences, Inc. and in the matter of Opposition by Alphapharm, Jun. 2, 2015.
Declaration of Helen Grimes in the matter Australian patent application 2011253996 in the name of Gilead Sciences, Inc. and in the matter of Opposition by Alphapharm, Jun. 2, 2015.
Statement of Grounds and Particulars filed by Alphapharm Pty Ltd in the Australian Patent Office for Application No. 2011253996, dated Mar. 17, 2015, 12 pages.
Australian Product Information for EMTRIVA (emtricitabine) Capsules, Apr. 2014, 22 pages.
Australian Product Information for Viread® (tenofovir disoproxil fumarate) 300 mg Tablets, Oct. 2013, 44 pages.
Australian Product Information for 3TC® Tablets and Oral Solution , Feb. 2015, 26 pages.
Australian Product Information for REYATAZ® (atazanavir sulfate), Dec. 2014, 52 pages.
Australian Product Information for STOCRIN® (efavirenz), Mar. 2015, 29 pages.
Australian Product Information for KALETRA, Jan. 30, 2015, 39 pages.
Abstracts of the 10th Conference on Retroviruses and Opportunistic Infections Feb. 10-14, 2003, p. 10, Abstract 564b (A-564b), 5 pages.
Braunwald et al, Harrison's Principles of Internal Medicine (McGraw-Hill, 15th ed, 2001),pp. 1852-1913 (Harrison's).

(56) **References Cited**

OTHER PUBLICATIONS

Cadman et al GMHC Treat Issues vol. 12 No. 3, 1998, pp. 5-9 (1998).
Rudnic, E., Remington 20th Edition, Chapter 45, pp. 996-1035, (2000).
"AIDS," Monthly Index of Medical Specialties, pp. 194-198 (2002).
"Anti-HIV Drug Updates—Three Drugs on the Near Horizon," Project Inform Perspective 35:4-7 (2003).
"Atripla Fact Sheet", www.fda.gov, [Online], Jul. 12, 2006, pp. 1-2 retrieved from the internet www.fda.gov/cder/drug/inforpage/atripla/factsheet.htm [retrieved on Jan. 31, 2007.
"Gilead Buys Triangle in $464M Deal" Pharma Marketletter, 1 page (Dec. 9, 2002).
"Gilead Captures Triangle for $464 Million," Chemical Market Reporter 262(21):1 page (Dec. 9, 2002).
"Gilead set to acquire Triangle for $464m," BT Catalyst 17(1):1 page (Jan. 1, 2003).
"HIV Treatment Information," Project Inform, (on line), Jan. 2006, pp. 1-3, retrieved from http://www.projinf.org/bn/news_013006.html [retrieved on Jan. 31, 2007].
"Rescriptor," Patient Prescribing Information Leaflet, 7 pages (2001).
"Scientific Discussion," EMEA, pp. 1/28-3/28, European Medicines Agency: (Feb. 2005).
Adis Data Information, "Emtricitabine/Tenofovir Disoproxil Fumarate," Drugs in R & D 5(3):160-161 (2004).
Alexander et al., "Investigation of (Oxodioxolenyl)methyl carbamates as nonchiral bioreversible prodrug moieties for chiral amines," J. Med. Chem. 39:480-486, 1996.
Amended Answer and Counterclaim against Gilead Sciences, Inc. filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Jul. 20, 2009).
Anderson, "Antiviral dynamics and sex differences of zidovudine and lamivudine triphosphate concentrations in HIV-infected individuals," AIDS 17:2159-2168 (2003).
Ansel et al., "Pharmaceutical Dosage Forms and Rug Delivery Systems," 7th Edition, Lippincott Williams & Wilkins, pp. 209-213 (1999).
Answer and Counterclaim against Gilead Sciences, Inc. filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Feb. 5, 2009).
Answer filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (May 10, 2010).
Answer filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01851 (May 17, 2010).
Arimilli et al., "Orally bioavailable acylic nucleoside phosphonate prodrugs: Adefovir, Dipivoxil and Bis(POC)PMPA," vol. 3 (accepted for publication), Adv. Antiviral Drug Design, 1998.
Arimilli et al., "Synthesis, in vitro biological evaluation and oral bioavailability of 9-[2-(phosphonomethoxy)propyl]adenine (PMPA) prodrugs," Antiviral Chem. & Chemo. 8(6):557-567, 1997.
Arribas et al., "Tenofovir Disoproxil Fumarate, Entricitabine and Efavirenz Compared with Zidovudine/Lamivudine and Efavirenz in Treatment-Naive Patients 144-Week Analysis," JAIDS 47(1):74-78 (2008).
Balzarini et al, "Differential antiherpesvirus and antiretrovirus effects of the (S) and (R) enantiomers of acyclic nucleoside phosphonates: potent and selective in vitro and in vivo antiretrovirus activities of (R)-9-(2-phosphonomethoxypropyl)-2,6-diaminopurine." Antimicrob Agents Chemother. Feb. 1993; 37(2): 332-338.
Banker, Modem Pharmaceutics, Drugs and the Pharmaceutical Sciences, p. 340, Mercel Dekker, Inc. 1996.
Bartlett et al., "Overview of the effectiveness of triple combination therapy in antiretroviral-naive HIV-1 infected adults," AIDS 15:1369-1377 (2001).
Beauchamp et al., "Amino acid ester prodrugs of acyclovir," Antivir. Chem. and Chemoth. 3(3):157-64, 1992.
Benzaria et al., "New prodrugs of 9-(2-phosphonomethoxyethyl)adenine (PMEA): Synthesis and stability studies," Nucls. & Nuclt. 14(3-5):563-565, 1995.
Benzaria et al., "Synthesis, in Vitro Antiviral Evaluation, and Stability Studies of Bis(S-acy1-2-thioethyl) Ester Derivatives of 9[2-(Phosphonometyoxy)ethyl]adenine (PMEA) as Potential PMEA Prodrugs with Improved Oral Bioavailability," J. Med. Chem. 39:4958-4965 (1996).
Berge et al., "Pharmaceutical salts," J. Pharm. Sci. 66(1):1-19, 1977.
Blackburn et al., "DNA and RNA structure," pp. 15-81, Nucleic Acids in Chemistry and Biology, 1996.
Bristol Myers Squibb "Sustiva®," http://www.fda.gov/medwaTCH/SAFETY/2005/Sustiva_PI61005.pdf, pp. 3-40 (retrieved Jan. 31, 2007).
Bundgaard et al., "Design and Application of Prodrugs," pp. 113-191, Textbook of Drug Design and Development, 1991, 1 page.
Byrn (editor), Solid State Chemistry of Drugs, 2cd Edition, p. 22, 1999.
Colla et al., "Synthesis and antiviral activity of water-soluble esters of acyclovir [9-[(2-Hydroxyethoxy)methyl]guanine]," J. Med. Chem. 26:602-04, 1983.
Communication about intention to grant a European patent for Patent Publication EP 1583542 (Application No. 04701819.7) issued by the European Patent Office (Nov. 5, 2007).
Communication and Annex for Patent Publication EP 1583542 (Application No. 04701819.7) issued by the European Patent Office (Jul. 17, 2007).
Communication and Annex for Patent Publication EP 1583542 (Application No. 04701819.7) issued by the European Patent Office (Jul. 26, 2006).
Communication and Annex for Patent Publication EP 1583542 (Application No. 04701819.7) issued by the European Patent Office (Oct. 24, 2005).
Communication from the Examining Division of the EPO for EP 1890681 B1 (Application No. 06773194.3) (May 13, 2009).
Communication of further notices of opposition pursuant to Rule 79(2) EPC for EP 1583542 B1 (Application No. 04701819.7) and Request to File Observations (Apr. 23, 2009).
Communication of Intent to Grant EP 1890681 B1 (Application No. 06773194.3) and Druckexemplar issued by the European Patent Office (Jul. 15, 2008).
Communication of notices of opposition pursuant to Rule 79(2) EPC for EP 1890681 B1 (Application No. 06773194.3) and Request to File Observations (Nov. 12, 2009).
Communication pursuant to Article 94(3) EPC for Patent Publication EP 1923063 (Application No. 08152527.1) issued by the European Patent Office (Sep. 4, 2009).
Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc. and Emory University Case No. 10-CV-01798 (Mar. 5, 2010).
Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc. Case No. 08-CV-10838 (Dec. 12, 2008).
Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Mar. 5, 2010).
Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company. Case No. 10-CV-01851 (Mar. 9, 2010).
Conference Call Transcript—Gilead Sciences Conference call to Discuss Triangle Pharmaceuticals Acquisition. Event Date/Time Dec. 4, 2002/ 9:00 AM ET (11 pages).
Correction of an Error in the Decision According to Rule 140 EPC for EP 1890681 B1 (Application No. 06773194.3) (Jul. 5, 2011).
Dando et al., "Emtricitabine/Tenofovir Disoproxil Fumarite," Drugs 64(18):2075-2082 (2004).
Davidsen et al., "N-(Acyloxyalkyl)pyridinium salts as soluble prodrugs of a potent platelet activating factor antagonist," J. Med. Chem. 37(26):4423-4429, 1994.

(56) **References Cited**

OTHER PUBLICATIONS

De Clercq et al., "(S)-9-(2,3-dihydroxypropyl)adenine: An aliphatic nucleoside analog with broad spectrum antiviral activity," Science 200:563-565, 1978.
De Clercq, "Antiviral drugs: current state of the art," J. Clin. Virol. 22:73-89 (2001).
De Clercq et al., "New Developments in Anti-HIV Chemotherapy," Curr. Med. Chem. 8(13):1543-1572 (2001).
De Clercq et al., "New developments in anti-HIV chemotherapy," Farmaco 56(1-2):3-12 (2001).
De Clercq, "Highlights in the Development of New Antiviral Agents," Mini-Rev. Med. Chem. 2(2):163-175 (2002).
De Clercq, "New developments in anti-HIV chemotherapy," Biochem Biophys Acta 1587(2-3):258-275 (2002).
De Lombaert et al., "N-Phosphonomethyl Dipeptides and their Phosphonate Prodrugs, a New Generation of Neutral Endopeptidase (NEP, EC 3.4.24.11) Inhibitor," J. Med. Chem. 37:498-511 (1994).
Decision of Hearing of the Indian Patent Office for Patent Application No. 3383/DELNP/2005 (Mar. 25, 2009).
Decision of Rejection for Application No. 7000806/1999 issued by the Korean Intellectual Property Office (Jan. 20, 2006) (translation).
Decision of Rejection for Patent Application No. 7000636/2000 issued by the Korean Intellectual Property Office (May 10, 2006) (translation).
Decision of Rejection for Patent Application No. 7007002/2008 issued by the Korean Intellectual Property Office (Jan. 7, 2009) (translation).
Decision of Rejection for Patent Application No. 86110757 issued by the Intellectual Property Office of Taiwan (Nov. 11, 1999) (translation).
Decision of Rejection for Patent Application No. 87112168 issued by the Taiwanese Intellectual Property Office (Apr. 6, 2001) (translation).
Decision of the Opposition Division for EP Patent EP 1583542 B1 (Application No. 04701819.7), Claims, Grounds for the Decision and Provision of the minutes (Jan. 31 and Feb. 14, 2011).
Decision to Grant Patent Application No. 06773194.3 (EP 1890681B1) issued by the European Patent Office (Dec. 11, 2008).
Declaration of Colleen Tracy in Support of a Motion for Leave to File Amended Complaint Case No. 10-CV-01796 (Apr. 11, 2011).
Declaration of James Galbraith in Opposition of a Motion for Leave to File Amended Complaint Case No. 10-CV-01796 (Apr. 25, 2011).
Delehanty et al. Slides from the oral presentation for "A Randomized Study of Three Doses of FTC Versus 3TC in HIV-Infected Patients," 6th CROI (Jan. 31-Feb. 4, 1999) Chicago.
Delehanty et al., "A Phase I/II Randomized, Controlled Study of FTC Versus 3TC in HIV-Infected Patients," 6th CROI (Jan. 31-Feb. 4, 1999) Chicago, Session 5, Abstract 16.
Drugs and the Pharmaceutical Sciences, vol. 1999, p. 60 (Mark Gibson, ed), 2009.
Engel, R., "Phosphonates as analogues of natural phosphates," Chem. Rev. 77(3):349-367, 1977.
Examination Report for Patent Application No. 540728 issued by the Intellectual Property Office of New Zealand (Apr. 24, 2007).
Examination Report Patent Application No. 564045 issued by the Intellectual Property Office of New Zealand (Oct. 6, 2009).
Examiner's First Report on Patent Application No. 2009200414 issued by the Australian Patent Office (Feb. 24, 2010).
Examiner's First Report on Patent Application No. 85827/98 issued by the Australian Patent Office (Feb. 28, 2001).
Examiner's First Report Patent Application No. 20026257795 issued by the Australian Patent Office (Sep. 29, 2009).
Examiner's Remarks for Patent Application No. a 2008 00493 issued by the Ukrainian Patent Office (2010).
Examiner's Second Report on Patent Application No. 85827/98 issued by the Australian Patent Office (Mar. 27, 2002).
Examiner's First Report on Patent Application No. 2004206821 issued by the Australian Patent office (Aug. 28, 2007).
Examiner's Remarks for Patent Application No. 200501134/28 issued by the Eurasian Patent Office (received Jul. 30, 2009) (translation).
Examiner's Second Report on Patent Application No. 2004206821 issued by the Australian Patent office (Aug. 20, 2008).
Extended European Search Report for Patent Publication EP 1923063 (Application No. 08152527.1) issued by the European Patent Office (Mar. 10, 2009).
Farquhar et al., "Biologically Reversible Phosphate-Protective Groups," J. Pharm. Sci. 72:324-325 (1983).
Farquhar et al., "Synthesis and antitumor evaluation of Bis[(pivaloxy)methyl] 2'-deoxy-5-fluorouridine 5'-monophosphate (FdUMP): a strategy to introduce nucleotides into cells," J. Med. Chem. 37(23):3902-03, 1994.
Fasman et al., pp. 385-394, Practical Handbook of Biochem. and Molec. Biol., 1989.
FDA: "Guidance for industry fixed dose combination and co-packaged drug products for treatment of HIV," www.fda.org. [on line], May 2004, pp. 1-17, (retrieved from http://www.fda.gov/oc/initiatives/hiv/hivguidance.html>[retrieved on Jan. 31, 2007].
Fell et al., "The tensile strength of lactose tablets" J. Pharm. Pharmacol. 20:657-659 (1968).
Feng et al. 2009 "The triple combination of tenofovir, emtricitabine and efavirenz show synergistic anti-HIV-1 activity in vitro: a mechanism of action study," Retrovirology 6:44, http://www.retrovirology.com/content/6/1/44.
Feng, J. et al, "Mechanistic studies show that 9-)-FTC-TP is a better inhibitor of HIV-1 reverse transcriptase than 3TC-TP," FASEB 13:1511-1517 (1999).
Final Office Action for Patent Application No. 86110757 issued by the Intellectual Property Office of Taiwan (Sep. 5, 2000) (translation).
Final Office Action for Patent Application. No. 89123708 issued by the Intellectual Property Office of Taiwan (Apr. 12, 2001) (translation).
First Amended Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Jul. 1, 2009).
First Examination Report for Application No. 564102 Issued by the Intellectual Property Office of New Zealand (Oct. 6, 2009).
First Examination Report for Patent Application No. 3383/DELNP/2005 from the Indian Patent Office (Jul. 31, 2007).
First Examination Report for Patent Application No. 602/DEL/2007 issued by the Indian Patent Office (Nov. 18, 2009).
First Office Action for Patent Application No. 200410046290.X issued by the Chinese Patent Office (Jun. 17, 2005) (translation).
First Office Action for Patent Application No. 200480002190.5 issued by the Patent Office of the People's Republic of China (Aug. 4, 2006).
First Office Action for Patent Application No. 200510099916.8 issued by the Chinese Patent Office (Jun. 15, 2007) (translation).
First Office Action for Patent Application No. 97197460.8 issued by the Chinese Patent Office (Jan. 16, 2004) (translation).
First Official Action for Patent Application No. a 2008 00555 issued by the Ukrainian Patent Office (2011) (translation).
Fiske et al., "Pharmacokinetics, safety and tolerability of single escalating doses of DMP 266, an HIV non-nucleoside reverse transcriptase inhibitor, in healthy volunteers," Pharm. Res. 14(11 Suppl.):S609 (1997).
Flaherty et al., "Synthesis and selective monoamine oxidase B-inhibiting properties of 1-Methyl-1,2,3,6-tetrahydropyrid-4-yl carbamate derivatives: potential prodrugs of (R)-and (S)-Nordeprenyl," J. Med. Chem. 39:4759-4761, 1996.
Folkmann et al., "Acyloxymethyl carbonochloridates. New intermediates in prodrug synthesis," Synthesis, pp. 1159-1166, 1990.
Frampton et al., "Emtricitabine: A Review of Its Use in the Management of HIV Inspection," Drugs 65(10):1427-1448 (2005).
Freeman et al.., "3 Prodrug Design for Phosphate and Phosphonates," Progress in Medicinal Chemistry 34:112-147 (1997).
Fridland, "Tenofovir," Curr. Opin. Anti-Infect. Invest. Drugs 2(3):295-301 (2000).

(56) **References Cited**

OTHER PUBLICATIONS

Fung et al., "Tenofovir Disoproxil Fumarate: A Nucleotide Reverse Transcriptase Inhibitor for the Treatment of HIV Infection," Clin. Therapeutics 24(10):1515-1548 (2002).
Further Examination Report for Patent Application No. 540728 issued by the Intellectual Property Office of New Zealand (Feb. 11, 2008).
Further Examination Report for Patent Application No. 540728 issued by the Intellectual Property Office of New Zealand (Jun. 18, 2008).
Generics [UK] Limited, Notice of Opposition of EP Patent EP 1583542B1 (Application No. 04701819.7) (Mar. 18, 2009).
Generics [UK] Limited, Written Submission in preparation for Oral Proceedings for EP Patent EP 1583542 B1 (Application No. 04701819.7) (Sep. 17, 2010).
Gerhartz (editor) Ullmann's Encyclopedia of Industrial Chemistry vol. B2, Unit Operations I, 5th Edition, p. 3-7.
Gilead "Truvada®," http://www.fda.gov/medwatch/SAFETY/2005/Oct_PI/Truvada_PI.pdf, pp. 1-29 (retrieved Jan. 31, 2007).
Gilead Sciences Inc., Appeal Grounds against the Decision of the Opposition Division for EP 1890681 B1 (Application No. 06773194.3) (Oct. 17, 2011).
Gilead Sciences Inc., Notice of Appeal against the Decision of the Opposition Division for EP 1890681 B1 (Application No. 06773194.3) (Aug. 16, 2011).
Gilead Sciences Inc., Written Submission in preparation for Oral Proceedings for the Opposition of EP 1890681 B1 (Application No. 06773194.3) (Feb. 4, 2011).
Gilead Sciences Inc., Written Submission in preparation for Oral Proceedings for the Opposition of EP 1890681 B1 (Application No. 06773194.3) (Mar. 2 & 4, 2011).
Gilead Sciences Inc., Written Submission in preparation to/during Oral Proceedings for EP Patent EP 1583542 B1 (Application No. 04701819.7) (Sep. 17, 2010).
Gilead Sciences, Inc., "Data Comparing Viread (R) and Emtriva (R) to Combivir (R) as Part of Combination HIV Therapy Published in New England Journal of Medicine," p. 1-5, Press Release, 2006.
Gilead Sciences, Inc., "Gilead Sciences to Acquire Triangle Pharmaceuticals for $464 Million; Gilead to Launch Coviracil in 2003; Will Develop Co-Formulation of Viread and Coviracil," 2 pages, Press Release (2002).
Gilead Sciences, Inc., "U.S. FDA Approves Gilead Sciences' Emtriva A one-capsule, Once-Daily Medication for the Treatment of HIV," pp. 3-7, Press Release, 2003.
Gilead Sciences, Inc., Physician Insert for Truvada, pp. 1-30 (2007).
Gilead, Bristol-Myers Squibb "Atripla™," http://www.fda.gov/cder/foi/;abe;/2006/0219371b1.pdf, pp. 4-53 (retrieved Jan. 31, 2007).
Gilead: "Bristol-Myers Squibb and Gilead announce data supporting bioequivalence for single PIII fixed dose regimen of Sustiva®(efavirenz) and Truvada® (emtricitabine and tenofovir fumarate)" Gilead Press Release (online Jan. 9, 2006), pp. 1-5, retrieved from http://www.gilead.com/press.
Gilead: "Gilead provides update on development of fixed-dose regimen of Truvada (emtricitabine and tenofovir disoproxil fumarate) and Sustiva (efavirenz)," Gilead Press Release, [on line], Apr. 26, 2005, pp. 1-3, retrieved from http://www.gilead.com/press.
Hammer et al., "Ether, carbonate and urethane deoxynucleoside derivatives as prodrugs," Acta Chemica Scandinavia 50:609-622, 1996.
Harris et al., "Genotypic Analysis of HIV-1 Infected ART Naive Patients Receiving Emtricitabine (FTC) or Lamivudine (3TC) in a Double Blind Equivalence Trial," 5th International Workshop on Drug Resistance and Treatment Strategies, Jun. 4-8, No. 104 (2001).
Havlir et al., "In Vivo Antagonism with Zidovudine plus Stavudine Combination Therapy," J. Infect. Disease 182:321-325 (2000).
Hazen et al., "Relative Anti-HIV-1 Efficacy of Lamivudine and Emtricitabine in Vitro Is Dependent on Cell Type," J. AIDS 32:255-258 (2003).
Hostetler et al., "Greatly Enhanced Inhibition of Human Immunodeficiency Virus Type 1 Replication in CEM and HT 4-6C Cells by 3'-Deoxythymidine Diphosphate Dimyristoylglycerol, a Lipid Prodrug of 3'-Deoxythymidine," Antimicro. Agent Chemo. 36(9):2025-2029 (1992).
Hostetler et al.., "Synthesis and Antiretroviral Activity of Phospholipid Analogs of Azidothymidine and Other Antiviral Nucleosides," J. Biol. Chem. 265(11):6112-6117 (1990).
Ibbotson et al., Drugs 2003, 63(11), 1089-1096.
Ikeda et al., "Studies of prodrugs III. A convenient and practical preparation of Amphicillin prodrugs," Chem. Pharm. Bull. 32:4316-4322, 1984.
Information about the Results of Oral Proceedings for EP Patent EP 1583542 B1 (Application No. 04701819.7), Claims, Amended Claims and Minutes of the Oral Proceeding (Nov. 19, 2010).
International Preliminary Report on Patentability for PCT/US2004/000832 (Dec. 29, 2004).
International Preliminary Report on Patentability for PCT/US2006/023222 (Oct. 8, 2007).
International Preliminary Report on Patentability for PCT/US2006/023223 (Oct. 8, 2007).
International Search Report and Written Opinion mailed Jul. 12, 2004 for PCT/US2004/000832, Int'l. Filing Date Jan. 13, 2004.
International Search Report for PCT/US1997/013244 (Oct. 20, 1997).
International Search Report for PCT/US1998/015254 (Nov. 25, 1998).
International Search Report for PCT/US2006/023222 (Feb. 23, 2007).
International Search Report for PCT/US2006/023223 (Feb. 23, 2007).
Ishida and Asao, "Self-association and unique DNA binding properties of the anti-cancer agent TAS-103, a dual inhibitor of topoisomerases I and II," Biochem. Biophys. Acta 1587(2-3):155-163 (2002).
Iyer et al., "Synthesis of acyloxyalkyl acylphosphonates as potential prodrugs of the antiviral Trisodium phosphonoformate (Foscarnet sodium)," Tet. Lett. 30(51): 7141-7144, 1989.
Jones et al.., "Minireview: nucleotide prodrugs," Antiviral Res. 27:1-17 (1995).
Kearney et al., "Effect of Demographic Variables on the Pharmacokinetics of Tenofovir DF in HIV-Infected Patients and Healthy Subjects," 41st ICAAC Abstracts, Chicago, IL, Sep. 22-25, 2001, Abstract A-504.
Khamnei et al., "Neighboring Group Catalysis in the Design of Nucleotide Prodrugs," J. Med. Chem. 39:4109-4115 (1996).
King et al. "Potency of Nonnucleoside Reverse Transcriptase Inhibitors (NNRTIs) Used in Combination with Other Human Immunodeficiency Virus NNRTIs, NRTIs, or Protease Inhibitors," Antimicrobial Agents and Chemotherapy 46(6):1640-1646 (2002).
Kleinebudde et al. European journal of Pharmaceutics and Biopharmaceutics, 2004, 58, 317-326.
Krise et al., "Prodrugs of phosphates, phosphonates, and phosphinates," Advanced Drug Delivery Reviews 19:287-310, 1996.
Kucera et al., "Novel Membrane-Interactive Ether Lipid Analogs That Inhibit Infectious HIV-1 Production and Induce Defective Virus Formation," AIDS Res. & Hum. Retro. 6:491-501 (1990).
Lachman, et al. (1987) "The Theory and Practice of Industrial Pharmacy", Varghese Publishing House, Dadar Bombay, pp. 330-331.
Landgrebe, J. A., "Crystallization and filtration," Theory and Practice in the Organic Laboratory, 3rd Edition, pp. 65-77, 1982.
Letter Regarding the Opposition Procedure for EP Patent EP 1583542 B1 (Application No. 04701819.7) (Mar. 11, 2010).
Lieberman et al., "," Pharmaceutical Dosage Forms 1:177-178 (1989).
Lindahl et al., "Synthesis of an acyloxymethyl prodrug of the Inositol phosphate alpha-Trinositol," J. Carbohydrate Chemistry 15(5):549-554, 1996.
Liu et al., "Thymidylate synthase as a translational regulator of cellular gene expression," Biochem. Biophys. Acta 1587(2-3):174-182 (2002).

(56) **References Cited**

OTHER PUBLICATIONS

Loveday, "Nucleoside reverse transcriptase inhibitor resistance," JAIDS 26:S10-S24 (2001).
Maillard et al., "Adenosine receptor prodrugs: Synthesis and biological activity of derivatives of potent A1-selective agonists," J. Pharm. Sci. 83(1):46-53, 1994.
Margot et al., "Development of HIV-1 Drug Resistance Through 144 Weeks in Antiretroviral-Naive Subjects on Emtricitabine, Tenofovir Disoproxil Fumarate, and Efavirenz Compared with Lamivudine/Zidovudine and Efavirenz in Study GS-01-934," JAIDS 52(2):209-221 (2009).
Margot et al., "Genotypic and phenotypic analyses of HIV-1 in antiretroviral-experienced patients treated with tenofovir DF," AIDS 16:1227-1235 (2002).
Margot et al., "Resistance development over 144 weeks in treatment-naive patients receiving tenofovir disoproxil fumarate or stavudine with lamivudine and efavirenz in Study 903," HIV Medicine 7:442-450 (2006).
Masho et al., "Review of Tenofovir-Emtricitabine," Ther. Clin. Risk Manag. 3(6):1097-1104 (2007).
McColl et al., "Pooled Analysis of Recent Emtricitabine and Lamivudine Clinical Trials Reveals Differences in Rates of Development of the M184V/I Mutation," Poster Number: PE7.3/17, 10th European AIDS Conference (EACS) Nov. 17-20, 2005, Dublin Ireland.
McIntee et al., "Probing the mechanism of action and decomposition of amino acid phosphomonoester amidates of antiviral nucleoside prodrugs," J. Med. Chem. 40(2):3323-31, 1997.
Memorandum of Law in Opposition of a Motion for Leave to File Amended Complaint Case No. 10-CV-01796 (Apr. 25, 2011).
Miller et al., Sixth International Congress on Drug Therapy in HIV Infection, Nov. 17- 21, 2002 (1 page).
Mills et al., "Artemis: Efficacy and Safety of Darunavir/ritonavir (DRV/r) 800/100mg Once-daily vs Lopinavfr/ritonavir (LPV/r) in Treatment-naive, HIV-1-infected Patients at 96 wks," 48th Annual ICAAC/IDSA, 46th Annual Meeting, Washington, D.C. Oct. 25-28, 2008, Presentation Number: H-1250c.
Minutes of the Oral Proceedings before the Opposition Division for EP 1890681 B1 (Application No. 06773194.3) and Appendices (Apr. 5, 2011).
Molina et al., "A Lopinavir/Ritonavir-Based Once-Daily Regimen Results in Better Compliance and Is Non-inferior to a Twice-Daily Regimen Through 96 Weeks," AIDS Research and Human Retroviruses 23(12):1505-1514 (2007).
Molina et al., "Once-Daily Combination Therapy with Emtricitabine, Didanosine, and Efavirenz in Human Immunodeficiency Virus-Infected Patients," J. Infect. Dis. 182:599-602 (2000).
Mulato et al., "Anti-HIV activity of adefovir (PMEA) and PMPA in combination with antiretroviral compounds: in vitro analyses," Antiviral Res. 36(2):91-97 (1997).
Murry et al., "Reversion of the M184V Mutation in Simian Immunodeficiency Virus Reverse Transcriptase is Selected by Tenofovir, Even in the Presence of Lamivudine," J. Virol. 77(2):1120-1130 (2003).
Naesens et al., "Antiretroviral activity and pharmacokinetics in mice of oral Bis(Pivaloyloxymethyl)-9-(2-phosphonylmethoxyethyl)adenine, the Bis(Pivaloyloxymethyl)ester prodrug of 9-(2-Phosphonylmethoxyethyl)adenine," Antimicro AG & Chemo. 40(1)22-28, 1996.
Newman and Byrn, "Solid-state analysis of the active pharmaceutical ingredient in drug products" Drug Discovery Today, 8(19) 898-905 (2003).
Notari, "Prodrug Design," Pharmaceutical Therapy, 14:25-53, 1981.
Notice of Appeal of the Decision of the Opposition Division for EP Patent EP 1583542 B1 (Application No. 04701819.7) (Mar. 29, 2011).
Office Action for Korean Patent Application No. 7013069/2005 issued by the Korean Intellectual Property Office (May 22, 2007).
Office Action for Patent Application No. 10-2000-7000636 issued by the Korean Intellectual Property Office (Aug. 19, 2005) (translation).
Office Action for U.S. Appl. No. 08/900,752 issued by the United States Patent and Trademark Office (Apr. 16, 1998).
Office Action for Patent Application No. 11-510067 issued by the Japanese Patent Office (Dec. 11, 2007) (translation).
Office Action for Patent Application No. 2,261,619 issued by the Canadian Patent Office (Dec. 22, 2004).
Office Action for Patent Application No. 2,298,059 issued by the Canadian Intellectual Property Office (Apr. 25, 2007).
Office Action for Patent Application No. 2,512,475 issued by the Canadian Patent Office (Jan. 10, 2008).
Office Action for Patent Application No. 2,611,520 issued by the Canadian Patent Office (Jun. 7, 2010).
Office Action for Patent Application No. 2006-500939 issued by the Japanese Patent Office (Mar. 25, 2010).
Office Action for Patent Application No. 2006-500939 issued by the Japanese Patent Office (Nov. 9, 2009).
Office Action for Patent Application No. 7007002/2008 issued by the Korean Intellectual Property Office (Jun. 11, 2008) (translation).
Office Action for Patent Application No. 7009376/2009 issued by the Korean Intellectual Property Office (Oct. 9, 2009) (translation).
Office Action for Patent Application No. 87112168 issued by the Taiwanese Intellectual Property Office (Mar. 1, 2004) (translation).
Office Action for Patent Application No. 87112168 issued by the Taiwanese Intellectual Property Office (May 6, 2002) (translation).
Office Action for Patent Application No. 87112168 issued by the Taiwanese Intellectual Property Office (Oct. 20, 2000) (translation).
Office Action for Patent Application No. 93112403 issued by the Taiwanese Intellectual Property Office (Apr. 27, 2005) (translation).
Official Action for Application No. 095120445 issued by the Taiwanese Intellectual Property Office (Nov. 29, 2011) (translation).
Official Action for Application No. 2008-517083 issued by the Japanese Patent Office (Aug. 2, 2011) (translation).
Official Action for Ex Parte Reexamination of U.S. Pat. No. 5,922,695 (Dec. 11, 2007).
Official Action for Ex Parte Reexamination of U.S. Pat. No. 5,935,946 (Jan. 16, 2008).
Official Action for Ex Parte Reexamination of U.S. Pat. No. 5,977,089 (Jan. 16, 2008).
Official Action for Ex Parte Reexamination of U.S. Pat. No. 6,043,230 (Dec. 11, 2007).
Official Action for Patent Application No. 10-1999-7000806 issued by the Korean Intellectual Property Office (Apr. 28, 2005) (translation).
Official Action for Patent Application No. 10-508318 issued by the Japanese Patent Office (Apr. 10, 2007) (translation).
Official Action for Patent Application No. 200501134/28 issued by the Eurasian Patent Office (Dec. 25, 2008).
Official Action for Patent Application No. 200501134/28 issued by the Eurasian Patent Office (Oct. 15, 2006).
Official Action for Patent Application No. 333687 issued by the Intellectual Property Office of New Zealand (Mar. 2, 1999).
Official Action for Patent Application No. 7001077/2008 issued by the Korean Intellectual Property Office (Sep. 13, 2010).
Official Action for Patent Application No. 93100813 issued by the Taiwanese Intellectual Property Office (Jun. 22, 2005) (translation).
Official Action for Patent Application. No. 200800033/27 issued by the Eurasian Patent Office (2010) (translation).
Opinion on Patent Application No. 1-2005-00812 issued by the Vietnamese Patent Office (Jul. 27, 2008).
Opponents Comments to the Reply Statement by the Applicant relating to Patent Application No. 3383/DELNP/2005 (Aug. 14, 2008).
Order Granting Request for Ex Parte Reexamination of U.S. Pat. No. 5,922,695 (Jul. 13, 2007).
Order Granting Request for Ex Parte Reexamination of U.S. Pat. No. 5,935,946 (Jul. 13, 2007).
Order Granting Request for Ex Parte Reexamination of U.S. Pat. No. 5,977,089 (Jul. 13, 2007).
Order Granting Request for Ex Parte Reexamination of U.S. Pat. No. 6,043,230 (Jul. 13, 2007).

(56) **References Cited**

OTHER PUBLICATIONS

Osol et al., Editor, Remington's Pharmaceutical Sciences, Sixteenth Edition, pp. 1554-1557, 1980.
Pallella et al., J. Med. Chem. 338:853-860 (1998).
Parikh, Handbook of Pharmaceutical Granulation Tech., NY, Marcel Dekker Inc., 1996.
Pharmaceutical Dosage Forms and Drug Delivery Systems, 6th Ed. pp. 171-174 (1995).
Pharmaceutical Technology (2005), Big Pharma Companies Team Up to Develop Once-Daily Triple-Combination HIV Drug,vol. 29, No. 4.
Piantadosi et al., "Synthesis and Evaluation of Novel Ether Lipd Nucleoside Conjugates for Anti-HIV-I Activity," J. Med. Chem. 34:1408-1414 (1991).
Plaintiff's Reply to Teva USA's Amended Counterclaim, filed by Gilead Sciences, Emory University, Case No. 08-CV-10838 (Aug. 10, 2009).
Plaintiff's Reply to Teva USA's Second Amended Counterclaim, filed by Gilead Sciences, Emory University,Case No. 08-CV-10838 (Oct. 15, 2009).
Plaintiff's Reply to Teva's Counterclaim, filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Feb. 25, 2009).
Pozniak et al., "Tenofovir Disoproxil Fumarate, Emtricitabine, and Efavirenz Versus Fixed-Dose Zidovudine/Lamivudine and Efavirenz in Antiretroviral-Naive Patients," JAIDS 43(5):535-540 (2006).
Pre-Grant Opposition Petition against Brazilian Patent Application PI 0406760-6 (Aug. 20, 2010).
Puech et al., "Intracellular delivery of nucleoside monophosphates through a reductase-mediated activation process," Antiviral Res. 22:155-174 (1993).
Pujari et al., "Safety and long term effectiveness of generic fixed-dose formulations of nevirpine-based HAART amongst antiretroviral-naïve HIV-infected patients in India," World Health Organization, [on line], Dec. 16, 2003, pp. 99-116; (Retrieved from: http://libdoc.who.int/publications/2003/a86263.pdf.
Rejection Decision for Patent Application No. 200480002190.5 issued by the Patent Office of the People's Republic of China (Jan. 15, 2010).
Rejection of Patent Application No. 93100813 issued by the Taiwanese Intellectual Property Office (Feb. 22, 2006) (translation).
Reply of the Patent Proprietor to the Notice of Opposition of EP 1890681 B1 (Application No. 06773194.3) (Jun. 22, 2010).
Reply of the Patent Proprietor to the Notices of Opposition of EP Patent EP 1583542 B1 (Application No. 04701819.7) (Jan. 4, 2010).
Request for Ex Parte Reexamination of U.S. Pat. No. 5,922,695 (submitted Apr. 30, 2007).
Request for Ex Parte Reexamination of U.S. Pat. No. 5,935,946 (submitted Apr. 30, 2007).
Request for Ex Parte Reexamination of U.S. Pat. No. 5,977,089 (submitted Apr. 30, 2007).
Request for Ex Parte Reexamination of U.S. Pat. No. 6,043,230 (submitted Apr. 30, 2007).
Response to the Written Opinion of the ISA for PCT/US2006/023222 (May 10, 2007).
Response to the Written Opinion of the ISA for PCT/US2006/023223 (May 10, 2007).
Revised International Search Report for PCT/US2004/000832 (Aug. 5, 2004).
Revocation of European Patent EP 1890681 B1 (Application No. 06773194.3) (Jun. 8, 2011).
Richman, "Antiretroviral activity of emtricitabine, a potent nucleoside reverse transcriptase inhibitor," Antivir. Ther. 6(2):83-88 (2001).
Richman, "HIV Chemotherapy," Nature 410:995-1001 (2001).
Ristig et al., "Tenofovir Disoproxil Fumarate Therapy for Chronic Hepatitis B in Human Immunodeficiency Virus/Hepatitis B Virus—Coinfected Individuals for Whom Interferon-α and Lamivudine Therapy Have Failed," J. Infect. Dis.186:1844-1847 (2002).
Robinson et al., "Discovery of the Hemifumarate and (alpha-L-Alanyloxy)methylester as prodrugs of an antirheumatic oxindole: Prodrugs for enolic OH group," J. Med. Chem. 39:10-18, 1996.
Rousseau et al., "Prototype trial design for rapid dose selection of antiretroviral drugs: an example using emtricitabine (Coviracil)," Journal of Antimicrobial Chemotherapy 48:507-513 (2001).
Safadi et al., "Phosphoryloxymethyl carbamates and carbonates—Novel water soluble prodrugs for amines and hindered alcohols," Pharm. Res. 10(9):1350-1355, 1993.
Sakamoto et al., "Studies on prodrugs II. Preparation and characterization of (5-substituted 2-oxo-1, 3-dioxolen-4-yl)methyl esters of Ampicillin," Chem. Pharm. Bull. 32(6):2241-2248, 1983.
Samara et al., "Pharmacokinetic analysis of Diethylcarbonate prodrugs of Ibuprofen and Naproxen," Biopharmaceutics & Drug Disposition 16:201-210, 1995.
Sanne et al., "Genotypic Analysis of HIV-1 Infected ART-Naive Patients Receiving Emtricitabine (FTC) or Lamivudine (3TC) in a Double Blind Equivalence Trial," Poster No. 4433, presented at the XIV International AIDS Conference Jul. 7-12, 2002, Barcelona, Spain.
Sanne et al., "Two Randomized, Controlled, Equivalence Trials of Emtricitabine (FTC) to Lamivudine (3TC)," Poster 4432 presented at the XIV International AIDS Conference, Jul. 7-12, 2002, Barcelona, Spain.
Schinazi et al., "Characterization of Human Immunodeficiency Viruses Resistant to OxathiolaneCytosine Nucleosides," Antimicrobial Agents and Chemotherapy 374:875-881 (1993).
Schinazi et al., "Selective Inhibition of Human Immunodeficiency Viruses by Racemates and Enantiomers of cis-5-Fluoro-1-[2-(Hydroxymethyl)-1,3-Oxathiolan-5-yl]Cytosine," Antimricobial Agents and Chemotherapy 36(11):2423-2431(1992).
Schinazi et al., Letter to the Editor "Assessment of the Relative Potency of Emtricitabine and Lamivudine," J. AIDS 34(2)243-245 (2003).
Search and Examination Report for Appln No. AP/P/2005/003348 issued by the African Regional Intellectual Property Organization, 5 pages. (Apr. 10, 2008).
Second Amended Answer and Counterclaim against Gilead Sciences, Inc. filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Filed Oct. 9, 2009).
Second Amended Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Sep. 25, 2009).
Second Office Action for Application No. 200680026180.4 issued by the State Intellectual Property Office of the People's Republic of China (Oct. 20, 2011) (translation).
Second Office Action for Patent Application No. 200510099916.8 issued by the Chinese Patent Office (Nov. 16, 2007) (translation).
Second Office Action for Patent Application No. 97197460.8 issued by the Chinese Patent Office (Aug. 5, 2005) (translation).
Second Official Action for Patent Application No. a 2005 07947 issued by the Ukrainian Patent Office (2006) (translation).
Serafinowska et al., "Synthesis and in vivo Evaluation of prodrug of 9-{2-(Phosphonomethoxy) ethoxy} adenine," J. Med. Chem. 38:1372-1379, 1995.
Shaw et al., "Metabolism and pharmacokinetics of novel oral prodrugs of 9-[(R)-2-(phosphonomethoxy)propyl]adenine (PMPA) in dogs," Pharm. Res. 14(12):1824-1829, 1997.
Siddiqui et al., "Design and Synthesis of Lipophilic Phosphoramidate D4T-MP Prodrugs Expressing High Potency Against HIV in Cell Culture: Structural Determinants for in Vitro Activity and QSAR," J. Med. Chem. 42(20):4122-4128 (1999).
Smith et al., "Randomized, double-blind, placebo-matched, multicenter trial of abavacir/lamivudine or tenofovideintricitabine with lopinavir/ritonavir for inital HIV treatment," AIDS 23:1547-1556 (2009).
Srinivas et al., "Metabolism and in vitro antiretroviral activities of Bis(Pivaloyloxymethyl) prodrugs of acyclic nucleoside phosphonates," Antimicro AG & Chemo. 37(10):2247-2250, 1993.

(56) **References Cited**

OTHER PUBLICATIONS

Srivastva et al., "Bioreversible phosphate protective groups: Synthesis and stability of model acyloxymethyl phosphates," Bioorg. Chem. 12:118-129, 1984.
Starret et al., "Synthesis and in vitro evaluation of a phosphonate prodrug:bis(pivaloyloxymethyl) 9-(2-phophonylmethoxyethyl)adenine," Antiviral Res. 19:267-273, 1992.
Starret et al., "Synthesis, oral bioavailability determination, and in vitro evaluation of prodrugs of the Antiviral agent 9-[2-(phosphonomethoxy)ethyl)adenine (PMEA)," J. Med. Chem. 37:1857-1864, 1994.
Substantive Examination Report for Patent Application No. W00 2005 02145 from the Indonesian Patent Office (2010).
Sueoka et al., "Pharmacokinetics of Alkoxycarbonyloxy ester prodrugs of PMPA in dogs," Abstract, American Association of Pharmaceutical Science, Western Regional Meeting, Apr. 24-25, 1997.
Summons to Attend Oral Proceedings and Annex to the Communication for EP Patent EP1583542B1 (Application No. 04701819.7) (May 21, 2010).
Summons to Attend Oral Proceedings and Annex to the Communication for the Opposition of EP 1890681 B1 (Application No. 06773194.3) (Oct. 14, 2010).
Tamari, "A Decade in HIV Treatment: What Is the State of the Art and How Did We Arrive," Clinical Excellence for Nurse Practitioners 5(1):4-12 (2001).
Teva Pharmaceutical Industries Ltd., Notice of Opposition of EP Patent EP1583542B1 (Application No. 04701819.7) (Mar. 13, 2009).
Teva Pharmaceutical Industries Ltd., Written Submission in preparation for Oral Proceedings for EP Patent EP1583542B1 (Application No. 04701819.7) (Sep. 16, 2010).
Teva Pharmaceuticals Industries Ltd., Notice of Opposition of EP 1890681B1 (Application No. 06773194.3) (Oct. 7, 2009).
Teva Pharmaceuticals Industries Ltd., Written Submission in preparation for Oral Proceedings for the Opposition of EP 1890681B1 (Application No. 06773194.3) (Mar. 17, 2011).
Third Amended Answer filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Filed Aug. 6, 2010).
Third Amended Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Jul. 6, 2010).
Third Office Action for Patent Application No. 97197460.8 issued by the Chinese Patent Office (Jun. 9, 2006) (translation).
Third Official Action for Patent Application No. a 2005 07947 issued by the Ukrainian Patent Office (2007) (translation).
Thornber "Isosterism and Molecular Modification in Drug Design" Chem. Soc. Reviews 18: 563-580, 1979.
Tisdale et al., "Rapid in vitro selection of human immunodeficiency virus type 1 resistant to 3'-thiacytidine inhibitors due to a mutation in the YMDD region of reverse transcriptase," Proc. Natl. Acad. Sci. USA 90:5653-5656 (1993).
Tsai et al., "Effects of (R)-9-(2-phophonylmethoxypropyl)adenine monotherapy on chronic SIV infection in macaques," Aids Res. & Hum. Retro. 13(8):707-712, 1997.
Tsai et al., "Prevention of SIV infection in macaques by (R)-9-(2-phosphonylmethoxypropyl)adenine," Science 270:1197-1199, 1995.
U.S. Department of Health and Human Services (2004) "Guidance for Industry Fixed Dose Combination and Co-Packaged Drug Products for Treatment of HIV—Draft Guidance" pp. 1-21.
Ueda et al., "Vinyl compounds of nucleic acid bases I. Synthesis of N-vinyluracil, N-vinylthymine, and N-vinyladenine," Die Makromolekulare Chemie 120:13-20, 1968.
USP 24 The United States Pharmacopeia (2000).
Wainberg et al. "In vitro selection and characterization of HIV-1 with reduced susceptability to PMPA," Antiviral Therapy 4:87-94 (1999).
Walmsley et al., "Gemini. A Noninferiority Study of Saquinavir/Ritonavir Versus Lopinavir/Ritonavir as Initial HIV-1 Therapy in Adults," J. Acquir. Immune Defic. Syndr. 50(4):367-374 (2009).
Wang et al. "Lack of Significant Pharmacokinetic Interactions between Emtricitabine and Other Nucleoside Antivirals in Healthy Volunteers," 41st ICAAC Abstracts, Chicago, IL, Sep. 22-25, 2001, Abstract A-505.
Wang et al. "Pharmacokinetic and pharmacodynamic characteristics of emtrictabine support its once daily dosing," Int. Conf. AIDS, Jul. 7-12 14:abstract TUPeB4546 (2002).
Weller et al., "Orally active Fibrinogen receptor antagonists. 2. Amidoximes as prodrugs of amidines," J. Med. Chem. 39:3139-3147, 1995.
Written Opinion issued by the ISA for PCT/US2006/023223 (Feb. 23, 2007).
Written Opinion of the ISA for PCT/US2006/023222 (Feb. 23, 2007).
Yeni et al., "Antiretroviral Treatment for Adult HIV Infection in 2002," JAMA 288(2):222-235 (2002).
Yuan et al., "Degradation Kinetics of Oxycarbonyloxymethyl Prodrugs of Phosphonates in Solution," Pharm. Res. 18(2):234-237 (2001).
Zhang et al. "Phase transformation considerations during process development and manufacture of solid oral dosage forms" Adv Drug Del Reviews 56(30), 371-390 (2004).
"Annex I. Summary of Product Characteristics," for Epivir, 9 pages.
"Annex I. Summary of Product Characteristics," for Truvada film-coated tablets, 14 pages.
"graph," and "convolute of graphs,", cited as documents D54 and D55 in opposition proeedings of EP 04701819.7, Statement of Appeal Grounds filed Jun. 24, 2011.
"Lactose Anhydrous," p. 373 in Analytical Profiles of Drug Substances, vol. 20, Ed. Klaus Florey, Academic Press, Inc. (1990).
"Lactose," Handbook of Pharmaceutical Excipients, 3rd ed. Ed. A.H. Kibbe, American Pharmaceutical Association, pp. 276-285 (2000) pp. 276-285 (2000).
"New Uses for Tenofovir; More Questions about d4T," Project Inform Perspective 35:15-16 (2003).
Ait-Khaled, et al., "Zidovudine appears to prevent selection of K65R and L74V mutations normally selected by abacavir mono- or combination therapies not containing zidovudine" Antiviral Therapy, 2002, 7:S107 (Abstract).
Arimilli et al., "Nucleotide Analogues," U.S. Appl. No. 60/022,708, 40 pages, filed Jul. 26, 1996.
Arzneiformenlehre. Ein Lehrbuch für Pharmazeuten, List et al., Eds., Wissenschaftliche Verlagsgesellschaft mbH, pp. 79 and 477 (1985). An English translation is not readily available. Applicants believe this reference discloses that preparations with lactose can undergo the Maillard reaction with amines. Such preparations result in discolorations. Amines and reducing sugars may also form N-glycosylamines which may react further in a Maillard reaction.
BioWorld Today, "About BioWorld," 1 page, http://www.bioworld.com/servlet/com.accumedia.web.Dispatcher?next=aboutUs (2010).
Borroto-Esoda et al., "In vitro evaluation of the anti-HIV activitiy and metaboloc interactions of tenofovir and emtricitabine," Antiviral Therapy 11:377-384 (2006).
Brogan et al., "Cost-Effectiveness of Nucleoside Revers Transcriptase Inhibitor Pairs in Efavirenz-Based Regimens for Treatment-Naïve Adults with HIV Infection in the United States," Value in Health 14:657-664 (2011).
Communication concerning Correction of the EP Specification for Patent Publication EP 1583542 (Application No. 04701819.7) issued by the European Patent Office (Jul. 22, 2008).
Communication of Notice of Opposition of EP 1583542 B1 (Application No. 04701819.7)—first information to the patent proprietor (Mar. 24, 2009).
Communication of Notice of Opposition of EP 1583542 B1 (Application No. 04701819.7)—first information to the patent proprietor (Mar. 26, 2009).

(56) **References Cited**

OTHER PUBLICATIONS

Communication pursuant to Article 94(3) EPC for Patent Publication EP 1923063 (Application No. 08152527.1) issued by the European Patent Office (Mar. 26, 2012).
Crowley, "Drug-Excipient Interactions," Pharm. Tech., 6 pages (2001).
Dahl et al., Amended Transmittal of U.S. Appl. No. 10/540,794, Compositions and methods for combination antiviral therapy, filed Mar. 20, 2006.
De Clercq, "New Anti-HIV Agents and Targets," Medicinal Research Reviews 22(6):531-565 (2002).
Decision to Grant a European patent for EP Appln No. 04701819.7 and Druckexemplar (May 23, 2008).
Department of Health and Human Services, "Guidelines for the Use of Antiretroviral Agents in HIV-1-infected Adults and Adolescents," pp. 1, 33-49 (Nov. 3, 2008).
Examiner's Second Report on Patent Application No. 2009200414 issued by the Australian Patent Office (Aug. 29, 2011).
European Search Report, EP 2386294 (Application No. 11167101.2), 15 pages (Dec. 29, 2011).
Eyjolfsson, "Lisinopril-Lactose Incompatibility," Drug Devel. Indust. Pharm. 24(8):797-798 (1998).
First Examination Report for Patent Application No. 6665/DELNP/2008 issued by the Indian Patent Office (Jun. 30, 2011).
FTC 101 Virology analysis, TPI Document No. 14022 (2002).
Gallant, et al., "Early Non-Response to Tenofovir DF (TDF) + Abacavir (ABC) and Lamivudine (3TC) in a Randomized Trial Compared to Efavirenz (EFV) + ABC and 3TC: ESS30009 Unplanned Interim Analysis," Abstr Intersci Conf Antimicrob Agents Chemother Intersci Conf Antimicrob Agents Chemother, Abstract No. H-1722a (2003).
Giron, "Applications of thermal analysis in the pharmaceutical industry," J. Pharm. Biomed. Anal. 4(6):755-770 (1986).
Glaxo Marketing Material, Epivir + Ziagen, 6 pages (2003).
Huff, "Five New Drugs Enter the Homestretch," The Body: The Complete HIV/AIDS Resource, 3 pages (2002).
Information About the Results of the Oral Proceedings for EP 1890681 B1 (Application No. 06773194.3) (Apr. 5, 2011).
Jamsek, et al. "Poor Virological Responses and Early Emergence of Resistance in Treatment Naïve, HIV-infected Patients Receiving a Once Daily Triple Nucleoside Regimen of Didanosine, Lamivudine, and Tenofovir DF" 11th Conf Retrovir Oppor Infect, Abstract No. 51 (2004).
Kusmierek et al., "Kinetics and Mechanisms of Hydrolytic Reactions of Methylated Cytidines under Acidic and Neutral Conditions," Acta Chem. Scand. 43:196-202 (1989).
Lanier, et al. "Prediction of NRTI Optins by Linking Reverse Transcriptase Genotype to Phenotypic Breakpoints" 10th Conf Retrovir Oppor Infect, Abstract No. 586 (2003).
Lindahl, "Instability and decay of the primary structure of DNA," Nature 362:709-715 (1993).
Lu, et al., "Determination of Clinical Cut-Offs for Reduced Response to Tenofovir DF therapy in Antiretroviral-Experienced Patients," Antiviral Therapy 7(1):S104, Abstract No. 125 (2002).
Marcelin et al., "Resistance profiles of emtricitabine and lamivudine in tenofovir-containing regimens," J. Antimicrob. Chemother. 67:1475-1478 (2012).
Margot et al., "In Vitro Human Immunodeficiency Virus Type 1 Resistance Selections with Combinations of Tenofovir and Emtricitabine or Abacavir and Lamivudine," Antimicrobial Agents and Chemotherapy 50(12):4087-4095 (2006).
Merck Index, 13th Edition, p. ONR-65 (2001).
Molina et al., "Castle: Atazanavir-Ritonavir vs Lopinavir-Ritonavir in Antiretroviral-Naïve HIV-1 Infected Patients: 96 Week Efficacy & Safety," 48th Annual ICAAC/IDSA 46th Annual Meeting, Washington , D.C., Presentation No. H-1250d (Oct. 25-28, 2008).
National Institutes of Health, "Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents," 9 pages (2011/2012).
Notice of Allegation and Detailed Statement in respect of Tenofovir Disoproxil Fumarate and Emtricitabine (Truvada®) and Canadian Patent Nos. 2,261,619 and 2,298,059, 56 pages (Nov. 22, 2011).
Notice of Allegation and Detailed Statement in respect of Truvada and Canadian Patent Nos. 2,512,475, 37 pages (Nov. 22, 2011).
Office Action for Korean Patent Application No. 7013069/2005 issued by the Korean Intellectual Property Office (Jan. 23, 2008).
Office Action, 10 pages, from U.S. Appl. No. 12/195,161 (mailed May 7, 2010).
Office Action, 10 pages, from U.S. Appl. No. 12/204,174 (mailed Oct. 1, 2009).
Office Action, 7 pages, from U.S. Appl. No. 10/540,794 (mailed Sep. 21, 2006).
Office Action, 8 pages, from U.S. Appl. No. 10/540,794 (mailed Oct. 31, 2007).
Office Action, 8 pages, from U.S. Appl. No. 12/204,174 (mailed Jun. 4, 2010).
Office Action, 9 pages, from U.S. Appl. No. 10/540,794 (mailed May 16, 2007).
Official Action and Preliminary Notice of Allowance from the Eurasian Patent Office for Application No. 200501134/28 (May 14, 2010).
Official Action for Application No. 2008-517083 issued by the Japanese Patent Office (Mar. 23, 2012) (translation).
Official Action for Patent Application No. 93100813 issued by the Taiwanese Intellectual Property Office (Mar. 16, 2011) (translation).
Official Action, 2 pages, from JP appl. No. 2010-175808 (mailed Nov. 6, 2012) translation attached.
Official Communication for Patent Application No. 2100/DEL/2007 issued by the Indian Patent Office (Jan. 16, 2013).
Opposition filed in Indian Patent Appl. No. 9661/DELNP/2007 by Cipla Limited (Jun. 30, 2009).
Osborne, "Gilead plans $300M notes sale to recoup some merger costs.(Gilead Sciences Inc.)," Bioworld Today, 13(239), 1 page (Dec. 16, 2002).
Osborne, "Gilead, Triangle Plan Merger: $464 Deal Pairs HIV Drugs," Bioworld Today 13(233):1,6 (2002).
Pharmaceutical Dosage Forms. Tablets. 2nd Ed., revised and expanded, Lieberman et al., eds., pp. 93 and 98 (1990).
Project Inform, "Perspective," pp. 1-28 (2003).
Quan et al., "Endogenous Reverse Transcriptase Assays Reveal Synergy between Combinations of the M184V and other Drug-Resistance-conferring Mutations in Interactions with Nucleoside Analog Triphosphates," J. Mol. Biol. 277:237-247 (1998).
Reply to the statement of appeal grounds, EP 1583542 B1 (Application No. 04701819.7), 50 pages (Oct. 18, 2011).
Request for Correction of EP Appln No. 04701819.7 (Jul. 8, 2008).
Request for Correction of EP Appln No. 04701819.7 (Jun. 27, 2008).
Response to the Noting of Loss of Rights pursuant to Rule 112(1) EPC dated Sep. 3, 2012, Patent Publication EP 2386294 (Application No. 11167101.2) (Nov. 13, 2012).
Response to the Noting of Loss of Rights pursuant to Rule 112(1)EPC dated Nov. 14, 2012, Patent Publication EP 1923063 (Application No. 08152527.1) (Jan. 24, 2013).
Response to the reply letter of Teva Pharmaceutical Industries Ltd. dated Oct. 18, 2011, EP 1583542 B1 (Application No. 04701819.7), 35 pages (Aug. 9, 2012).
Reversal of Rejection Decision for Application No. 200480002190.5 by the Patent Rexamination Board (Patent Office of the People's Republic of China) (Jun. 10, 2010).
Riaz and Ami, "Stability of Aminophylline," Pak. J. Pharm. Sci. 6(1):35-44 (1993).
Scrip, "Gilead Acquires Triangle for $464 Million," 2 pages (Dec. 6, 2002).
Second Office Action for Patent Application No. 200480002190.5 issued by the Patent Office of the People's Republic of China (May 11, 2011).
Smirnov et al., "A Comparative Study of the Kinetics of Cytarabine Hydrolytic Deamination in Aqueous Solutions," Pharm. Chem. J. 34(8):451-454 (2000).
Staszewski et al., NEJM, 1999, 341 5,1865-1873.

(56) **References Cited**

OTHER PUBLICATIONS

Teva Pharmaceutical Industries, Ltd, (Opponent), Opposition Brief against Israel Patent No. 169243 (dated Jul. 26, 2009).
Teva's Response to Patentee's Appeal, 5 pages, EP Pat. No. 1890681, EP Appl. No. 06773194.3 (mailed May 3, 2012).
Theory and Practice of Industrial Pharmacy, 3rd Edition, Lachman et al., Eds., Lea & Febiger, pp. 324-329 (1986).
Thoithi et al., "Investigation of the Kinetics of Degradation of Hexopyranosylated Cytosine Nucleosides Using Liquid Chromatography," Nucleosides, Nucleotides & Nucleic Acids 19(1 &2):189-203 (2000).
Truvada Patient Information Leaflet, 33 pages (2008).
Viread (Tenofovir Disoproxil Fumarate Tablets) Summary of Product Characteristics, EMEA, SmPC, 37 pages (Feb. 5, 2002).
Viread Label pp. 1-44 (2001).
Viread Patient Information Leaflet, 21 pages (2002).
Virji-Jeganathan, "BVV Stock Table Highlights," Bioventure View, 17(25): pp. 6-7 (Dec. 10, 2002).
Wang "FTC: A Potent and Selective Anti-HIV and Anti-HBV Agent Demonstrating Desirable Pharmacokinetic (PK Characteristics," Abstracts of the IDSA, 36th Annual Meeting, Session 58, Poster 415, Hepatits A, B, and C in HIV-Infected Persons Friday, 4-6 pm (1998).
Wirth et al., "Maillard Reaction of Lactose and Fluoxetine Hydrochloride, a Secondary Amine," J. Pharm. Sci. 87(1):31-39 (1998).
Zalac et al., "Paracetamol-Propyphenazone Interaction and Formulation Difficulties Associated with Eutectic Formation in Combination Solid Dosage Forms," Chem. Pharm. Bull. 47(3):302-307 (1999).
Affirmation of Andrew W. Williams in Support of Plaintiffs Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co's Opening Claim Construction Brief and Exhibits Case No. 10-CV-01851 (Dec. 2, 2011).
Answer and Counterclaim filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 09-CV-04463 (Aug. 10, 2009).
Answer to Amended Complaint filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jun. 29, 2011).
Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc. Case No. 09-CV-04463 (May 8, 2009).
Declaration of Paul A. Bartlett in Support of Plaintiffs' Claim Constructions and Exhibits filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Nov. 4, 2011).
Declaration of Michael J. Freno in Support of Teva's Opening Claim Construction Brief and Exhibits filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Nov. 4, 2011).
Declaration of Adam C. LaRock in Support of Plaintiffs' Claim Constructions and Exhibits filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Nov. 4, 2011).
Declaration of Adam C. LaRock in Support of Plaintiffs' Rebuttal Claim Constructions and Exhibits filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Dec. 16, 2011).
Declaration of Natalie Lieber and Exhibits filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Dec. 2, 2011).
Declaration of Natalie Lieber in Support of Plaintiffs' Rebuttal Claim Constructions and Exhibits filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Jan. 13, 2012).
Declaration of Daniel P. Margolis in Support of Teva's Opening Claim Construction Brief and Exhibits filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Dec. 2, 2011).
Declaration of Daniel P. Margolis in Support of Teva's Rebuttal Claim Construction Brief and Exhibits filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Dec. 16, 2011).
Declaration of Daniel P. Margolis in Support of Teva's Rebuttal Claim Construction Brief and Exhibits filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jan. 13, 2012).
Declaration of Allan S. Myerson in Support of Plaintiffs' Claim Constructions and Exhibits filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Dec. 2, 2011).
Declaration of Robin D. Rogers, Ph.D. in Support of Teva's Claim Constructions and Exhibits Case No. 10-CV-01851 (Jan. 13, 2012).
Declaration of Karen C. Shen in Support of Teva's Opening Claim Construction Brief and Exhibits Case No. 10-CV-01851 (Dec. 2, 2011).
Declaration of Slaven Jesic in Support of Teva's Rebuttal Claim Construction Brief and Exhibits Case No. 10-CV-01851 (Jan. 13, 2012).
Declaration of Andrew W. Williams in Support of Plaintiffs Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co's Rebuttal Claim Construction Brief and Exhibits Case No. 10-CV-01851 (Jan. 13, 2012).
Defendants' Memorandum in Opposition to Plaintiff's Pretrial Memorandum filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jan. 28, 2013).
Defendants' Memorandum in Opposition to Plaintiffs' Pretrial Memorandum filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01851 (May 24, 2013).
Defendants' Notice Pursuant to 35 U.S.C. § 282 filed by Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co. Case No. 10-CV-01851 (May 22, 2013).
Defendants' Pretrial Memorandum filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jan. 14, 2013).
Defendants' Pretrial Memorandum filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01851 (May 10, 2013).
Defendants' Proposed Findings of Fact and Conclusions of Law filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jan. 14, 2013).
Defendants' Proposed Findings of Fact and Conclusions of Law filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01851 (May 10, 2013).
Letter to Judge Richard J. Sullivan Case No. 10-CV-01796 (Jan. 13, 2012).
Fourth Amended Answer filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Oct. 17, 2011).
Fourth Amended Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. and Exhibits filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Oct. 3, 2011).
First Amended Complaint for Patent Infringement filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Jun. 15, 2011).
Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co's Opening Claim Construction Brief Case No. 10-CV-01851 (Dec. 2, 2011).
Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co's Rebuttal Claim Construction Brief Case No. 10-CV-01851 (Jan. 13, 2012).
Order signed by Judge Richard J. Sullivan Case No. 08-CV-10838 (May 26, 2010).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01798 (May 26, 2010).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01796 (Dec. 5, 2011).
Order signed by Judge Richard J. Sullivan Case No. 08-CV-10838 (Dec. 19, 2011).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01796 (Apr. 26, 2012).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01796 (Jan. 18, 2013).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01796 (Jan. 30, 2013).

(56) **References Cited**

OTHER PUBLICATIONS

Order signed by Judge Richard J. Sullivan Case No. 10-CV-01851 (May 14, 2013).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01851 (May 24, 2013).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01851 (Jun. 5, 2013).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01851 (Jul. 19, 2013).
Plaintiff's Opposition to Defendants' Pretrial Memorandum by Gilead Sciences, Inc. Case No. 10-CV-01796 (Jan. 28, 2013).
Plaintiffs' Opening Claim Construction Brief filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Nov. 4, 2011).
Plaintiff's Opening Claim Construction Brief filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Dec. 2, 2011).
Plaintiffs' Opening Pretrial Brief filed by Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co. Case No. 10-CV-01851 (May 10, 2013).
Plaintiff's Pretrial Memorandum filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Jan. 14, 2013).
Plaintiff's Proposed Findings of Fact and Conclusions of Law filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Jan. 14, 2013).
Plaintiffs' Proposed Findings of Fact and Conclusions of Law filed by Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co. Case No. 10-CV-01851 (May 10, 2013).
Plaintiffs' Rebuttal Claim Construction Brief filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Dec. 16, 2011).
Plaintiffs' Rebuttal Claim Construction Brief filed by Gilead Sciences, Inc., Emory University Case No. 10-CV-01796 (Jan. 13, 2012).
Plaintiffs' Reply to Teva USA's Counterclaim filed by Gilead Sciences, Inc., Emory University Case No. 09-CV-04463 (Aug. 31, 2009).
Plaintiffs' Response to Defendants' Pretrial Memorandum filed by Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co. Case No. 10-CV-01851 (May 24, 2013).
Request for Leave to Submit Supplemental Expert Witness Affidavit of Jerry L. Atwood, Ph.D. on behalf of Plaintiffs filed by Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co. Case No. 10-CV-01851 (May 24, 2013).
Stipulation and Agreement Regarding U.S. Pat. Nos. 5,922,965, 5,935,946, 5,977,089, and 6,043,230 Case No. 10-CV-01796 (Oct. 9, 2012).
Teva's Opening Claim Construction Brief filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Nov. 4, 2011).
Teva's Opening Claim Construction Brief filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Dec. 2, 2011).
Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd.'s Opening Claim Construction Brief Case No. 10-CV-01851 (Dec. 2, 2011).
Teva's Rebuttal Claim Construction Brief filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Dec. 16, 2011).
Teva's Rebuttal Claim Construction Brief filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jan. 13, 2012).
Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd.'s Rebuttal Claim Construction Brief Case No. 10-CV-01851 (Jan. 13, 2012).
Transcript of Proceedings held on Apr. 26, 2012 Case No. 10-CV-01796.
Transcript of Proceedings held on Oct. 3, 3012, 2012 Case No. 10-CV-01796.
Bloor S., et al, Patterns of HIV drug resistance in routine clinical practice; a survey of almost 12000 samples from the USA in 1999, Antiviral Therapy 5(3):132.
Carpenter, C.C.J., et al., "Antiretroviral Therapy for HIV Infection in 1997: Updated Recommendations of the International AIDS Society—USA Panel," The Journal of the American Medical Association 277(24):1962-1969 (Jun. 25, 1999).
Cherry, et al., "Mutations at codon 184 in simian immunodeficiency virus reverse transcriptase confer resistance to the (−) enantiomer of 2,,3'-dideoxy-3,-thiacytidine," Antimicrob., Agents Chemother. 41:2763-2765 (1997) http://aac.asm.org/content/41/12/2763.full.pdf+html (retrieved on Jan. 29, 2014).
Delehanty J., et al., "Selection of FTC dose based on viral kinetics and pharmacokinetics in an accelerated clinical trial design,"12th World AIDS Conference, Geneva, Switzerland, Abstract No. 12208 (Jul. 1998) http://www.aegis.org/DisplayContent/print.aspx?Section1D=341007 (retrieved on Jan. 30, 2014).
Flexner, C., "HIV-Protease Inhibitors," The New England Journal of Medicine 338(18):1281-1292 (Apr. 30, 1998).
Gosselin, et al., "Anti-human immunodeficiency virus activities of the (3-L enantiomer of 2',3'-dideoxycytidine and its 5-fluoro derivative in vitr,"Antimicrob Agents Chemother 38: 1292-1297 (1994) http://aac.asm.org/content/38/6/1292.full.pdf (retrieved on Jan. 30, 2014).
Harrigan, P.R., et al., "Phenotypic susceptibilities to tenofovir in a large panel of clinically derived human immunodeficiency virus type 1 isolates," Antimicrob Agents Chemother. 46:1067-1072 (2002) http://aac.asm.Org/content/46/4/1067.full.pdf+html (retrieved on Jan. 30, 2014).
Miller, Margot N.A., et al., "Antiviral activity of tenofovir (PMPA) against nucleoside-resistant clinical HIV samples,"Nucleosides Nucleotides Nucleic Acids,; 20:1025-1028 (2001).
Miller M.D., et al., Human Immunodeficiency Virus Type 1 Expressing the Lamivudine-Associated M184V Mutation in Reverse Transcriptase Shows Increased Susceptibility to Adefovir and Decreased Replication Capability in Vitro, The Journal of Infectious Diseases 179:92-100 (1999) http://jid.oxfordjournals.org/content/179/1/92.full.pdf (retrieved on Jan. 30, 2014).
Robinson B.S., et al. BMS-232632, a Highly Potent Human Immunodeficiency Virus Protease Inhibitor That Can Be Used in Combination with Other Available Antiretroviral Agents.—Antimicrobial Agents and Chemotherapy 44(8):2093-2099 (Aug. 2000) http://www.ncbi.nhn.nih.gov/pmc/articles/PMC90019/pdf/acQ02093.pdf (retrieved on Jan. 30, 2014).
Srinivas R.V. et al., "Antiviral Activities of 9-R-2-Phosphonomethoxypropyl Adenine (PMPA) and Bis(isopropyloxymethylcarbonyl)PMPA against Various Drug-Resistant Human Immunodeficiency Virus Strains," Antimicrob. Agents Chemother 42(6):1484-1487 (Jun. 1988) http://aac.asm.org/content/42/6/1484.full.pdf+html (retrieved on Jan. 30, 2014).
Staszewski S., et al., "Efficacy and safety of tenofovir disoproxil fumarate (TDF) versus stavudine (d4T) when used in combination with lamivudine (3TC) and efavirenz (EFV) in HIV-1 infected patients naive to antiretroviral therapy (ART): 48-week interim results," XIV International AIDS Conference, Barcelona, Spain, Abstracts No. 9804:7-12 (Jul. 2002) http://www.iasocietv.org/Default.aspx?page1d=12&abstract1d=9804 (retrieved on Jan. 30, 2014).
Van Rompay, K. K., et al., "Virulence and reduced fitness of simian immunodeficiency virus with the M184V mutation in reverse transcriptase," J. Virology, 76(12):6083-6092 (2002) http://www.ncbi.nlm.nih.gov/pmc/articles/PMC136201/pdf/0196.pdf (retrieved on Jan. 30, 2014).
Van Rompay, et al., "9-[2-(Phosphonomethoxy)propyl]adenine therapy of established simian immunodeficiency virus infection in infant rhesus macaques," Antimicrob. Agents Chemother. 40:2586-2591 (1996) http://www.ncbi.nlm.nih.gov/pmc/articles/PMC163581/pdf/402586.pdf (retrieved on Jan. 31, 2014).
Zao, Biokad Objection against the application of Eurasian Patent No. 015145 in the Russian Federation dated Jul. 10, 2013.
Patent Examination Report No. 1, Australia patent appl. No. 2011253996, 2 pages (Nov. 15, 2012).
Office Action for Patent Application No. 200680026180.4 issued by the Chinese Patent Office (May 29, 2013) (translation).
Summons to attend Oral Proceedings EPO for Application No. 067731595.0 dated Jul. 9, 2013.

(56) **References Cited**

OTHER PUBLICATIONS

Communication and Annex from PO for Application No. 067731595.0 dated Jul. 9, 2013.
Response to Summons and Annex rom EPO for Application No. 067731595.0 dated Sep. 25, 2013.
Brief Communication from EPO for Application No. 067731595.0 dated Nov. 10, 2013.
Communication from EPO for Application No. 067731595.0 dated Nov. 15, 2013.
Minutes of Oral Proceedings from EPO for Application Application No. 067731595.0 dated Nov. 25, 2013.
Communication from EPO for Application No. 067731595.0 dated Dec. 4, 2013.
Excerpt of The Merck Veterinary Manual (Clarence M. Fraser et al. eds., 7th ed., 1991).
Excerpts from Daniel S. Kemp & Frank Vellaccio, Organic Chemisty (Worth Publishers, Inc. 1980).
Excerpts from K. Peter C. Vollhardt, Organic Chemistry (W.H. Freeman and Company 1987).
Excerpts from the Reexamination of U.S. Pat. No. 6,043,230.
Excerpts of Teva Pharmaceuticals USA, Inc.'s Detailed Statement of the Factual and Legal Bases for Its Opinion That U.S. Pat. Nos. 5,814,639, 5,914,331, 5,922,695, 5,935,946, 5,977,089. and 6,043,230 are Invalid, Unenforceable or Not Infringed by the Manufacture, Use or Sale of Its Emtricitabine & Tenofovir Disoproxil Fumarate Tablets, 200 mg/300 mg dated Jan. 28, 2010 ("2010 Detailed Statement").
Excerpts of Panel on Antiretroviral Guidelines for Adults and Adolescents, Department of Health and Human Services, Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents 1-167 (20 11).
Excerpts of Teva Pharmaceuticals USA, Inc.'s Detailed Statement of the Factual and Legal Bases for Its Opinion That U.S. Pat. Nos. 6,642,245 and 6,703,396 are Invalid, Unenforceable or Not Infringed by the Manufacture, Use or Sale of Its Emtricitabine & Tenofovir Disoproxil Fumarate Tablets, 200 mg/300 mg dated Nov. 3, 2008 ("2008 Detailed Statement"); "Teva Pharmaceuticals USA, Inc.'s Detailed Statement of the Factual and Legal Bases for Its Opinion That U.S. Pat. Nos. 6,642,245 and 6, 703,396 Are Invalid, Unenforceable or Not Infringed by the Manufacture, Use or Sale of Its Efavirenz/Emtricitabine/Tenofovir Disoproxil Fumarate Tablets, 600 mg/200 mg/300 mg" dated Mar. 30, 2009 ("2009 Detailed Statement"); and Teva Pharmaceuticals USA, Inc.'s Detailed Statement of the Factual and Legal Bases for Its Opinion That U.S. Pat. Nos. 5,814,639, 5,914,331, 5,922,695, 5,935,946, 5,977,089 and 6,043,230 are Invalid, Unenforceable or Not Infringed by the Manufacture, Use or Sale of Its Emtricitabine & Tenofovir Disoproxil Fumarate Tablets, 200 mg/300 mg dated Jan. 28, 2010 ("2010 Detailed Statement").
Excerpts of the Gilead Sciences, Inc., Q2, 2011 Earnings Results Conference Call and Webcast presentation, pp. 16 & 19 (Jul. 26, 2011).
Excerpts of the Gilead Sciences, Inc., Q2, 2011 Earnings Results Conference Call and Webcast presentation, p. 16-18 (Jul. 26, 2011).
Fischl, Margaret A. et al., Prolonged Zidovudine Therapy in Patients with AIDS and Advanced AIDS-Related Complex, 262 J.A.M.A. 2405 (1989).
Harada, Shinji et al., *Infection of HTLV-IIIILAV in HTLV-I-Carrying Cells MT-2 and MT-4 and Application in a Plaque Assay*, 229 Science 563 (1985).
Hoong, Lee K. et al., Enzyme-Mediated Enantioselective Preparation of Pure Enantiomers of the Antiviral Agent 2',3'-Dideoxy-5-.fluoro-3 '-thiacytidine (FTC) and Related Compounds, 57 J. Organic Chemistry 5563 (1992).
Larder, Brendan A. et al., HIV with Reduced Sensitivity to Zidovudine (AZT) Isolated During Prolonged Therapy, 243 Science 1731 (1989).
Norton, Telly M. et al., Efficacy of Acyclovir Against Herpesvirus Infection in Quaker Parakeets, 52(12) Am. J. Vet. Res. 2007-09 (Dec. 1991).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01796 (Jan. 17, 2012).
Stella, Valentino J. et al., *Prodrugs and Site-Specific Drug Delivery*, 23 Journal of Medicinal Chemistry 1275 (1980).
Teva Pharmaceuticals USA, Inc.'s Detailed Statement of the Factual and Legal Bases for Its Opinion That U.S. Pat. Nos. 5,922,695, 5,935,946, 5,977,089, and 6,043,230 are Invalid, Unenforceable or Not Infringed by the Manufacture, Use or Sale OfTenofovir Disoproxil Fumarate Tablets, 300 mg dated Jan. 25, 2010("2010 Detailed Statement").
Castello and Mattocks, "Discoloration of Tablets Containing Amines and Lactose," J. Pharm. Sci. 51(92):106-108 (Feb. 1962).
"Pill" Encarta Dictionary, 2 pages (2009), retrieved from encarta.msn.com on Mar. 16, 2009.
"Time-Release," Compact Oxford English Dictionary, 1 page (2009), retrieved from www.askoxford.com/concise_oed on Mar. 12, 2009.
Petition for Inter Partes Review of U.S. Pat. No. 5,922,695, for Case No. IPR2014-00885, filed Jun. 4, 2014, 75 pages.
Corrected Petition for Inter Partes Review of U.S. Pat. No. 5,922,695, for Case No. IPR2014-00885, filed Jun. 24, 2014, 74 pages.
Related Matters for Case No. IPR2014-00885, filed Jun. 25, 2014, 6 pages.
Patent Owner Preliminary Response for Case No. IPR2014-00885, filed Sep. 18, 2014, 69 pages.
Moyle, Graeme and Gazzard,Brian, "Current Knowledge and Future Prospects for the Use of HIV Protease Inhibitors," 51:701-712, Drugs, May 1996.
Danner, et al., "A Short-Term Study of the Safety, Pharmacokinetics, and Efficacy of Ritonavir, and Inhibitor of HIV-1 Protease," 333:1528-1533, The New England Journal of Medicine, Dec. 1995.
Link, Derek; "The Collapse of Early Intervention at the Ninth International AIDS Conference," 7(6) Treatment Issues, 1993.
Bechtel-Boenning,Christine;"State of the Art Antiviral Treatment of HIV Infection," 31:1-13, NIH Nursing Clinics of North America, Mar. 1996.
Leary, Warren E.; "F.D.A. Panel Urges Fast Action on Approving a New AIDS Drug," N.Y. Times, Nov. 8, 1995.
HIV/AIDS Historical Time Line 1995-1999, FDA, 6 pages.
Vistide® (cidofovir injection) Package Insert, 2 pages.
Lacy et al., "Evaluation of the Subchronic Toxicity of an Oral Prodrug of the Anti-HIV Nucleotide Analog (9-2-Phosphonylmethoxyethyl)Adenine (PMEA)," 15(1) at 179 Abstract 958, Abstracts of the 34th Annual Meeting of the Society of Toxicology, 1995.
Lalezari et al., "(S)-1-[3-Hydroxy-2-(Phosphonylmethoxy)propyl] cytosine (Cidofovir): Results of Phase I/II Study of a Novel Antiviral Nucleotide Analogue," 171:788-796, The Journal of Infectious Diseases, 1995.
Foscavir® (foscarnet sodium) Package Insert, 2 pages.
Lee, William A. and Martin, John C., "Perspectives on the development of acyclic nucleotide analogs as antiviral drugs", 71:254-259, Antiviral Research, 2006.
Park et al., "Acyclovir Permeation Enhancement Across Intestinal and Nasal Mucosae by Bile Salt-Acylcarnitine Mixed Micelles," 9:1262-1267, Pharmaceutical Research, 1992.
Fix et al., "Acylcarnitines: drug absorption-enhancing agents in the gastrointestinal tract," 251:G332-G340, Am. J. Physiol., 1986.
Fleisher et al., "Improved oral drug delivery: solubility limitations overcome by the use of prodrugs," 19:115-130, Advanced Drug Delivery Reviews, 1996.
Starrett et al., "Synthesis, Oral Bioavailability Determination, and in Vitro Evaluation of Prodrugs of the Antiviral Agent 9-[2-(Phosphonomethoxy)ethyl]adenine (PMEA)," 37:1857-1864, Journal of Medicinal Chemistry, 1994.
Negi et al., "Studies on Orally Active Cephalosporins: Synthesis and Structure-Activity Relationships of New 3-Substituted Carbamoyloxymethyl Cephalosporins," 47:1507-1525, The Journal of Antibiotics, 1994.
Safadi et al., "Phosphoryloxymethyl Carbamates and Carbonates Novel Water—Soluble Prodrugs for Amines and Hindered Alcohols," 10:1350-55, Pharmaceutical Research, 1993.

(56) **References Cited**

OTHER PUBLICATIONS

Samara et al., "Pharmacokinetic Analysis of Diethylcarbonate Prodrugs of Ibuprofen and Naproxen," 16:201-210, Biopharmaceutics & Drug Disposition, 1995.
World Health Organization Model List of Essential Medicines, 18th Ed. (2013), 47 pages.
Institution Decision Case No. IPR2014-00885, filed Dec. 9, 2014, 23 pages.
Mylan's Updated Exhibit List, Case No. IPR2014-00885, dated Jan. 7, 2015, 9 pages.
Rehearing Request, Case No. IPR2014-00885, dated Jan. 8, 2015, 17 pages.
U.S. Pat. No. 5,922,695C1 (Ex Parte Reexamination Certificate) to Murty N. Arimilli, Kenneth C. Cundy, Joseph P. Dougherty, Choung U. Kim, Reza Oliyai and Valentino J. Stella, issued on Oct. 14, 2008 (Mylan's Updated Exhibit List Jan. 7, 2015.
U.S. Pat. No. 5,922,695 Prosecution File History U.S. Appl. No. 08/900,746, filed Jul. 25, 1997.
U.S. Pat. No. 5,922,695 Reexamination File History Reexamination Request U.S. Appl. No. 90/008,555, filed Apr. 30, 2007.
U.S. Pat. No. 5,977,089C1 (Ex Parte Reexamination Certificate) to Murty N. Arimilli, Kenneth C. Cundy, Joseph P. Dougherty, Choung U. Kim, Reza Oliyai and Valentino J. Stella, issued on Nov. 25, 2008.
U.S. Pat. No. 5,977,089 Reexamination File History Reexamination Request U.S. Appl. No. 90/008,550, filed Apr. 30, 2007.
U.S. Pat. No. 6,043,230 (Ex Parte Reexamination Certificate) to Murty N. Arimilli, Kenneth C. Cundy, Joseph P. Dougherty, Choung U. Kim, Reza Oliyai and Valentino J. Stella, issued on Oct. 14, 2008.
U.S. Pat. No. 6,043,230 Prosecution File History U.S. Appl. No. 09/314,606, filed May 19, 1999.
U.S. Pat. No. 6,043,230 Reexamination File History Reexamination Request U.S. Appl. No. 90/008,549, filed Apr. 30, 2007.
U.S. Pat. No. 5,935,946C1 (Ex Parte Reexamination Certificate) to John D. Munger, Jr., John C. Rohloff and Lisa M. Schultze, issued on Oct. 14, 2008.
U.S. Pat. No. 5,935,946 Prosecution File History U.S. Appl. No. 08/900,752, filed Jul. 25, 1997.
U.S. Pat. No. 5,935,946 Reexamination File History Reexamination Request U.S. Appl. No. 90/008,556, filed Apr. 30, 2007.
Gilead Sciences, Inc. v. *Teva Pharmaceuticals USA, Inc.* et al., 10cv-07196-RJS (S. Dist. NY (Foley Square)) (consolidation of 08-cv-10838 ; 12-cv-06351; 12-cv-06294; 12-cv-07910), dated Feb. 4, 2014, 1618 pages.
Fischl, M.A., et al., "The Efficacy of Azidothymidine (AZT) in the Treatment of Patients with AIDS and AIDS Related Complex," New England J. Med. 317:185 (1987).
Richman, D. et al., "The Toxicity of Azidothymidine (AZT) in the Treatment of Patients with AIDS and AIDS Related Complex," New England J. Med. 317:192 (1987).
Larder, B.A., Darby, G. and Richman, D., "HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy," Science 243:1731 (1989).
Kieburtz, KD, et al "Extended follow-up of peripheral neuropathy in patients with AIDS and AIDS related complex treated with dideoxyinosine," J. Acquir. Immune Defic. Syndr. 5:60 (1992).
Allan, J.D., et al. "Long term follow-up of didanosine administered orally twice daily to patients with advanced human immunodeficiency virus infection and hematologic intolerance of zidovudine," Clin. Infect. Dis. 16(S1):S46 (1993).
Simpson, D.M. and Tagliati, M., "Nucleoside Analogue Associated Peripheral Neuropathy in Human Immunodeficiency Virus Infection," J. Acquir. Immune Defic. Syndr. 9:153 (1995).
Van Leeuwen, R., et al., "Evaluation of Safety & Efficacy of 3TC (Lamivudine) in Patients with Asymptomatic or Mildly Symptomatic Human Immunodeficiency Virus Infection: A Phase I/II Study," J. Infect. Dis. 171:1166 (1995).
Beck, EJ., et al. "Survival and the use and costs of hospital services for London AIDS patients treated with AZT," Int. J. STD AIDS 7:507 (1996).
Boucher, C., et al., "High level Resistance to (-31) Enantiomeric 2',Deoxy3'-Thiacytidine in Vitro is Due to One Amino Acid Substitution in the Catalytic Site of Human Immunodeficiency Virus Type 1 Reverse Transcriptase," Antimicro. Agents Chemother. 37:2231 (1993).
Mayers, D., "Rational Approaches to Resistance: Nucleoside Analogues," AIDS 10(S1):S913 (1996).
Cleland, A. Watson, HG, Robertson, P., Ludlum, CA and Brown, HA, "Evolution of zidovudine resistance genotype in HumaImmunodeficiency Virus I in infected patients," AIDS 12:618 (1996).
Anderson, BD, Shirasaka, T, Kojima E, Yarchoan, R and Mitsuya H, "Identification of drug related genotypic changes in HIV1 from serum using the selective polymerase chain reaction," Antiviral Res. 25:24558 (1994).
Volberding, P., "The Need for Additional Options in the Treatment of Human Immunodeficiency Virus Infection," J. Infect. Diseases 171(S2):150 (1995).
Holy, A., Rosenberg, I., Dvorakova, H. and DeClercq, E., "Synthesis and Evaluation of Acyclic Nucleotide Analogs," Nucleosides & Nucleotides 7:667 (1988).
Srinivas, R., Robbins, B., Connelly,M, Gong, Y., Bischofberger, B., Fridland, A., "Metabolism and in Vitro Antiretroviral Activities of Bis(Pivaloyloxymethyl) Prodrugs of Acyclic Nucleoside Phosphonates," Antimicro. Agents Chemother. 37:2247 (1993).
Heljtink, R.A., et al , "Inhibitory effects of acyclic nucleoside phosphonates on human hepatitis B virus and duck hepatitis B virus infections in tissue culturQ," Antimicrobial. Agents Chemotherap. 38:2180 (1994).
Tsai, C., et al., "Prevention of SIV Infection in Macaques by (R)9(2Phosphonylmethoxypropyl)adenine," Science 270:1197 (1995).
Cohen, J., "New Drug Shows Promise in Monkeys," Science 220:1121 (1995).
Gong, Y., Marshall, D., Srinivas, R., Fridland, A., "Susceptibilities of zidovudineresistant variants of human immunodeficiency virus type 1 to inhibitor by acyclic nucleoside phosphonates," Antimicrobial Agents Chemother. 38:1683 (1994).
Examiner's Amendment and Notice of Allowability (dated Apr. 14, 1998), 5 pages.
Kubo, et al. "Nonpeptide angiotensin II receptor antagonists. Synthesis and biological activity of benzimidazolecarboxylic acids." J Med Chem. 1993 vol. 36, Issue No. 15, pp. 2343-2349.
Balzarini, et al. "Differential Antiherpesvirus and Antiretrovirus Effects of the (S) and (R) Enantiomers of Acyclic Nucleoside Phosphonates: Potent and Selective in Vitro and in Vivo Antiretrovirus Activities of (R)-9-(2-Phosphonomethoxypropyl)-2,6-Diaminopurine" Antimicrobial Agents and Chemotherapy, 1993, vol. 37, No. 2, p. 332-338.
James, John S. "AIDS Treatment News, PMPA in Perspective" AIDS Treatment News Issue No. 236, Dec. 1, 1995.
Editorials, "Carnitine Deficiency" Lancet 335:631-33 (1990).
Islam, I., Hinshaw, R., Chong, K-T., Kato,A., Borchardt, R. and Fisher, J.F. "Synthesis and Antiviral Activity of [2-[[4-[3-[(1-Methylethyl)amino]-2-Pyridyl]—Piperazinyl]Carbonyl]-1H-Indol-5-yl] (BHAP) Acylsphingosine HIV Reverse Transcriptase Inhibitors," Bioorg. Chem. 23:499-511 (1995).
De Clercq, E., "Antiviral Therapy for Human Immunodeficiency Virus Infections," Clin. Microbiol. Rev. 8:200-239 (1995).
Bundgaard, H., "Design of prodrugs," pp. 4-5, (1985) 1 page.
Holme, E. et al., "Carnitine deficiency induced by pivampicillin and pivmecillinam therapy" Lancet 2(8661):469-73 (1989).
"Federal Food and Drug Administration Guidance (Drugs) Development of New Stereoisomeric drugs", Chirality, vol. 4, Issue 5, pp. 338-340, 1992.
Bighley, L.D., et al. "Salt forms of drugs and absorption" in Encyclopedia of Pharm. Tech., Eds. J. Swarbrick and J.C. Boylan, vol. 13 (Marcel Dekker, Inc., New York) (1996), 49 pages.
Gould, Phillip L. "Salt selection for basic drugs", International Journal for Pharmaceutics, (1986) vol. 33, pp. 201-217.
Bisoprolol Fumarate Label (Final Printed Labeling, May 1, 1992).

(56) **References Cited**

OTHER PUBLICATIONS

Bischofberger, N, et al. "Bis(POC)PMPA, an orally bioavailable prodrug of the antiretroviral agent PMPA" Conf. on Retroviruses and Opportunistic Infections 4th:104 (abstract No. 214) (Jan. 22-26, 1997).
Expert Declaration of J. Allen McCutchan, M.D., M.Sc. (Dated May 29, 2014).
Expert Declaration of Jed F. Fisher Ph.D. (Dated Jun. 3, 2014).
Bordwell, F.G. "Equilibrium Acidities in Dimethyl Sulfoxide Solution" Acc. Chem. Res. (1988) vol. 21, pp. 456-463.
Miyazaki, T., Yanagida, S., Itoh, A., and Okahara, M., "Synthesis and Alkali-cation Complexing Properties of 12-Crown-4 Derivatives", The Chemical Society of Japan, Bull. Chem. Soc. Jpn., (1982) vol. 55, pp. 2005-2009.
Petition for Inter Partes Review of U.S. Pat. No. 5,935,946 for Case No. IPR2014-00886, filed Jun. 4, 2014, 75 pages.
Corrected Petition for Inter Partes Review of U.S. Pat. No. 5,922,695, for Case No. IPR2014-00886, filed Jun. 24, 2014, 74 pages.
Related Matters for Case No. IPR2014-00886, filed Jun. 25, 2014, 6 pages.
Patent Owner Preliminary Response for Case No. IPR2014-00886, filed Sep. 18, 2014, 70 pages.
Institution Decision Case No. 1PR2014-00886, filed Dec. 9, 2014, 21 pages.
Mylan's Updated Exhibit List, Case No. IPR2014-00886, dated Jan. 7, 2015, 9 pages.
Request for Rehearing IPR2014-00886, dated Jan. 16, 2014, 15 pages.
Physician's Desk Reference (51 ed. 1997), 28 pages.
Famvir® Package Insert, 16 pages.
Hepsera® Package Insert, 29 pages.
Bastin et al., Organic Process Research and Development, 4:427-435, 2000.
Van Westrenen et al., "The synthesis of polyhydroxycarboxylates. Part 6. N-Alkylation of amino compounds by a Michael-type addition with maleate," Recl. Tray. Chim. Pays-Bas, 109:474-478, 1990.
Stahl and Basel, Characterization and Improvement of the Stability Behaviour of Drug Substances in Stability Testing in the EC, Japan and the USA 56 (Dr. Wolfgang Grimm and Dr. Kurt Krummen, eds., 1993.
Petition for Inter Partes Review of U.S. Pat. No. 5,977,089, for Case No. IPR2014-00887, filed Jun. 4, 2014, 64 pages.
Corrected Petition for Inter Partes Review of U.S. Pat. No. 5,977,089, for Case No. IPR2014-00887, filed Jun. 24, 2014, 63 pages.
Related Matters for Case No. IPR2014-00887, filed Jun. 25, 2014, 6 pages.
Patent Owner Preliminary Response for Case No. IPR2014-00887, filed Sep. 18, 2014, 69 pages.
Institution Decision Case No. IPR2014-00887, filed Dec. 9, 2014, 17 pages.
Mylan's Updated Exhibit List, Case No. IPR2014-00887, dated Jan. 7, 2015, 9 pages.
Rehearing Request, Case No. IPR2014-00887, dated Jan. 8, 2015, 17 pages.
Petition for Inter Partes Review of U.S. Pat. No. 6,043,230, for Case No. IPR2014-00888, filed Jun. 4, 2014, 65 pages.
Corrected Petition for Inter Partes Review of U.S. Pat. No. 6,043,230, for Case No. IPR2014-00888, filed Jun. 24, 2014, 64 pages.
Related Matters for Case No. IPR2014-00888, filed Jun. 25, 2014, 6 pages.
Patent Owner Preliminary Response for Case No. IPR2014-00888, filed Sep. 28, 2014, 67 pages.
Institution Decision Case No. IPR2014-00888, filed Dec. 9, 2014, 20 pages.
Mylan's Updated Exhibit List, Case No. IPR2014-00888, dated Jan. 7, 2015, 9 pages.
Rehearing Request, Case No. IPR2014-00888, dated Jan. 8, 2015, 17 pages.
Complaint for Patent Infringement. Document filed by Emory University, Gilead Sciences, Inc, Case No. 1:12-cv-06293-RJS (Filed: Aug. 16, 2012), 14 pages.
Lupins Limited's Answer, Separate Defenses, and Counterclaims to Plaintiffs' Complaint. Document filed by Lupin Limited, Case No. 1:12-cv-06293-RJS (Filed Oct. 22, 2012), 21 pages.
Plaintiffs' Reply to Defendant's Counterclaims. Document filed by Emory University, Gilead Sciences, Inc., Case No. 1:12-cv-06293-RJS (Filed: Nov. 9, 2012), 10 pages.
Letter to Judge Richard J. Sullivan from Peter J. Curtin dated Sep. 3, 2013 Case No. 1:12-cv-06293-RJS (Filed: Sep. 4, 2013), 1 page.
Stipulation and Agreement Regarding U.S. Pat. Nos. 6,703,396, 6,642,245, 5,814,639 and 5,914,331: Case No. 1:12-cv-06293-RJS (Dated: Sep. 4, 2013), 6 pages.
Letter to Judge Richard J. Sullivan from David B. Bassett dated Sep. 16, 2014 re: Settlement. Document filed by Emory University, Gilead Sciences, Inc., Case No. 1:12-cv-06293-RJS (Filed: Sep. 16, 2014), 1 page.
Exhibit A to Letter addressed to Judge Richard J. Sullivan from David B. Bassett dated Sep. 16, 2014 re: Settlement. Document filed by Emory University, Gilead Sciences, Inc., Case No. 1:12-cv-06293-RJS (Filed: Sep. 16, 2014).
Order on Stipulation for Dismissal of Plaintiffs, Gilead Sciences, Inc. ("Gilead") and Emory University ("Emory"), and Defendant, Lupin Limited ("Lupin"), Case No. 1:12-cv-06293-RJS (Dated: Sep. 17, 2014), 2 pages.
Complaint for Patent Infringment. Document filed by Gilead Sciences, Inc., Case No. 1:12-cv-07910-RJS (Filed: Oct. 24, 2012), 9 pages.
Lupins Limited's Answer, Separate Defenses, and Counterclaims to Plaintiff's Complaint. Document filed by Lupin Limited, Case No. 1:12-cv-07910-RJS (Filed: Dec. 20, 2012), 23 pages.
Plaintiff's Reply to Defendant's Counterclaims. Document filed by Gilead Sciences, Inc., Case No. 1:12-cv-07910-RJS (Filed: Jan. 9, 2013), 9 pages.
Order Case No. 1:12-cv-07910-RJS (Dated: Jul. 24, 2013), 1 page.
Complaint for Patent Infringment. Document filed by Gilead Sciences, Inc. Case No. 2:14-cv-00796-JRG-RSP (Filed: Jul. 24, 2014), 14 pages.
Joint Motion for Order on Stipulation for Dismissal. Case No. 2:14-cv-00796-JRG-RSP (Filed: Sep. 16, 2014), 3 pages.
Order on Stipulation for Dismissal. Signed by Magistrate Judge Roy S. Payne on Sep. 23, 2014. (nkl,) Case No. 2:14-cv-00796-JRG-RSP (Dated: Sep. 23, 2014).
Complaint for Patent Infringment. Document filed by Gilead Sciences, Inc. Case No. 1:12-cv-06351-RJS. (Filed:Aug. 20, 2012), 23 pages.
First Amended Complaint for Patent Infringment.Document filed by Gilead Sciences, Inc., Case No. 1:12-cv-06351-RJS (Filed: Sep. 11, 2012), 47 pages.
Cipla Limited's Answer and Separate Defenses to Plaintiff's First Amended Complaint. Document filed by Cipla Limited, Case No. 1:12-cv-06351-RJS) (Filed: Dec. 10, 2012), 30 pages.
Second Amended Complaint for Patent Infringement. Document filed by Gilead Sciences, Inc., Case No. 1:12-cv-06351-RJS (Filed: Oct. 17, 2013), 24 pages.
Defendant Cipla's Unneccesary Opening Claim Construction Brief. Document filed by Cipla Limited, Case No. 1:12-cv-06351-RJS) (Filed: Oct. 25, 2013), 25 pages.
Declaration of Aaron M. Johnson in Support of Defendant Cipla's Unneccesary Opening Claim Construction Brief. Case No. 1:12-cv-06351-RJS (Filed: Oct. 25, 2013), 3 pages.
Stella, et al., "Prodrugs—Do They Have Advantages in Clinical Practice?" *Drugs*, 29:455-473 (1985).
Exhibit 10 to Declaration of Aaron M. Johnson in Support re: 46 Claim Construction Statement. Document filed by Cipla Limited, Case No. 1:12-cv-06351-RJS (Filed: Oct. 25, 2013), 3 pages.
"prophylaxis" *Steadman's Medical Dictionary* (1995), 3 pages.
Plaintiff's Opening Claim Construction Brief. Document filed by Gilead Sciences, Inc., (Filed: Oct. 25, 2013), 18 pages.

(56) **References Cited**

OTHER PUBLICATIONS

Declaration of Jason Johnson. Document filed by Gilead Sciences, Inc., Case No. 1:12-cv06351-RJS (Filed: Oct. 25, 2013), 3 pages.
Exhibit A to Declaration of Jason Johnson in Support of Plaintiff's Opening Claim Construction Brief. Document filed by Gilead Sciences, Inc., Case No. 1:12-cv-06351-RJS (Filed: Oct. 25, 2013), 25 pages.
Exhibit B to Declaration of Jason Johnson in Support of Plaintiff's Opening Claim Construction Brief. Document filed by Gilead Sciences, Inc., Case No. 1:12-cv-06351-RJS (Filed: Oct. 25, 2013), 9 pages.
Exhibit C to Declaration of Jason Johnson in Support of Plaintiff's Opening Claim Construction Brief. Document filed by Gilead Sciences, Inc., Case No. 1:12-cv-06351-RJS (Filed: Oct. 25, 2013), 3 pages.
Gilead Scienes, Inc. Q4 2012 Earnings Results Conference call and Webcast presentation (Feb. 4, 2013), 3 pages.
Gilead Scienes, Inc. Q2 2011 Earnings Results Conference call and Webcast presentation (Jul. 26, 2011), 4 pages.
Exhibit G to of Jason Johnson in Support of Plaintiff's Opening Claim Construction Brief. Document filed by Gilead Sciences, Inc., Case No. 1:12-cv-06351-RJS (Filed: Oct. 25, 2013), 2 pages.
Declaration of Amy Patick in Support of Plaintiff's Opening Claim Construction Document filed by Gilead Sciences, Inc.,. Case No. 1:12-cv-06351-RJS (Filed:Oct. 25, 2013), 13 pages.
Exhibit 1 to Declaration of Amy Patick. Document filed by Gilead Sciences, Inc.,. Case No. 1:12-cv-06351-RJS (Filed:Oct. 25, 2013), 15 pages.
Defendant Cipla Limited's Answer and Separate Defenses to Plaintiff's Second Amended Complaint. Case No. 1:12-cv-06351-RJS (Filed: Nov. 1, 2013), 16 pages.
Defendant Cipla's Reply to Plaintiff's Opening Claim Construction Brief. Document filed by Cipla Limited. Case No. 1:12-cv-06351-RJS (Filed: Nov. 22, 2013), 15 pages.
Second Declaration of Aaron M. Johnson in Support of Defendant Cipla's Reply to Plaintiff's Opening Claim Construction Brief. Document filed by Cipla Limited, Case No. 1:12-cv-06351-RJS (Filed: Nov. 22, 2013) , 2 pages.
Honess, R.W. and Watson, D. H.; "Unity and Diversity in the Herpesviruses," *J. Gen. Virol.* (1977), 37:15-37.
Chang, C., et al, "Expression of the precore region of an avian hepatitis B virus is not required for viral replication," *J. Virol.* 1987, 61(10): 3322.
Plantiff's Rebuttal Claim Construction Brief. Document filed by Gilead Sciences, Inc., Case No. 1:12-cv-06351-RJS (Filed: Nov. 22, 2013), 10 pages.
Declaration of Jason Johnson in Support of Plantiff's Rebuttal Claim Construction Brief. Document filed by Gilead Sciences, Inc., (Case No. 1:12-cv-06351-RJS (Filed: Nov. 22, 2013), 2 pages.
Exhibit A to Declaration of Jason Johnson in Support of Plantiff's Rebuttal Claim Construction Brief. Document filed by Gilead Sciences, Inc., Case No. 1:12-cv-06351-RJS (Filed: Nov. 22, 2013), 5 pages.
Letter addressed to Judge Richard J. Sullivan from Christopher P. Borello dated Jul. 28, 2014, Document filed by Gilead Sciences, Inc., Case No. 1:12-cv-06351-RJS (Filed: Jul. 28, 2014), 1 page.
Order on Stipulation for Dismissal Case No. 1:12-cv-06351-RJS—(Dated: Jul. 29, 2014).
Complaint for Patent Infringment. Filed by Gilead Sciences, Inc., Case No. 1:12-CV-06294-RJS (Filed: Aug. 16, 2012), 19 pages.
Lupin Limited's Answer, Separate Defenses, and Counterclaims to Plaintiff's Complaint. Filed by Lupin Limited Case No. 1:12-CV-06294-RJS (Filed: Oct. 22, 2012), 22 pages.
Plaintiff's Reply to Defendant's Counterclaims. Filed by Gilead Sciences, Inc., Case No. 1:12-CV-06294-RJS (Filed: Nov. 9, 2012), 9 pages.
Order: Filed in Associated Cases:1:12-cv-06294-RJS, 1:12-cv-07910-RJS (Dated: Jul. 23, 2013), 1 page.
Stipulation and Agreement Regarding U.S. Pat. Nos. 5,922,695, 5,935,946, 5,977,089 and 6,043,230: 1. (Dated: Aug. 27, 2013), 4 pages.
Order (Dated: May 30, 2014), 1 page.
Order (Dated May 30, 2014), 4 pages.
Complaint for Patent Infringement. Filed by Gilead Sciences, Inc., Emory University, Case No. 1:14-cv-05352-RJS (Filed Jul. 18, 2014), 12 pages.
Lupin Limited's Answer, Separate Defenses, and Counterclaims to Plaintiff's Complaint. Filed by Lupin Limited.For Case No. 1:14-cv-05352-RJS (Dated: Aug. 22, 2014), 16 pages.
Letter addressed to Judge Richard J. Sullivan from David B. Bassett dated Sep. 16, 2014. Document filed by Emory University, Gilead Sciences, Inc.,Case No. 1:14-cv-05352-RJS (Filed Sep. 16, 2014), 1 page.
Order on Stipulation for Dismissal: Case No. 1:12-cv-06293-RJS (Dated: Sep. 16, 2014), 2 pages.
Complaint for Patent Infringement. Filed by Emory University, Gilead Sciences, Inc., Case No. 1:14-cv-03928-RJS (Filed: Jun. 2, 2014), 18 pages.
Letter addressed to Judge Richard J. Sullivan from Colleen Tracy dated Jun. 27, 2014. Document filed by Emory University, Gilead Sciences, Inc., Case No. 1:14-cv-03928-RJS (Filed: Jun. 27, 2014), 2 pages.
Notice of Voluntary Dismissal Pursuant to F.R.C.P. 41(a)(1)(a)(i) Case No. 1:14-cv-03928-RJS (Dated: Jun. 27, 2014), 1 page.
Complaint for Patent Infringement. Filed by Emory University, Gilead Sciences, Inc., Case No. 1:12-CV-06350 (Filed: Aug. 20, 2012), 14 pages.
Cipla Limited's Answer and Separate Defences to Plaitiffs' Complaint. Filed by Cipla Limited, Case No. 1:12-CV-06350-RJS (Filed: Dec. 10, 2012), 10 pages.
Letter addressed to Judge Richard J. Sullivan from Christopher P. Borello dated Jul. 28, 2014. Document filed by Emory University, Gilead Sciences, Inc., 1:12-cv-06350-RJS (Dated: Jul. 28, 2014), 1 page.
Order on Stipulation for Dismissal. Case No. 1:12-cv-06350-RJS (Dated: Jul. 29, 2014), 2 pages.
Complaint for Patent Infringement. Filed by Gilead Sciences, Inc. and Emory University, Case No. 1:14-cv-00099-IMK (Filed: Jun. 10, 2014), 19-pages.
Appendix 1 to Complaint Filed by Gilead Sciences, Inc. and Emory University, Case No. 1:14-cv-00099-IMK (Filed: Jun. 10, 2014), 19 pages.
Amended Complaint for Patent Infringement. Filed by Gilead Sciences, Inc. and Emory University, Case No. 1:14-cv-00099-IMK (Filed: Jun. 27, 2014), 18 pages.
Answer, Defenses, and Counterclaims of Mylan Pharmaceuticals Inc and Mylan Inc., Filed by Mylan Inc., Mylan Pharmaceuticals Inc., Case No. 1:14-cv-00099-IMK (Filed Aug. 12, 2014), 36 pages.
Plaintiff's Answer to Defendants' Counterclaims. Filed by Gilead Sciences, Inc. and Emory University, Case No. 1:14-cv-00099-IMK (Filed: Sep. 2, 2014), 10 pages.
Amended Answer, Defenses, and Counterclaims of Mylan Pharmaceuticals Inc and Mylan Inc., Filed by Mylan Inc., Mylan Pharmaceuticals Inc., Case No. 1:14-cv-00099-IMK (Filed: Sep. 2, 2014), 36 pages.
Stipulation Concerning Defendants Amended Answer, Defenses and Counterclaims, Case No. 1:14-cv-00099-IMK (Filed: Oct. 2, 2014), 2 pages.
Stipulation Regarding Mylan Inc., Filed by Mylan Inc., Case No. 1:14-cv-00099-IMK (Filed: Dec. 15, 2014), 3 pages.
Second Amended Complaint for Patent Infringement, Filed by Gilead Sciences, Inc. and Emory University,Case No. 1:14-cv-00099-IMK (Filed: Jan. 21, 2015), 21 pages.
Answer, Defenses, and Counterclaims of Mylan Pharmaceuticals Inc and Mylan Inc. To Plaintiffs' Second Amended Complaint, Filed by Mylan Inc., Mylan Pharmaceuticals Inc., Case No. 1:14-cv-00099-IMK (Filed: Feb. 9, 2015), 51 pages.
Joint Stipulation Regarding Claim Construction. Case No. 1:14-cv-00099-IMK (Filed: Feb. 19, 2015), 4 pages.

(56) **References Cited**

OTHER PUBLICATIONS

Gilead's Opening Brief in Support of Its Proposed Claim Constructions for U.S. Pat. Nos. 8,592,397 and 8,716,264. Case No. 1:14-cv-00099-IMK (Filed: Feb. 19, 2015), 27 pages.
Declaration of David B. Bassett in Support of Gilead's Opening Brief in Support of Its Proposed Claim Construction. Case No. 1:14-cv-00099-IMK (Filed: Feb. 19, 2015), 134 pages.
Declaration of Patrick J. Sinko, Ph.D., R.Ph., in Support of Gilead's Opening Brief in Support of Its Proposed Claim Construction. Case No. 1:14-cv-00099-IMK (Filed: Feb. 19, 2015), 171 pages.
Declaration of Carlo-Federico Perno, M.D. Ph.D. in Support of Gilead's Claim Construction., Case No. 1:14-cv-00099-IMK (Filed: Feb. 19, 2015), 14 pages.
Exhibit 1 Declaration of Carlo-Federico Perno, M.D. Ph.D. in Support of Gilead's Claim Construction. Case No. 1:14-cv-00099-IMK (Filed: Feb. 19, 2015), 42 pages.
Exhibit 2 Declaration of Carlo-Federico Perno, M.D. Ph.D. in Support of Gilead's Claim Construction.Case No. 1:14-cv-00099-IMK (Filed: Feb. 19, 2015), 2 pages.
Exhibit 3 Declaration of Carlo-Federico Perno, M.D. Ph.D. in Support of Gilead's Claim Construction. Case No. 1:14-cv-00099-IMK (Filed: Feb. 19, 2015), 3 pages.
Muma, R. D., et al, "Zidovudine adherence among individuals with HIV infection." *AIDS Care.* 1995;7(4):439-47.
Richman, D.D., "Antiretroviral drug resistance: mechanisms, pathogenesis, clinical significance." *Antiviral Chemotherapy* 4, 383-395 J. Mills, Ed., Plenum Press, New York, 1996.
Johnson, V., "Combination therapy for HIV-1 infection-overview: preclinical and clinical analysis of antiretroviral combinations" *Antiviral Research* 29 (1996) 35-39.
Akanbi, M.O., et al, "Combination nucleoside/nucleotide reverse transcriptase inhibitors for treatment of HIV infection" *Expert Opin Pharmacother.* Jan. 2012;13(1):65-79.
De Clercq, E., "Anti-HIV drugs: 25 compounds approved within 25 years after the discovery of HIV" *Int J Antimicrob Agents.* Apr. 2009;33(4):307-20.
Heffernan, J.M. and Wahl, L. M., "Natural variation in HIV infection: Monte Carlo estimates that include CD8 effector cells" *Journal of Theoretical Biology* 243 (2006) 191-204.
Ho, D. D., "Viral Counts Count in HIV Infection" vol. 272, 1124-1125, May 24, 1996.
Mellors, J. W., et al, "Prognosis in HIV-1 Infection Predicted by the Quantity of Virus in Plasma" *Science*,vol. 272, 1167-1170, May 24, 1996.
Plaintiffs' Motion to Strike Mylan's Third Separate Defense. Document filed by Gilead Sciences, Inc. and Emory University, Case No. 1:14-cv-00099-IMK (Filed: Mar. 2, 2015), 3 pages.
Declaration of David Bassett in Support of Plaintiffs' Motion to Strike Mylan's Third Separate Defense. Document filed by Gilead Sciences, Inc. and Emory University, Case No. 1:14-cv-00099-IMK (Filed: Mar. 2, 2015), 12 pages.
Memorandum in Support of Plaintiffs' Motion to Strike Mylan's Third Separate Defense. Document filed by Gilead Sciences, Inc. and Emory University, Case No. 1:14-cv-00099-IMK (Filed: Mar. 2, 2015), 15 pages.
Plaintiffs' Answer to Defentant Mylan Pharmaceuticals Inc.'s Amended Counter Claims. Document filed by Gilead Sciences, Inc. and Emory University, Case No. 1:14-cv-00099-IMK (Filed: Mar. 2, 2015), 12 pages.
Defendants Mylan Inc. and Mylan Pharmaceuticals Inc.'s Answering Claim Construction Brief in Support of Their Proposed Claim Constructions for U.S. Pat. Nos. 8,592,397 and 8,716,264. Document filed by Mylan Inc., Mylan Pharmaceuticals Inc., Case No. 1:14-cv-00099-IMK (Filed: Mar. 12, 2015), 28 pags.
Declaration of Karen M. Cassidy in Support of Defendants Mylan Inc. and Mylan Pharmaceuticals Inc.'s Answering Claim Construction Brief in Support of Their Proposed Claim Constructions for U.S. Pat. Nos. 8,592,397 and 8,716,264. Document filed by Mylan Inc., Mylan Pharmaceuticals Inc., Case No. 1:14-cv-00099-IMK (Filed: Mar. 12, 2015), 199 pages.
Declaration of Choloe Lynne Thio, M.D. in Support of Defendants Mylan Inc. and Mylan Pharmaceuticals Inc.'s Answering Claim Construction Brief in Support of Their Proposed Claim Constructions for U.S. Pat. Nos. 8,592,397 and 8,716,264. Document filed by Mylan Inc., Mylan Pharmaceuticals Inc., Case No. 1:14-cv-00099-IMK (Filed: Mar. 12, 2015), 184 pages.
Declaration of Frank Chrzanowsiu, Ph.D. in Support of Defendants Mylan Inc. and Mylan Pharmaceuticals Inc.'s Answering Claim Construction Brief in Support of Their Proposed Claim Constructions for U.S. Pat. Nos. 8,592,397 and 8,716,264. Document filed by Mylan Inc., Mylan Pharmaceuticals Inc., Case No. 1:14-cv-00099-IMK (Filed: Mar. 12, 2015), 32 pages.
Gilead's Motion to Defer Consideration of Mylan's Indefiniteness Argument or, Alternatively, for Leave to File a Reply. Case No. 1:14-cv-00099-IMK (Filed: Mar. 19, 2015), 8 pages.
Declaration of David B. Bassett in Support of Gilead's Motion to Defer Consideration of Mylan's Indefiniteness Argument or, Alternatively, for Leave to File a Reply., Case No. 1:14-cv-00099-IMK (Filed: Mar. 19, 2015), 30 pages.
Mylan Inc.'s and Mylan Pharmaceuticals Inc.'s Response to Plaintiffs' Motion to Strike, Case No. 1:14-cv-00099-IMK (Filed: Mar. 19, 2015), 3 pages.
Motion Requesting Expedited Briefing for Gilead's Motion to Defer Consideration of Mylan's Indefiniteness Argument or, Alternatively, for Leave to File a Reply., Case No. 1:14-cv-00099-IMK (Filed: Mar. 19, 2015), 3 pages.
Defendants Mylan Inc. and Mylan Pharmaceuticals Inc.'s Response to Gilead's Motion to Defer Consideration of Mylan's Indefiniteness Argument or, Alternatively, for Leave to File a Reply. Case No. 1:14-cv-00099-IMK (Filed: Mar. 26, 2015), 9 pages.
Declaration of William O. Adams in Support of Defendants Mylan Inc. and Mylan Pharmaceuticals Inc.'s Response to Gilead's Motion to Defer Consideration of Mylan's Indefiniteness Argument or, Alternatively, for Leave to File a Reply. Document filed by Mylan Inc., Mylan Pharmaceuticals Inc., Case No. 1:14-cv-00099-IMK (Filed: Mar. 26, 2015), 20 pages.
Reply Memorandum in Support of Gilead's Motion to Defer Consideration of Mylan's Indefiniteness Argument. Document filed by Emory University and Gilead Sciences, Inc., Case No. 1:14-cv-00099-IMK (Filed: Mar. 30, 2015), 6 pages.
Joint Stipulation Regarding Claim Construction. Signed by District Judge Irene M. Keeley on Mar. 31, 2015. (jss), Case No. 1:14-cv-00099-IMK (Filed: Mar. 31, 2015), 4 pages.
Notice of Opposition filed by Alphaphann Pty Ltd in the Australian Patent Office for Application No. 2011253996, dated Nov. 28, 2014, 1 page.
Coffin JM, Hughes SH, Varmus HE, editors, "Retroviruses," Cold Spring Harbor (NY) : Cold Spring Harbor Laboratory Press 1997.
White et al., "Molecular Mechanisms of Resistance to Human Immunodeficiency Virus Type 1 with Reverse Transcriptase Mutations K65R and K65R M184V and Their Effects on Enzyme Function and Viral Replication Capacity," Antimicrobial Agents and Chemotherapy, Nov. 2002, p. 2437-3446.
Viread insert, 2001, DNA 21-356, 20 pages.
Epivir Approval Package Mar. 23 ,1999, 22 pages.
Whitney, "The M184V Mutation in Reverse Transcriptase Can Delay Reversion of Attenuated Variants of Simian Immunodeficiency Virus," Journal of Vrology vol. 76 (17) p. 8958-8962.
Retrovir Product Information (2001), 23 pages.
Jemesk, Poor Virologic Responses and Early Emergence of Resistance in Treatment Naive, HIV-infected Patients Receiving a Once Daily Triple Nucleoside Regimen of Didanosine, Lamivudine, and Tenefoir DF, Program Abstr. Conf, Ertrovir, Oppor Infect 11th 2004 San Francisco Calf, Feb. 8-11, 2004. Abstract 51.
Farthing, "Early Virologic Failure in a Pilot Study Evaluating the Efficacy of Abacavir, Lamivudine and Tenofovir in the Treatment Naive HIV-Infected Patients," The 2nd IAS Conference on HIV Pathogenesis and Treatment Abstract No. 43, Jul. 2003.
Kagan, "Increasing prevalence of HIV-1 reverse transcriptase mutation K65R correlates with tenofovir utilization," 2004, AntiViral Therapy vol. 9 pp. 827-828.

(56) **References Cited**

OTHER PUBLICATIONS

Fisher , "The safety and efficacy of adefovir dipivoxil in patients with advanced HIV disease: a randomized, placebocontrolled trial," Aids vol. 15 p. 1695-1700.
Cihiar, "Human Renal Organic Anion Transporter 1 (Hoat1) and Its Role in the Nephrotoxicity of Antiviral Nucleotide Analogs," Nucleosides ,Nucleotides & nucleic Acids, 2001 vol. 20(4-7) p. 641-648.
Charpentier, "Evolution of the K65R, K103N and M184V/I reverse transcriptase mutations prevalence in HIV-1 infected patients experiencing virologic failure between 2005 and 2010," Abstract CROI 2012.
Grant and Hackh's "Chemical Dictionary" 5th ed. R. Grant (editors) 1987, 7 pages.
Foye's Principles of Medicinal Chemistry 5th ed. 2002, 4 pages.
Center for drug evaluation and research 21-356 Chemistry Review (Viread)—12 pages.
Thoithi et. al, "Investigation of the kinetics of degradation of hexopyranosylated cytosine nucleosides using liquid chromatography" Nucleosides ,Nucleotides & Nucleic Acids (2003).
Calculating the pH of an aquesou solution of TDF, 1 page.
Handbook of Pharmaceutical Excipients, Fourth Edition, edited by Rowe, Sheskey, and Weller, "Lactose and Water," 17 pages (Nov. 2002).
Kashuba, "Antiretroviral-Drug Concentrations in Semen: Implications for Sexual Transmission of Human Immunodeficiency Virus Type 1," Antimicrobial Agents and Chemotherapy 43(8):1817-1826 (1999).
The Emtriva Label, 19 pages.
Chemistry Reviews for NDA 21-752 Truvada, 27 pages.
Clinical pharmacology section of the ANCOBON, 6 pages (Mar. 2003).
Proloprim, 3 pages.
ICH Stability Testing of New Drug Substances and Products 2003, 98 pages.
Reynolds et al. "Available Guidance and Best Practices for Conducting Forced Degradation Studies" Pharm Technol 2002, 9 pages.
Carstensen, J.T., *Advanced Pharmaceutical Solids*, 2001, p. 273, 3 pages.
Novak, "Prevalence of Antiretroviral Drug Resistance Mutations in Chronically HIV-Infected, Treatment-Naïve Patients: Implications for Routine Resistance Screening before Initiation of Antiretroviral Therapy," Clin. Infect Dis. Feb. 1, 2005;40(3) 486-74.
Grant et al., "Preexposure Chemoprophylaxis for HIV Prevention in Men Who Have Sex with Men," New England Journal of Medicine 363:27, 2587 (2010).
Time Magazine, Dec. 9, 2010, "AIDS Drugs Lower the Risk of HIV Infection," by Alice Park.
EMEA (European Medicine Agency) Scientific Discussion on Truvada, 2005, 28 pages.
EMEA (European Medicine Agency) Scientific Discussion on Emtriva, 2005,—30 pages.
Pischetsrieder, "Formation of an Aminoreductone during the Maillard Reaction of Lactose with Nr-Acetyllysine or Proteins," J. Agric. Food Chem. 1998, 46, 928-931.
Bharate, "Interactions and incompatibilities of pharmaceutical excipients with active pharmaceutical ingredients: a comprehensive review" J. Excipients and Food Chem. 1 (3) (2010).
Expert Opinion Prof. Richard M. Novak relating to opposition proceedings being conducted in Israel against Gilead Sciences, Inc. ("The Applicant"), concerning Israel patent application No. IL 169243, Sep. 22, 2012, 40 pages.
Second Expert Opinion Prof. Richard M.Novak relating to opposition proceedings being conducted in Israel against Gilead Sciences, Inc. ("The Applicant"), concerning Israel patent application No. IL 169243, Apr. 9, 2014, 50 pages.
Expert Opinion Prof. Joseph Marian Fortunak relating to opposition proceedings being conducted in Israel against Gilead Sciences, Inc. ("The Applicant"), concerning Israel patent application No. IL 169243, Sep. 20, 2012, 30 pages.
Expert Opinion of Dr. G. Patrick Stahly filed in the Israeli Patent Office in opposition proceedings initiated by Teva Pharmaceutical Industries Ltd. against Israeli Patent Application No. 169,243, filed by GileadSciences Inc., 27 pages.
Truvada Summary of Product Characteristics, 35 pages.
Shapiro and Klein "The Deamination of Cytidine and Cytosine by Acidic Buffer Solutions. Mutagenic Implications," *Biochemistry*, vol. 5, No. 7, Jul. 1966, 2358.
Notari, "A Mechanism for the Hydrolytic Deamination of Cytosine Arabinoside in Aqueous Buffer," *Journal of Pharmaceutical Science*, vol. 56, No. 7, Jul. 1967, 804.
Notari, "intermolecular and Intramolecular Catalysis in Deamination of Cytosine Nucleosides," *Journal of Pharmaceutical Science*, vol. 59, No. 1, Jan. 1970, 28.
Lonnberg, "Competition between the hydrolysis and deandntion of cytidine and its 5-substituted deirivatives in aqueous acid," *Nucleic Acid Research*, vol. 13, Nov. 1985, 2451.
Dong, "Modeling of Autocatalytic Hydrolysis of Adefovir Dipivoxil in Solid Formulations," *Journal of the Pharmaceutical Society of Japan*, 131(4), 643.
Wong, "Major Degradation Product Identified in Several Pharmaceutical Formulkions against the Common Cold," *Anal. Chem.*, 2006, 78, 7891.
Aulton, *Pharmaceutics the Science of Dosage Form Design* (1988), 618.
Augsburger, "Tablet Formulation," *Encyclopedia of Pharmaceutical Technology* (3rd. ed.), 3646, 13 pages (2007).
Armstrong, "Tablet Manufacture by Direct Compression," Encyclopedia of Pharmaceutical Technology (3rd. ed.), 3673-3683 (2007).
Chan, "Excipients: Powders and Solid Dosage Forms," Encyclopedia of Pharmaceutical Technology (3rd. ed.), 1646-1655 (2007).
Airaksinen, "Role of Water in the Physical Stability of Solid Dosage Formulations," *Journal of Pharmaceutical Sciences*, vol. 94, No. 10, 19 pages.
Ahlneck and Zografi, "The molecular basis of moisture effects on the physical and chemical stability of drugs in the solid state," *International Journal of Pharmaceutics*, 62 (1990) 87-95.
Kaupp, "Solid-state reactions, dynamics in molecular crystals," *Current Opinion in Solid State & Material Science*, 6 (2002) 131-138.
Byrn, "Chemical reactivity in solid-state pharmaceuticals: formulation implications," *Advanced Drug Deliveries Reviews* 48 (2001), 115-136.
Li, "The Solid State Michael Addition of Indole to 4-Arylidene-3-methyl-5 pyrazolone," 6*J. Heterocyclic. Chem.*, 36, 697 (1999).
Frantz, "The trouble with making combination drugs,"News & Analysis section of *Nature Review Drug Discovery*, vol. 5, 881, 2 pages.
Summary of Preformulation Report for GS-4331 fumarate salt,1 page, Mar. 4, 1997.
Flemming, "Compaction of lactose drug mixtures: Quantification of the extent of incompatibility by FT-Raman spectroscopy," *European Journal of Pharmaceutics and Biopharmaceutics* 68, 802-810.
Desai, "Preformulation Compatibility Studies of tamsylate and Fluconazole Drugs with Lactose by DSC," *Journal of Thermal Analysis*, vol. 71, 651-658.
Gokhale, "Glycosylation of Aromatic Amines I: Characterization of Reaction Products and Kinetic Scheme," *AAPS PharmaSciTech*, vol. 10, No. 2, 317.
Gallo, "Frequent Detection and Isolation of Cytopathic Retroviruses (HTL V-III) from Patients with AIDS and at Risk for AIDS," *Science, New Series*, vol. 224, No. 4648 (May 4, 1984), 500-503.
Expert Opinion of Prof. Robert R. Redfield in opposition proceedings initiated by Teva Pharmaceutical Industries Ltd. against Israeli Patent Application No. 169,243, filed by Gilead Sciences Inc., 38 pages.
Redfield, "Frequent Transmission of HTLV-III Among Spouses of Patients with AIDS-Related Complex and AIDS", JAMA 1985; 253(11):1571-1573.
Redfield, "The Walter Reed Staging Classification for HTLV III, LAV Infection", *New England Journal of Medicine* 1986; 314: 131-132.

(56) **References Cited**

OTHER PUBLICATIONS

Baeten, "Antiretroviral Prophylaxis for HIV Prevention in Heterosexual Men and Women," New England Journal of Medicine 367:5, 399 (2012).

Thigpen, "Antiretroviral Preexposure Prophylaxis for Heterosexual HIV Transmission in Botsw," New England Journal of Medicine 367:5, 423 (2012).

Highlights of Prescribing Information for Truvada, 45 pages, Jul. 2012.

Grossman, et al., "Drug-Resistant HIV Infection among Drug-Naïve Patients in Israel," Journal of Clinical Infectious Disease 40, 294 (2005).

Bazmi, "In Vitro Selection of Mutations in the Human Immunodeficiency Virus Type 1 Reverse Transcriptase That Decrease Susceptibility to (2)-b-D-Dioxolane-Guanosine and Suppress Resistance to 39-Azido-39-Deoxythymidine," Antimicrobial Agents and Chemotherapy, 44, 1783 (2000).

Roge, "Genotypic and phenotypic changes in antiretroviral-naive patients experiencing failure on randomised treatment with abacavir, didanosine and stavudine," Antiviral Therapy, 7, S125 (2002).

Dart Virology Group and Trial Team "Virological response to a triple nucleoside/nucleotide analogue regimen over 48 weeks in HIV-1-infected adults in Africa," AIDS 20(10), 1391-1399 (2006).

Rey, "Virologic Response of Zidovudine, Lamivudine, and Tenofovir Disoproxil Fumarate Combination in Antiretroviral-Naive HIV-1-Infected Patients," *J. AIDS* 43(5), 530 (2006).

Kirkland, "Response to Lamivudine-Zidovudine plus Abacavir Twice Daily in Antiretroviral-Naive, Incarcerated Patients with HIV Infection Taking Directly Observed Treatment," Clinical Infectious Diseases, 34, 511 (2002).

Little, "Antiretroviral-Drug Resistance Among Patients Recently Infected With HIV," New England Journal of Medicine, 347(6), 385 (2002).

Rubio,"Increase in the frequency of mutation at codon 215 associated with zidovudine resistance in HIV-1-infected antiviral-naive patients from 1989 to 1996," AIDS, 11(9), 1184 (1997).

Winston, "The prevalence and determinants of the K65R mutation in HIV-1 reverse transcriptase in tenofovir-naive patients," AIDS, 16(15), 2087, Oct. 18, 2002.

Pillay, "HIV Type 1 Subtype C Drug Resistance among Pediatric and Adult South African Patients Failing Antiretroviral Therapy," AIDS Research Human Retroviruses, 24, 1449 (2008).

Loemba, "Genetic Divergence of Human Immunodeficiency Virus Type 1 Ethiopian Clade C Reverse Transcriptase (RT) and Rapid Development of Resistance agains," *Antimicrobial Agents and Chemotherapy*, 46(7), 2087, Jul. 2002.

Lambert, "Prevalence of pre-existing resistance-associated mutations to rilpivirine, emtricitabine and tenofovir in antiretroviral-naiv," *Journal of Antimicrobial Chemotherapy*, Jan. 29, 2013.

Anion, "The K65R Mutation Confers Increased DNA Polymerase Processivity to HIV-1 Reverse Transcriptase ," *J. Biological Chemistry*, 37, 15908, Aug. 16, 1996.

Miller, "Human Immunodeficiency Virus Type 1 Reverse Transcriptase Expressing the K7OE Mutation Exhibits a Decrease in Specific Activity and Processivity," Molecular Pharmacology, 54, 291 (1998).

The FDA's press release dated Jul. 2, 2002 reporting the new approved dosing for Epivir (3TC), titled "New Dosing approved for Epivir (lamivudine)," 1 page.

Raune,Pharmacodynamic effects of zidovudine 600 mg once daily versus zidovudine 300 mg twice daily in therapy-naïve HIVinfected patients (COD20002), Late Breaker Poster Exhibition: *The XIV International AIDS Conference* (2002), 1 page.

FDA Press Release Zerit XR, Dec. 31, 2002, titled "Approval of ZERIT® XR, a new formulation that allows once-a-day dosing Dec. 31," 1 page.

FDA Videx EC Approval Letter dated Oct. 31, 2000, 3 pages.

Callendar, "Pharmacokinetics of Oral Zidovudine Entrapped in Biodegradable Nanospheres in Rabbits," *Antimicrobial Agents and Chemotherapy* 43(4), 972, Apr. 1999.

Cohen, 45th *Interscience Conference on Antimicrobial Agents and Chemotherapy* Poster H-521, 1 page.

Elion, "Once-Daily Abacavir/Lamivudine/Zidovudine plus Tenofovir for the Treatment of HIV-1 Infection in Antiretroviral-Naive Subjects: A 48-Week Pilot Study," *HIV Clinical Trials*, 7(6), 324, 2006.

Meier, "Cidofovir-induced End-Stage Renal Failure,"Nephrology Dialysis Transplantation, 17, 148 (2002).

Verhelst, "Fanconi Syndrome and Renal Failure Induced by Tenofovir: A First Case Report," American Journal of Kidney Diseases, 40(6), 1331, Dec. 2002.

Coca, "Rapid Communication: Acute Renal Failure Associated with Tenofovir: Evidence of Drug-Induced Nephrotoxicity," American Journal of Medical Science 324(6), 342 Dec. 2002.

Bowonwatanuwong, "A Randomised, Open Label Study to Investigate Abacavir (Abc) and Lamivudine (3TC) As Once Daily (QD) Components of a Triple Combination Regimen (EPV40001).," 1st IAS Conference, Buenos Aires, Argentina, Jul. 8-11 2001, 1 page.

Statement in Response by Applicant in opposition proceedings initiated by Teva Pharmaceutical Industries Ltd. against Israeli Patent Application No. 169,243, filed by Gilead Sciences Inc., 27 pages.

Memorandum Opinion and Order Granting Plaintiffs' Motion to Defer Consideration of Mylan's Indefiniteness Argument, CV No. 1:14CV99, signed Apr. 6, 2015.

Statement concerning Request for Intervention for Case No. 3k:26-27, Istanbul Patent No. TR 2008/067, dated Apr. 13, 2015, 25 pages.

İlko İlaç San. ve Tic. A.Ş.'s Invalidation Petition for Turkish Patent No. 2008/06970 filed Sep. 10, 2014.

Gilead Sciences, Inc.'s Response to İlko İlaçç San. ye Tic. A.Ş.'s Invalidation Petition for Turkish Patent No. 2008/06970 filed Dec. 31, 2014.

İlko İlaç San. ye Tic. A.Ş.'s Second Invalidation Petition for Turkish Patent No. 2008/06970 field Feb. 26, 2015 (Translation).

* cited by examiner

COMPOSITIONS AND METHODS FOR COMBINATION ANTIVIRAL THERAPY

This application is a Continuation of U.S. application Ser. No. 14/227,653 filed Mar. 27, 2014, which is a continuation of U.S. patent application Ser. No. 12/204,174 now U.S. Pat. No. 8,716,264, filed Sep. 4, 2008, which is a continuation of U.S. patent application Ser. No. 10/540,794, filed Mar. 20, 2006, which is a national stage entry of PCT/US04/00832, filed Jan. 13, 2004 which claims the benefit of Provisional Application Nos. 60/440,246 and 60/440,308, both filed Jan. 14, 2003, the disclosures of each of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The invention relates generally to combinations of compounds with antiviral activity and more specifically with anti-HIV properties. In particular, it relates to chemically stable combinations of structurally diverse anti-viral agents.

BACKGROUND OF THE INVENTION

Human immunodeficiency virus (HIV) infection and related diseases are a major public health problem worldwide. Human immunodeficiency virus type 1 (HIV-1) encodes at least three enzymes which are required for viral replication: reverse transcriptase (RT), protease (Prt), and integrase (Int). Although drugs targeting reverse transcriptase and protease are in wide use and have shown effectiveness, particularly when employed in combination, toxicity and development of resistant strains have limited their usefulness (Palella, et al *N. Engl. J. Med.* (1998) 338:853-860; Richman, D. D. *Nature* (2001) 410:995-1001). Human immunodeficiency virus type 1 (HIV-1) protease (Prt) is essential for viral replication and is an effective target for approved antiviral drugs. The HIV Prt cleaves the viral Gag and Gag-Pol polyproteins to produce viral structural proteins (p17, p24, p7 and p6) and the three viral enzymes. Combination therapy with RT inhibitors has proven to be highly effective in suppressing viral replication to unquantifiable levels for a sustained period of time. Also, combination therapy with RT and Prt inhibitors (PI) have shown synergistic effects in suppressing HIV replication. Unfortunately, a high percentage, typically 30 to 50% of patients currently fail combination therapy due to the development of drug resistance, non-compliance with complicated dosing regimens, pharmacokinetic interactions, toxicity, and lack of potency. Therefore, there is a need for new HIV-1 inhibitors that are active against mutant HIV strains, have distinct resistance profiles, fewer side effects, less complicated dosing schedules, and are orally active. In particular, there is a need for a less onerous dosage regimen, such as once per day oral dosing, optimally with as few pills as possible.

The use of combinations of compounds can yield an equivalent antiviral effect with reduced toxicity, or an increase in drug efficacy. Lower overall drug doses can reduce the frequency of occurrence of drug-resistant variants of HIV. Many different methods have been used to examine the effects of combinations of compounds acting together in different assay systems (Furman WO 02/068058). Lower doses predict better patient compliance when pill burden decreases, dosing schedules are simplified and, optionally, if synergy between compounds occurs (Loveday, C. "Nucleoside reverse transcriptase inhibitor resistance" (2001) *JAIDS Journal of Acquired Immune Deficiency Syndromes* 26:S10—S24). AZT (Zidovudine™, 3'-azido, 3'-deoxythymidine) demonstrates synergistic antiviral activity in vitro in combination with agents that act at HIV-1 replicative steps other than reverse transcription, including recombinant soluble CD4 castanospermine and recombinant interferon-α. However, it must be noted that combinations of compounds can give rise to increased cytotoxicity. For example, AZT and recombinant interferon-α have an increased cytotoxic effect on normal human bone marrow progenitor cells.

Chemical stability of combinations of antiviral agents is an important aspect of co-formulation success and the present invention provides examples of such combinations.

There is a need for new combinations of orally-active drugs for the treatment of patients infected with certain viruses, e.g. HIV, that provide enhanced therapeutic safety and efficacy, impart lower resistance, and predict higher patient compliance.

SUMMARY OF THE INVENTION

The present invention provides combinations of antiviral compounds, in particular compositions and methods for inhibition of HIV. In an exemplary aspect, the invention includes a composition including tenofovir disoproxil fumarate and emtricitabine which has anti-HIV activity. The composition of tenofovir DF and emtricitabine is both chemically stable and either synergistic and/or reduces the side effects of one or both of tenofovir DF and emtricitabine. Increased patient compliance is likely in view of the lower pill burden and simplified dosing schedule.

The present invention relates to therapeutic combinations of [2-(6-amino-purin-9-yl)-1-methyl-ethoxymethyl]-phosphonic acid diisopropoxycarbonyloxymethyl ester fumarate (tenofovir disoproxil fumarate, tenofovir DF, TDF, Viread®) and (2R,5S,cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine, Emtriva™, (−)-cis FTC) and their use in the treatment of HIV infections including infections with HIV mutants bearing resistance to nucleoside and/or non-nucleoside inhibitors. The present invention is also concerned with pharmaceutical compositions and formulations of said combinations of tenofovir disoproxil fumarate and emtricitabine. Another aspect of the invention is a pharmaceutical formulation comprising a physiologically functional derivative of tenofovir disoproxil fumarate or a physiologically functional derivative of emtricitabine.

Therapeutic combinations and pharmaceutical compositions and formulations of the invention include the combination of PMEA or PMPA (tenofovir) compounds with emtricitabine or (2R,5S,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (3TC, lamivudine, Epivir™), and their use in the treatment of HIV infections.

One aspect of the invention is a method for the treatment or prevention of the symptoms or effects of an HIV infection in an infected animal which comprises administering to, i.e. treating, said animal with a therapeutically effective amount of a combination comprising [2-(6-amino-purin-9-yl)-1-methyl-ethoxymethyl]-phosphonic acid diisopropoxycarbonyloxymethyl ester fumarate (tenofovir DF, TDF) or a physiologically functional derivative thereof, and (2R,5S,cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine) or a physiologically functional derivative thereof.

Another aspect of the invention is a unit dosage form of a therapeutic combination comprising tenofovir disoproxil fumarate and emtricitabine, or physiological functional derivatives thereof. The unit dosage form may be formulated

for administration by oral or other routes and is unexpectedly chemically stable in view of the properties of the structurally diverse components.

Another aspect of the invention is directed to chemically stable combination antiviral compositions comprising tenofovir disoproxil fumarate and emtricitabine. In a further aspect of the invention, the chemically stable combinations of tenofovir disoproxil fumarate and emtricitabine further comprise a third antiviral agent. In this three-component mixture, the unique chemical stability of tenofovir disoproxil fumarate and emtricitabine is taken advantage of in order to enable the combination with the third antiviral agent. Particularly useful third agents include, by way of example and not limitation, those of Table A. Preferably, the third component is an agent approved for antiviral use in humans, more preferably, it is an NNRTI or a protease inhibitor (PI), more preferably yet, it is an NNRTI. In a particularly preferred embodiment, the invention is directed to a combination of the chemically stable mixture of tenofovir disoproxil fumarate and emtricitabine together with efavirenz.

Another aspect of the invention is a patient pack comprising at least one, typically two, and optionally, three active ingredients and other antiviral agents selected from tenofovir disoproxil fumarate and emtricitabine, and an information insert containing directions on the use of tenofovir disoproxil fumarate and emtricitabine together in combination.

Another aspect of the invention is a process for preparing the combinations hereinbefore described, which comprises bringing into association tenofovir DF and emtricitabine of the combination in a medicament to provide an antiviral effect. In a further aspect of the present invention, there is provided the use of a combination of the present invention in the manufacture of a medicament for the treatment of any of the aforementioned viral infections or conditions.

DETAILED DESCRIPTION OF THE INVENTION

While the invention will be described in conjunction with the enumerated claims, it will be understood that they are not intended to limit the invention to those claims. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the scope of the present invention as defined by the claims.

Definitions

Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings:

When tradenames are used herein, applicants intend to independently include the tradename product and the active pharmaceutical ingredient(s) of the tradename product.

The term "chemical stability" means that the two primary antiviral agents in combination are substantially stable to chemical degradation. Preferably, they are sufficiently stable in physical combination to permit commercially useful shelf life of the combination product. Typically, "chemically stable" means that a first component of the mixture does not act to degrade a second component when the two are brought into physical combination to form a pharmaceutical dosage form. More typically, "chemically stable" means that the acidity of a first component does not catalyzes or otherwise accelerate the acid decomposition of a second component. By way of example and not limitation, in one aspect of the invention, "chemically stable" means that tenofovir disoproxil fumarate is not substantially degraded by the acidity of emtricitabine. "Substantially" in this context means at least about less than 10%, preferably less than 1%, more preferably less than 0.1%, more preferably yet, less than 0.01% acid degradation of tenofovir disoproxil fumarate over a 24-hour period when the products are in a pharmaceutical dosage form.

The terms "synergy" and "synergistic" mean that the effect achieved with the compounds used together is greater than the sum of the effects that results from using the compounds separately, i.e. greater than what would be predicted based on the two active ingredients administered separately. A synergistic effect may be attained when the compounds are: (1) co-formulated and administered or delivered simultaneously in a combined formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation therapy, a synergistic effect may be attained when the compounds are administered or delivered sequentially, e.g. in separate tablets, pills or capsules, or by different injections in separate syringes. In general, during alternation therapy, an effective dosage of each active ingredient is administered sequentially, i.e. serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together. A synergistic antiviral effect denotes an antiviral effect which is greater than the predicted purely additive effects of the individual compounds of the combination.

The term "physiologically functional derivative" means a pharmaceutically active compound with equivalent or near equivalent physiological functionality to tenofovir DF or emtricitabine when administered in combination with another pharmaceutically active compound in a combination of the invention. As used herein, the term "physiologically functional derivative" includes any: physiologically acceptable salt, ether, ester, prodrug, solvate, stereoisomer including enantiomer, diastereomer or stereoisomerically enriched or racemic mixture, and any other compound which upon administration to the recipient, is capable of providing (directly or indirectly) such a compound or an antivirally active metabolite or residue thereof.

"Bioavailability" is the degree to which the pharmaceutically active agent becomes available to the target tissue after the agent's introduction into the body. Enhancement of the bioavailability of a pharmaceutically active agent can provide a more efficient and effective treatment for patients because, for a given dose, more of the pharmaceutically active agent will be available at the targeted tissue sites.

The compounds of the combinations of the invention may be referred to as "active ingredients" or "pharmaceutically active agents."

The term "prodrug" as used herein refers to any compound that when administered to a biological system generates the drug substance, i.e. active ingredient, as a result of spontaneous chemical reaction(s), enzyme catalyzed chemical reaction(s), and/or metabolic chemical reaction(s).

"Prodrug moiety" means a labile functional group which separates from the active inhibitory compound during metabolism, systemically, inside a cell, by hydrolysis, enzymatic cleavage, or by some other process (Bundgaard, Hans, "Design and Application of Prodrugs" in Textbook of Drug Design and Development (1991), P. Krogsgaard-Larsen and H. Bundgaard, Eds. Harwood Academic Publishers, pp. 113-191). Prodrug moieties can serve to enhance solubility, absorption and lipophilicity to optimize drug delivery, bio-

availability and efficacy. A "prodrug" is thus a covalently modified analog of a therapeutically-active compound.

"Alkyl" means a saturated or unsaturated, branched, straight-chain, branched, or cyclic hydrocarbon radical derived by the removal of one hydrogen atom from a single carbon atom of a parent alkane, alkene, or alkyne. Typical alkyl groups consist of 1-18 saturated and/or unsaturated carbons, such as normal, secondary, tertiary or cyclic carbon atoms. Examples include, but are not limited to: methyl, Me ($—CH_3$), ethyl, Et ($—CH_2CH_3$), acetylenic ($—C{\equiv}CH$), ethylene, vinyl ($—CH{=}CH_2$), 1-propyl, n-Pr, n-propyl ($—CH_2CH_2CH_3$), 2-propyl, i-Pr, i-propyl ($—CH(CH_3)_2$), allyl ($—CH_2CH{=}CH_2$), propargyl ($—CH_2C{\equiv}CH$), cyclopropyl ($—C_3H_5$), 1-butyl, n-Bu, n-butyl ($—CH_2CH_2CH_2CH_3$), 2-methyl-1-propyl, i-Bu, i-butyl ($—CH_2CH(CH_3)_2$), 2-butyl, s-Bu, s-butyl ($—CH(CH_3)CH_2CH_3$), 2-methyl-2-propyl, t-Bu, t-butyl ($—C(CH_3)_3$), 1-pentyl, n-pentyl, ($—CH_2CH_2CH_2CH_2CH_3$), 2-pentyl ($—CH(CH_3)CH_2CH_2CH_3$), 3-pentyl ($—CH(CH_2CH_3)_2$), 2-methyl-2-butyl ($—C(CH_3)_2CH_2CH_3$), cyclopentyl ($—C_5H_9$), 3-methyl-2-butyl ($—CH(CH_3)CH(CH_3)_2$), 3-methyl-1-butyl ($—CH_2CH_2CH(CH_3)_2$), 2-methyl-1-butyl ($—CH_2CH(CH_3)CH_2CH_3$), 1-hexyl ($—CH_2CH_2CH_2CH_2CH_2CH_3$), 5-hexenyl ($—CH_2CH_2CH_2CH_2CH{=}CH_2$) 1-hexyl ($—CH(CH_3)CH_2CH_2CH_2CH_3$), 3-hexyl ($—CH(CH_2CH_3)(CH_2CH_2CH_3)$), cyclohexyl ($—C_6H_{11}$), 2-methyl-2-pentyl ($—C(CH_3)_2CH_2CH_2CH_3$), 3-methyl-2-pentyl ($—CH(CH_3)CH(CH_3)CH_2CH_3$), 4-methyl-2-pentyl ($—CH(CH_3)CH_2CH(CH_3)_2$), 3-methyl-3-pentyl ($—C(CH_3)(CH_2CH_3)_2$), 2-methyl-3-pentyl ($—CH(CH_2CH_3)CH(CH_3)_2$), 2,3-dimethyl-2-butyl ($—C(CH_3)_2CH(CH_3)_2$), and 3,3-dimethyl-2-butyl ($—CH(CH_3)C(CH_3)_3$.

"Aryl" means a monovalent aromatic hydrocarbon radical of 6-20 carbon atoms derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system. Typical aryl groups include, but are not limited to, radicals derived from benzene, substituted benzene, naphthalene, anthracene, biphenyl, and the like.

"Arylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with an aryl radical. Typical arylalkyl groups include, but are not limited to, benzyl, 2-phenylethan-1-yl, 2-phenylethen-1-yl, naphthylmethyl, 2-naphthylethan-1-yl, 2-naphthylethen-1-yl, naphthobenzyl, 2-naphthophenylethan-1-yl and the like. The arylalkyl group 6 to 20 carbon atoms e.g., the alkyl moiety, including alkanyl, alkenyl or alkynyl groups, of the arylalkyl group is 1 to 6 carbon atoms and the aryl moiety is 5 to 14 carbon atoms.

"Substituted alkyl", "substituted aryl", and "substituted arylalkyl" mean alkyl, aryl, and arylalkyl respectively, in which one or more hydrogen atoms are each independently replaced with a substituent. Typical substituents include, but are not limited to, —X, —R, $—O^-$, —OR, —SR, $—S^-$, $—NR_2$, $—NR_3$, $=NR$, $—CX_3$, —CN, —OCN, —SCN, $—N{=}C{=}O$, —NCS, —NO, $—NO_2$, $=N_2$, $—N_3$, $NC(=O)R$, $—C(=O)R$, $—C(=O)NRR$—$S(=O)_2O^-$, $—S(=O)_2OH$, $—S(=O)_2R$, $—OS(=O)_2OR$, $—S(=O)_2NR$, —S(=O)R, $—OP(=O)O_2RR$, $—P(=O)O_2RR$ $—P(=O)(O^-)_2$, $—P(=O)(OH)_2$, $—C(=O)R$, $—C(=O)X$, —C(S)R, —C(O)OR, $—C(O)O^-$, —C(S)OR, —C(O)SR, —C(S)SR, —C(O)NRR, —C(S)NRR, —C(NR)NRR, where each X is independently a halogen: F, Cl, Br, or I; and each R is independently —H, alkyl, aryl, heterocycle, or prodrug moiety.

"Heteroaryl" and "Heterocycle" refer to a ring system in which one or more ring atoms is a heteroatom, e.g. nitrogen, oxygen, and sulfur. Heterocycles are described in: Katritzky, Alan R., Rees, C. W., and Scriven, E. *Comprehensive Heterocyclic Chemistry* (1996) Pergamon Press; Paquette, Leo A.; *Principles of Modern Heterocyclic Chemistry* W. A. Benjamin, New York, (1968), particularly Chapters 1, 3, 4, 6, 7, and 9; "The Chemistry of Heterocyclic Compounds, A series of Monographs" (John Wiley & Sons, New York, 1950 to present), in particular Volumes 13, 14, 16, 19, and 28. Exemplary heterocycles include but are not limited to substituents, i.e. radicals, derived from pyrrole, indole, furan, benzofuran, thiophene, benzothiophene, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-quinolyl, 3-quinolyl, 4-quinolyl, 2-imidazole, 4-imidazole, 3-pyrazole, 4-pyrazole, pyridazine, pyrimidine, pyrazine, purine, cinnoline, pthalazine, quinazoline, quinoxaline, 3-(1,2,4-N)-triazolyl, 5-(1,2,4-IV)-triazolyl, 5-tetrazolyl, 3-N)-oxazole, 5-(1-O, 3-N)-oxazole, 4-(1-S, 3-N)-thiazole, 5-(1-S, 3-N)-thiazole, 2-benzoxazole, 2-benzothiazole, 4-(1,2,3-N)-benzotriazole, and benzimidazole.

Stereochemical definitions and conventions used herein generally follow S. P. Parker, Ed., *McGraw-Hill Dictionary of Chemical Terms* (1984) McGraw-Hill Book Company, New York; and Eliel, E. and Wilen, S., Stereochemistry of Organic Compounds (1994) John Wiley & Sons, Inc., New York. Many organic compounds exist in optically active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L or R and S are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (−) are employed to designate the sign of rotation of plane-polarized light by the compound, with (−) or l meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, these compounds, called stereoisomers, are identical except that they are mirror images of one another. A specific stereoisomer is also referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture or a racemate. The terms "racemic mixture" and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.

The term "chiral" refers to molecules which have the property of non-superimposability of the mirror image partner, while the term "achiral" refers to molecules which are superimposable on their mirror image partner.

The term "stereoisomers" refers to compounds which have identical chemical constitution, but differ with regard to the arrangement of the atoms or groups in space.

"Diastereomer" refers to a stereoisomer with two or more centers of chirality and whose molecules are not mirror images of one another. Diastereomers have different physical properties, e.g. melting points, boiling points, spectral properties, and reactivities. Mixtures of diastereomers may separate under high resolution analytical procedures such as electrophoresis and chromatography.

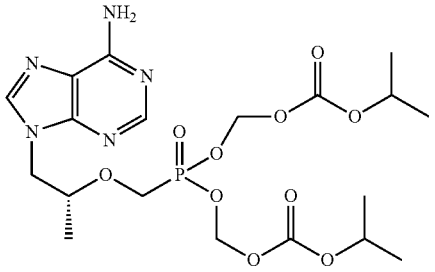
"Enantiomers" refer to two stereoisomers of a compound which are non-superimposable mirror images of one another.

Active Ingredients of the Combinations

The present invention provides novel combinations of two or more active ingredients being employed together. In some embodiments, a synergistic antiviral effect is achieved. In other embodiments, a chemically stable combination is obtained. The combinations include at least one active

ingredient selected from (1) tenofovir disoproxil fumarate and physiologically functional derivatives, and at least one active ingredient selected from (2) emtricitabine and physiologically functional derivatives. The term "synergistic antiviral effect" is used herein to denote an antiviral effect which is greater than the predicted purely additive effects of the individual components (a) and (b) of the combination.

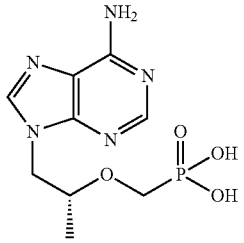
Tenofovir disoproxil fumarate (also known as Viread®, Tenofovir DF. Tenofovir disoproxil, TDF, Bis-POC-PMPA (U.S. Pat. Nos. 5,935,946, 5,922,695, 5,977,089, 6,043,230, 6,069,249) is a prodrug of tenofovir, and has the structure:



and including fumarate salt ($HO_2CCH_2CH_2CO_2^-$).

The chemical names for Tenofovir disoproxil include: [2-(6-amino-purin-9-yl)-1-methyl-ethoxymethyl]-phosphonic acid diisopropoxycarbonyloxymethyl ester; 9-[(R)-2-[[bis[[(isopropoxycarbonyl)oxy]methoxy]phosphinyl] methoxy]propyl]adenine; and 2,4,6,8-tetraoxa-5-phosphanonanedioic acid, 5-[[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy]methyl]-, bis(1-methylethyl) ester, 5-oxide. The CAS Registry numbers include: 201341-05-1; 202138-50-9; 206184-49-8. It should be noted that the ethoxymethyl unit of tenofovir has a chiral center. The R (rectus, right handed configuration) enantiomer is shown. However, the invention also includes the S isomer. The invention includes all enantiomers, diastereomers, racemates, and enriched stereoisomer mixtures of tenofovir (PMPA) and physiologically functional derivatives thereof.

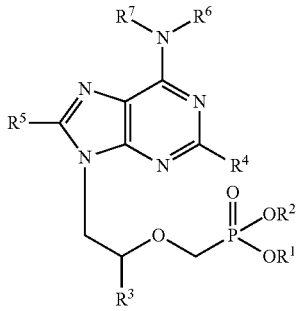
PMPA or tenofovir (U.S. Pat. Nos. 4,808,716, 5,733,788, 6,057,305) has the structure:



The chemical names of PMPA, tenofovir include: (R)-9-(2-phosphonylmethoxypropyl)adenine; and phosphonic acid, [[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy] methyl]. The CAS Registry number is 147127-20-6.

Tenofovir disoproxil fumarate (DF) is a nucleotide reverse transcriptase inhibitor approved in the United States in 2001 for the treatment of HIV-1 infection in combination with other antiretroviral agents. Tenofovir disoproxil fumarate or Viread® (Gilead Science, Inc.) is the fumarate salt of tenofovir disoproxil. Viread® may be named as: 9-[(R)-2-[[bis[[(isopropoxycarbonyl)oxy]methoxy]phosphinyl] methoxy]propyl]adenine fumarate (1:1); or 2,4,6,8-tetraoxa-5-phosphanonanedioic acid, 5-[[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy]methyl]-, bis(1-methylethyl) ester, 5-oxide, (2E)-2-butenedioate (1:1). The CAS Registry number is 202138-50-9.

Physiologically functional derivatives of tenofovir disoproxil fumarate include PMEA (adefovir, 9-((R)-2-(phosphonomethoxy)ethyl)adenine) and PMPA compounds. Exemplary combinations include a PMEA or PMPA compound in combination with emtricitabine or 3TC. PMEA and PMPA compounds have the structures:



where PMEA (R^3 is H) and PMPA (R^3 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, or CH_2OR^8 where R^8 is C_1-C_6 alkyl, C_1-C_6 hydroxyalkyl or C_1-C_6 haloalkyl. R^6 and R^7 are independently H or C_1-C_6 alkyl. R^4 and R^5 are independently H, NH_2, NHR or NR_2 where R is C_1-C_6 alkyl. R^1 and R^2 are independently H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_6-C_{20} arylalkyl, C_6-C_{20} substituted arylalkyl, acyloxymethyl esters $—CH_2OC(=O)R^9$ (e.g. POM) or acyloxymethyl carbonates $—CH_2OC(=O)OR^9$ (e.g. POC) where R^9 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl or C_6-C_{20} substituted aryl. For example, R_1 and R_2 may be pivaloyloxymethoxy, POM, $—CH_2OC(=O)C(CH_3)_3$; $—CH_2OC(=O)OC(CH_3)_3$; or POC, $—CH_2OC(=O)OCH(CH_3)_2$. Also for example, tenofovir has the structure where R^3 is CH_3, and R^1, R^2, R^4, R^5, R^6 and R^7 are H. Dialkyl phosphonates may be prepared according to the methods of: Quast et al (1974) *Synthesis* 490; Stowell et al (1990) *Tetrahedron Lett.* 3261; U.S. Pat. No. 5,663,159.

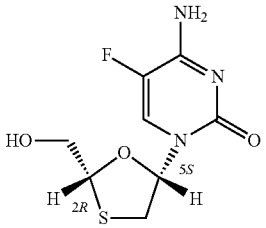
The PMPA compound may be enantiomerically-enriched or purified (single stereoisomer) where the carbon atom bearing R^3 may be the R or S enantiomer. The PMPA compound may be a racemate, i.e. a mixture of R and S stereoisomers.

Adefovir (9-(2-phosphonomethoxyethyl)adenine where R_1-R_7=H) is an exemplary PMEA compound (U.S. Pat. Nos. 4,808,716, 4,724,233). As the bis-pivalate prodrug, Adefovir dipivoxil, also known as bis-POM PMEA, (R_3-R_7=H, R_1 and R_2=$—CH_2OC(=O)C(CH_3)_3$, pivoxil, POM, pivaloyloxymethoxy), is effective against HIV and Hepatitis B infections (U.S. Pat. Nos. 5,663,159, 6,451,340). Adefovir dipivoxil has demonstrated minor to moderate synergistic inhibition of HIV replication in combination with other compounds with anti-HIV activity including PMPA, d4T, ddC, nelfinavir, ritonavir, and saquinavir (Mulato et al (1997) *Antiviral Research* 36:91-97).

The invention includes all enantiomers, diastereomers, racemates, and enriched stereoisomer mixtures of PMEA and PMPA, and physiologically functional derivatives thereof.

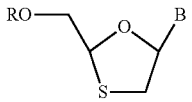
Emtricitabine ((−)-cis-FTC, Emtriva™), a single enantiomer of FTC, is a potent nucleoside reverse tran-

scriptase inhibitor approved for the treatment of HIV (U.S. Pat. Nos. 5,047,407, 5,179,104, 5,204,466, 5,210,085, 5,486,520, 5,538,975, 5,587,480, 5,618,820, 5,763,606, 5,814,639, 5,914,331, 6,114,343, 6,180,639, 6,215,004; WO 02/070518). The single enantiomer emtricitabine has the structure:



The chemical names for emtricitabine include: (−)-cis-FTC; β-L-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane; (2R,5S)-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine; and 4-amino-5-fluoro-1-(2-hydroxymethyl-[1,3]-(2R,5S)-oxathiolan-5-yl)-1H-pyrimidin-2-one. The CAS Registry numbers include: 143491-57-0; 143491-54-7. It should be noted that FTC contains two chiral centers, at the 2 and 5 positions of the oxathiolane ring, and therefore can exist in the form of two pairs of optical isomers (i.e. enantiomers) and mixtures thereof including racemic mixtures. Thus, FTC may be either a cis or a trans isomer or mixtures thereof. Mixtures of cis and trans isomers are diastereomers with different physical properties. Each cis and trans isomer can exist as one of two enantiomers or mixtures thereof including racemic mixtures. The invention includes all enantiomers, diastereomers, racemates, and enriched stereoisomer mixtures of emtricitabine and physiologically functional derivatives thereof. For example, the invention includes physiological functional derivatives such as the 1:1 racemic mixture of the enantiomers (2R,5S,cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine) and its mirror image (2S,5R,cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one, or mixtures of the two enantiomers in any relative amount. The invention also includes mixtures of cis and trans forms of FTC.

Physiologically functional derivatives of emtricitabine include 1,3 oxathiolane nucleosides having the structure:

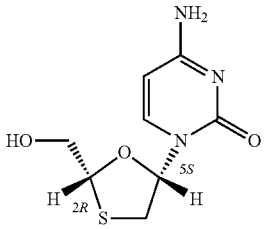


In the 1,3 oxathiolane nucleoside structure above, B is a nucleobase including any nitrogen-containing heterocyclic moiety capable of forming Watson-Crick hydrogen bonds in pairing with a complementary nucleobase or nucleobase analog, e.g. a purine, a 7-deazapurine, or a pyrimidine. Examples of B include the naturally occurring nucleobases: adenine, guanine, cytosine, uracil, thymine, and minor constituents and analogs of the naturally occurring nucleobases, e.g. 7-deazaadenine, 7-deazaguanine, 7-deaza-8-azaguanine, 7-deaza-8-azaadenine, inosine, nebularine, nitropyrrole, nitroindole, 2-aminopurine, 2-amino-6-chloropurine, 2,6-diaminopurine, hypoxanthine, pseudouridine, 5-fluorocytosine, 5-chlorocytosine, 5-bromocytosine, 5-iodocytosine, pseudocytosine, pseudoisocytosine, 5-propynylcytosine, isocytosine, isoguanine, 7-deazaguanine, 2-thiopyrimidine, 6-thioguanine, 4-thiothymine, 4-thiouracil, O^6-methylguanine, N^6-methyladenine, O^4-methylthymine, 5,6-dihydrothymine, 5,6-dihydrouracil, 4-methylindole, pyrazolo[3,4-D]pyrimidines (U.S. Pat. Nos. 6,143,877 and 6,127,121; WO 01/38584), and ethenoadenine (Farman (1989) in Practical Handbook of Biochemistry and Molecular Biology, pp. 385-394, CRC Press, Boca Raton, Fla.).

Nucleobases B may be attached in the configurations of naturally-occurring nucleic acids to the 1,3 oxathiolane moiety through a covalent bond between the N−9 of purines, e.g. adenin-9-yl and guanin-9-yl, or N−1 of pyrimidines, e.g. thymin-1-yl and cytosin-1-yl (Blackburn, G. and Gait, M. Eds. "DNA and RNA structure" in *Nucleic Acids in Chemistry and Biology*, 2nd Edition, (1996) Oxford University Press, pp. 15-81).

Also in the 1,3 oxathiolane nucleoside structure above, R is H, C_1-C_{18} alkyl, C_1-C_{18} substituted alkyl, C_2-C_{18} alkenyl, C_2-C_{18} substituted alkenyl, C_2-C_{18} alkynyl, C_2-C_{18} substituted alkynyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_2-C_{20} heterocycle, C_2-C_{20} substituted heterocycle, phosphonate, phosphophosphonate, diphosphophosphonate, phosphate, diphosphate, triphosphate, polyethyleneoxy, or a prodrug moiety.

Physiologically functional derivatives of emtricitabine also include 3TC (lamivudine, Epivir®), a reverse transcriptase inhibitor approved in the United States for the treatment of HIV-1 infection in combination with AZT as Combivir® (GlaxoSmithKline). U.S. Pat. Nos. 5,859,021; 5,905,082; 6177435; 5627186; 6417191. Lamivudine (U.S. Pat. Nos. 5,587,480, 5,696,254, 5,618,820, 5,756,706, 5,744,596, 568,164, 546,6806, 5,151,426) has the structure:



For example and for some therapeutic uses, 3TC may be a physiologically functional derivative of emtricitabine in combination with tenofovir DF or a physiologically functional derivative of tenofovir DF.

It will be appreciated that tenofovir DF and emtricitabine, and their physiologically functional derivatives may exist in keto or enol tautomeric forms and the use of any tautomeric form thereof is within the scope of this invention. Tenofovir DF and emtricitabine will normally be utilized in the combinations of the invention substantially free of the corresponding enantiomer, that is to say no more than about 5% w/w of the corresponding enantiomer will be present.

Prodrugs

The invention includes all prodrugs of tenofovir and emtricitabine. An exemplary prodrug of tenofovir is tenofovir disoproxil fumarate (TDF, Viread®). A large number of structurally-diverse prodrugs have been described for phosphonic acids (Freeman and Ross in *Progress in Medicinal Chemistry* 34: 112-147 (1997). A commonly used prodrug class is the acyloxyalkyl ester, which was first used as a prodrug strategy for carboxylic acids and then applied to phosphates and phosphonates by Farquhar et al (1983) *J.*

Pharm. Sci. 72: 324; also U.S. Pat. Nos. 4,816,570, 4,968,788, 5,663,159 and 5,792,756. Subsequently, the acyloxyalkyl ester was used to deliver phosphonic acids across cell membranes and to enhance oral bioavailability. A close variant of the acyloxyalkyl ester strategy, the alkoxycarbonyloxyalkyl ester, may also enhance oral bioavailability as a prodrug moiety in the compounds of the combinations of the invention. Aryl esters of phosphorus groups, especially phenyl esters, are reported to enhance oral bioavailability (DeLambert et al (1994) *J. Med. Chem.* 37: 498). Phenyl esters containing a carboxylic ester ortho to the phosphate have also been described (Khamnei and Torrence, (1996) *J. Med. Chem.* 39:4109-4115). Benzyl esters are reported to generate the parent phosphonic acid. In some cases, substituents at the ortho- or para-position may accelerate the hydrolysis. Benzyl analogs with an acylated phenol or an alkylated phenol may generate the phenolic compound through the action of enzymes, e.g. esterases, oxidases, etc., which in turn undergoes cleavage at the benzylic C—O bond to generate the phosphoric acid and the quinone methide intermediate. Examples of this class of prodrugs are described by Mitchell et al (1992) *J. Chem. Soc. Perkin Trans. I*2345; Brook et al WO 91/19721. Still other benzylic prodrugs have been described containing a carboxylic ester-containing group attached to the benzylic methylene (Glazier et al WO 91/19721). Thio-containing prodrugs are reported to be useful for the intracellular delivery of phosphonate drugs. These proesters contain an ethylthio group in which the thiol group is either esterified with an acyl group or combined with another thiol group to form a disulfide. Deesterification or reduction of the disulfide generates the free thio intermediate which subsequently breaks down to the phosphoric acid and episulfide (Puech et al (1993) *Antiviral Res.,* 22: 155-174; Benzaria et al (1996) *J. Med. Chem.* 39: 4958). Cyclic phosphonate esters have also been described as prodrugs of phosphorus-containing compounds.

Prodrug esters in accordance with the invention are independently selected from the following groups: (1) mono-, di-, and tri-phosphate esters of tenofovir or emtricitabine or any other compound which upon administration to a human subject is capable of providing (directly or indirectly) said mono-, di, or triphosphate ester; (2) carboxylic acid esters (3) sulphonate esters, such as alkyl- or aralkylsulphonyl (for example, methanesulphonyl); (4) amino acid esters (for example, alanine, L-valyl or L-isoleucyl); (5) phosphonate; and (6) phosphonamidate esters.

Ester groups (1)-(6) may be substituted with; straight or branched chain C_1-C_{18} alkyl (for example, methyl, n-propyl, t-butyl, or n-butyl); C_3-C_{12} cycloalkyl; alkoxyalkyl (for example, methoxymethyl); arylalkyl (for example, benzyl); aryloxyalkyl (for example, phenoxymethyl); C_5-C_{20} aryl (for example, phenyl optionally substituted by, for example, halogen, C_1-C_4 alkyl, C_1-C_4 alkoxy, or amino; acyloxymethyl esters —$CH_2OC(=O)R^9$ (e.g. POM) or acyloxymethyl carbonates —$CH_2OC(=O)OR^9$ (e.g. POC) where R^9 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl or C_6-C_{20} substituted aryl. For example, ester groups may be: —$CH_2OC(=O)C(CH_3)_3$, —$CH_2OC(=O)OC(CH_3)_3$ or —$CH_2OC(=O)OCH(CH_3)_2$.

An exemplary aryl moiety present in such esters comprises a phenyl or substituted phenyl group. Many phosphate prodrug moieties are described in U.S. Pat. No. 6,312,662; Jones et al (1995) *Antiviral Research* 27:1-17; Kucera et al (1990) *AIDS Res. Hum. Retro Viruses* 6:491-501; Piantadosi et al (1991) *J. Med. Chem.* 34:1408-14; Hosteller et al (1992) *Antimicrob. Agents Chemother.* 36:2025-29; Hostetler et al (1990) *J. Biol. Chem.* 265:611127; and Siddiqui et al (1999) *J. Med. Chem.* 42:4122-28.

Pharmaceutically acceptable prodrugs refer to a compound that is metabolized in the host, for example hydrolyzed or oxidized, by either enzymatic action or by general acid or base solvolysis, to form an active ingredient. Typical examples of prodrugs of the active ingredients of the combinations of the invention have biologically labile protecting groups on a functional moiety of the active compound. Prodrugs include compounds that can be oxidized, reduced, aminated, deaminated, esterified, deesterified, alkylated, dealkylated, acylated, deacylated, phosphorylated, dephosphorylated, or other functional group change or conversion involving forming or breaking chemical bonds on the prodrug.

Chemical Stability of a Pharmaceutical Formulation

The chemical stability of the active ingredients in a pharmaceutical formulation is of concern to minimize the generation of impurities and ensure adequate shelf-life. The active ingredients, tenofovir disoproxil fumarate and emtricitabine, in the pharmaceutical formulations of the invention have relatively low pKa values, indicative of the potential to cause acidic hydrolysis of the active ingredients. Emtricitabine, with a pKa of 2.65 (Emtriva™ Product Insert, Gilead Sciences, Inc. 2003, available at gilead.com) is subject to hydrolytic deamination of the 5-fluoro cytosine nucleobase to form the 5-fluoro uridine nucleobase. Tenofovir disoproxil fumarate, with a pKa of 3.75 (Yuan L. et al "Degradation Kinetics of Oxycarbonyloxymethyl Prodrugs of Phosphonates in Solution", *Pharmaceutical Research* (2001) Vol. 18, No. 2, 234-237), is subject also to hydrolytic deamination of the exocyclic amine of the adenine nucleobase, and to hydrolysis of one or both of the POC ester groups (U.S. Pat. No. 5,922,695). It is desirable to formulate a therapeutic combination of tenofovir disoproxil fumarate and emtricitabine, and the physiological functional derivatives thereof, with a minimum of impurities and adequate stability.

The combinations of the present invention provide combination pharmaceutical dosage forms which are chemically stable to acid degradation of: (1) a first component (such as tenofovir disoproxil fumarate, and physiological functional derivatives; (2) a second component (such as emtricitabine, and physiological functional derivatives; and (3) optionally a third component having antiviral activity. The third component includes anti-HIV agents and include: protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), and integrase inhibitors. Exemplary third active ingredients to be administered in combination with first and second components are shown in Table A. First and second components are as defined in the above section entitled: ACTIVE INGREDIENTS OF THE COMBINATIONS.

Salts

Any reference to any of the compounds in the compositions of the invention also includes any physiologically acceptable salt thereof. Examples of physiologically acceptable salts of tenofovir DF, emtricitabine and their physiologically functional derivatives include salts derived from an appropriate base, such as an alkali metal (for example, sodium), an alkaline earth (for example, magnesium), ammonium and NX_4^+(wherein X is C_1-C_4 alkyl), or an organic acid such as fumaric acid, acetic acid, succinic acid. Physiologically acceptable salts of an hydrogen atom or an amino group include salts of organic carboxylic acids such as acetic, benzoic, lactic, fumaric, tartaric, maleic, malonic, malic, isethionic, lactobionic and succinic acids; organic

sulfonic acids, such as methanesulfonic, ethanesulfonic, benzenesulfonic and p-toluenesulfonic acids; and inorganic acids, such as hydrochloric, sulfuric, phosphoric and sulfamic acids. Physiologically acceptable salts of a compound of an hydroxy group include the anion of said compound in combination with a suitable cation such as Na^+ and NX_4^+ (wherein X is independently selected from H or a C_1-C_4 alkyl group).

For therapeutic use, salts of active ingredients of the combinations of the invention will be physiologically acceptable, i.e. they will be salts derived from a physiologically acceptable acid or base. However, salts of acids or bases which are not physiologically acceptable may also find use, for example, in the preparation or purification of a physiologically acceptable compound. All salts, whether or not derived from a physiologically acceptable acid or base, are within the scope of the present invention.

Administration of the Formulations

While it is possible for the active ingredients of the combination to be administered alone and separately as monotherapies, it is preferable to administer them as a pharmaceutical co-formulation. A two-part or three-part combination may be administered simultaneously or sequentially. When administered sequentially, the combination may be administered in one, two, or three administrations.

Preferably, two-part or three-part combinations are administered in a single pharmaceutical dosage form. More preferably, a two-part combination is administered as a single oral dosage form and a three-part combination is administered as two identical oral dosage forms. Examples include a single tablet of tenofovir disoproxil fumarate and emtricitabine, or two tablets of tenofovir disoproxil fumarate, emtricitabine, and efavirenz.

It will be appreciated that the compounds of the combination may be administered: (1) simultaneously by combination of the compounds in a co-formulation or (2) by alternation, i.e. delivering the compounds serially, sequentially, in parallel or simultaneously in separate pharmaceutical formulations. In alternation therapy, the delay in administering the second, and optionally a third active ingredient, should not be such as to lose the benefit of a synergistic therapeutic effect of the combination of the active ingredients. By either method of administration (1) or (2), ideally the combination should be administered to achieve peak plasma concentrations of each of the active ingredients. A one pill once-per-day regimen by administration of a combination co-formulation may be feasible for some HIV-positive patients. Effective peak plasma concentrations of the active ingredients of the combination will be in the range of approximately 0.001 to 100 μM. Optimal peak plasma concentrations may be achieved by a formulation and dosing regimen prescribed for a particular patient. It will also be understood that tenofovir DF and emtricitabine, or the physiologically functional derivatives of either thereof, whether presented simultaneously or sequentially, may be administered individually, in multiples, or in any combination thereof. In general, during alternation therapy (2), an effective dosage of each compound is administered serially, where in co-formulation therapy (1), effective dosages of two or more compounds are administered together.

Formulation of the Combinations

When the individual components of the combination are administered separately they are generally each presented as a pharmaceutical formulation. The references hereinafter to formulations refer unless otherwise stated to formulations containing either the combination or a component compound thereof. It will be understood that the administration of the combination of the invention by means of a single patient pack, or patient packs of each formulation, within a package insert diverting the patient to the correct use of the invention is a desirable additional feature of this invention. The invention also includes a double pack comprising in association for separate administration, formulations of tenofovir disoproxil fumarate and emtricitabine, or a physiologically functional derivative of either or both thereof.

The combination therapies of the invention include: (1) a combination of tenofovir DF and emtricitabine or (2) a combination containing a physiologically functional derivative of either or both thereof.

The combination may be formulated in a unit dosage formulation comprising a fixed amount of each active pharmaceutical ingredient for a periodic, e.g. daily, dose or subdose of the active ingredients.

Pharmaceutical formulations according to the present invention comprise a combination according to the invention together with one or more pharmaceutically acceptable carriers or excipients and optionally other therapeutic agents. Pharmaceutical formulations containing the active ingredient may be in any form suitable for the intended method of administration. When used for oral use for example, tablets, troches, lozenges, aqueous or oil suspensions, dispersible powders or granules, emulsions, hard or soft capsules, syrups or elixirs may be prepared (*Remington's Pharmaceutical Sciences* (Mack Publishing Co., Easton, Pa.). Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents including antioxidants, sweetening agents, flavoring agents, coloring agents and preserving agents, in order to provide a palatable preparation. Tablets containing the active ingredient in admixture with non-toxic pharmaceutically acceptable excipient which are suitable for manufacture of tablets are acceptable. These excipients may be, for example, inert diluents, such as calcium or sodium carbonate, lactose, lactose monohydrate, croscarmellose sodium, povidone, calcium or sodium phosphate; granulating and disintegrating agents, such as maize starch, or alginic acid; binding agents, such as cellulose, microcrystalline cellulose, starch, gelatin or acacia; and lubricating agents, such as magnesium stearate, stearic acid or talc. Tablets may be uncoated or may be coated by known techniques including microencapsulation to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate alone or with a wax may be employed.

Formulations for oral use may be also presented as hard gelatin capsules where the active ingredient is mixed with an inert solid diluent, for example pregelatinized starch, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, such as peanut oil, liquid paraffin or olive oil.

Aqueous suspensions of the invention contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients include a suspending agent, such as sodium carboxymethylcellulose, methylcellulose, hydroxypropyl methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as a naturally occurring phosphatide (e.g., lecithin), a condensation product of an alkylene oxide with a fatty acid (e.g., polyoxyethylene stearate), a condensation product of ethyl-

ene oxide with a long chain aliphatic alcohol (e.g., heptadecaethyleneoxycetanol), a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol anhydride (e.g., polyoxyethylene sorbitan monooleate). The aqueous suspension may also contain one or more preservatives such as ethyl or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents, such as sucrose, sucralose or saccharin.

Oil suspensions may be formulated by suspending the active ingredient in a vegetable oil, such as arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oral suspensions may contain a thickening agent, such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents, such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid, BHT, etc.

Dispersible powders and granules of the invention suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, a suspending agent, and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those disclosed above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions or liposome formulations. The oily phase may be a vegetable oil, such as olive oil or arachis oil, a mineral oil, such as liquid paraffin, or a mixture of these. Suitable emulsifying agents include naturally-occurring gums, such as gum acacia and gum tragacanth, naturally occurring phosphatides, such as soybean lecithin, esters or partial esters derived from fatty acids and hexitol anhydrides, such as sorbitan monooleate, and condensation products of these partial esters with ethylene oxide, such as polyoxyethylene sorbitan monooleate. The emulsion may also contain sweetening and flavoring agents. Syrups and elixirs may be formulated with sweetening agents, such as glycerol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, a flavoring or a coloring agent.

The pharmaceutical compositions of the invention may be in the form of a sterile injectable preparation, such as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, such as a solution in 1,3-butane-diol or prepared as a lyophilized powder. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile fixed oils may conventionally be employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid may likewise be used in the preparation of injectables.

The pharmaceutical compositions of the invention may be injected parenterally, for example, intravenously, intraperitoneally, intrathecally, intraventricularly, intrastemally, intracranially, intramuscularly or subcutaneously, or they may be administered by infusion techniques. They are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art.

The pharmaceutical compositions of the invention may also be administered intranasally or by inhalation and are conveniently delivered in the form of a dry powder inhaler or an aerosol spray presentation from a pressurized container or a nebuliser with the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFC 134a), carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurized container or nebuliser may contain a solution or suspension of the composition, e.g. using a mixture of ethanol and the propellant as the solvent, which may additional contain a lubricant, e.g. sorbitan trioleate. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated to contain a powder mix of a compound of the formula (I) and a suitable powder base such as lactose or starch. Aerosol or dry powder formulations are preferably arranged so that each metered dose or "puff" contains from 20 μg to 20 mg of a composition for delivery to the patient. The overall daily dose with an aerosol will be in the range of from 20 μg to 20 mg which may be administered in a single dose or, more usually, in divided doses throughout the day.

The amount of active ingredient that may be combined with the carrier material to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a time-release formulation intended for oral administration to humans may contain approximately 1 to 1000 mg of active material compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95% of the total compositions (weight:weight). The pharmaceutical composition can be prepared to provide easily measurable amounts for administration. For example, an aqueous solution intended for intravenous infusion may contain from about 3 to 500 μg of the active ingredient per milliliter of solution in order that infusion of a suitable volume at a rate of about 30 mL/hr can occur. As noted above, formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be administered as a bolus, electuary or paste.

The combinations of the invention may conveniently be presented as a pharmaceutical formulation in a unitary dosage form. A convenient unitary dosage formulation contains the active ingredients in any amount from 1 mg to 1 g each, for example but not limited to, 10 mg to 300 mg. The synergistic effects of tenofovir DF in combination with emtricitabine may be realized over a wide ratio, for example 1:50 to 50:1 (tenofovir DF:emtricitabine). In one embodiment, the ratio may range from about 1:10 to 10:1. In another embodiment, the weight/weight ratio of tenofovir to emtricitabine in a co-formulated combination dosage form, such as a pill, tablet, caplet or capsule will be about 1, i.e. an approximately equal amount of tenofovir DF and emtricitabine. In other exemplary co-formulations, there may be more or less tenofovir than FTC. For example, 300 mg

tenofovir DF and 200 mg emtricitabine can be co-formulated in a ratio of 1.5:1 (tenofovir DF: emtricitabine). In one embodiment, each compound will be employed in the combination in an amount at which it exhibits antiviral activity when used alone. Exemplary Formulations A, B, C, D, E, and F (Examples) have ratios of 12:1 to 1:1 (tenofovir DF:emtricitabine). Exemplary Formulations A, B, C, D, E, and F use amounts of tenofovir DF and emtricitabine ranging from 25 mg to 300 mg. Other ratios and amounts of the compounds of said combinations are contemplated within the scope of the invention.

A unitary dosage form may further comprise tenofovir DF and emtricitabine, or physiologically functional derivatives of either thereof, and a pharmaceutically acceptable carrier.

It will be appreciated by those skilled in the art that the amount of active ingredients in the combinations of the invention required for use in treatment will vary according to a variety of factors, including the nature of the condition being treated and the age and condition of the patient, and will ultimately be at the discretion of the attending physician or health care practitioner. The factors to be considered include the route of administration and nature of the formulation, the animal's body weight, age and general condition and the nature and severity of the disease to be treated. For example, in a Phase I/II monotherapy study of emtricitabine, patients received doses ranging from 25 mg to 200 mg twice-a-day for two weeks. At each dose regimen greater or equal to 200 mg, a 98-percent (1.75 log 10) or greater viral suppression was observed. A once-a-day dose of 200 mg of emtricitabine reduced the viral load by an average of 99 percent (1.92 log 10). Viread® (tenofovir DF) has been approved by the FDA for the treatment and prophylaxis of HIV infection as a 300 mg oral tablet. Emtriva™ (emtricitabine) has been approved by the FDA for the treatment of HIV as a 200 mg oral tablet.

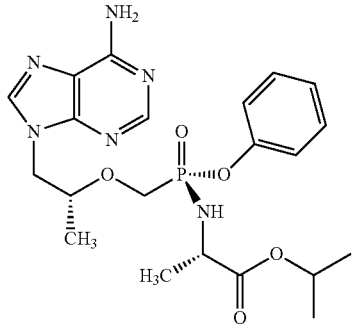
It is also possible to combine any two of the active ingredients in a unitary dosage form for simultaneous or sequential administration with a third active ingredient. The three-part combination may be administered simultaneously or sequentially. When administered sequentially, the combination may be administered in two or three administrations. Third active ingredients have anti-HIV activity and include protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), and integrase inhibitors. Exemplary third active ingredients to be administered in combination with tenofovir DF, emtricitabine, and their physiological functional derivatives, are shown in Table A.

TABLE A

5,6 dihydro-5-azacytidine
5-aza 2'deoxycytidine
5-azacytidine
5-yl-carbocyclic 2'-deoxyguanosine (BMS200,475)
9 (arabinofuranosyl)guanine; 9-(2' deoxyribofuranosyl)guanine
9-(2'-deoxy 2'fluororibofuranosyl)-2,6-diaminopurine
9-(2'-deoxy 2'fluororibofuranosyl)guanine
9-(2'-deoxyribofuranosyl)-2,6 diaminopurine
9-(arabinofuranosyl)-2,6 diaminopurine
Abacavir, Ziagen ®
Acyclovir, ACV; 9-(2-hydroxyethoxylmethyl)guanine
Adefovir dipivoxil, Hepsera ®
amdoxivir, DAPD
Amprenavir, Agenerase ®
araA; 9-β-D-arabinofuranosyladenine (Vidarabine)
atazanivir sulfate (Reyataz ®)
AZT; 3'-azido-2',3'-dideoxythymdine, Zidovudine, (Retrovir ®)
BHCG; (.+−.)-(1a,2b,3a)-9-[2,3-bis(hydroxymethyl)cyclobutyl]guanine
BMS200,475; 5-yl-carbocyclic 2'-deoxyguanosine
Buciclovir; (R) 9-(3,4-dihydroxybutyl)guanine
BvaraU; 1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil (Sorivudine)
Calanolide A
Capravirine
CDG; carbocyclic 2'-deoxyguanosine
Cidofovir,
HPMPC; (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine
Clevudine, L-FMAU; 2'-Fluoro-5-methyl-β-L-arabino-furanosyluracil
Combivir ® (lamivudine/zidovudine)
Cytallene; [1-(4'-hydroxy-1',2'-butadienyl)cytosine]
d4C; 3'-deoxy-2',3'-didehydrocytidine
DAPD; (−)-β-D-2,6-diaminopurine dioxolane
ddA; 2',3'-dideoxyadenosine
ddAPR; 2,6-diaminopurine-2',3'-dideoxyriboside
ddC; 2',3'-dideoxycytidine (Zalcitabine)
ddI; 2',3'-dideoxyinosine, didanosine, (Videx ®, Videx ® EC)
Delavirdine, Rescriptor ®
Didanosine, ddI, Videx ®; 2',3'-dideoxyinosine
DXG; dioxolane guanosine
E-5-(2-bromovinyl)-2'-deoxyuridine
Efavirenz, Sustiva ®
Enfuvirtide, Fuzeon ®
F-ara-A; fluoroarabinosyladenosine (Fludarabine)
FDOC; (−)-β-D-5-fluoro-1-[2-(hydroxymethyl)-1,3-dioxolane]cytosine
FEAU; 2'-deoxy-2'-fluoro-1-β-D-arabinofuranosyl-5-ethyluracil
FIAC; 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine
FIAU; 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouridine
FLG; 2',3'-dideoxy-3'-fluoroguanosine
FLT; 3'-deoxy-3'-fluorothymidine
Fludarabine; F-ara-A; fluoroarabinosyladenosine
FMAU; 2'-Fluoro-5-methyl-β-L-arabino-furanosyluracil
FMdC
Foscarnet; phosphonoformic acid, PFA
FPMPA; 9-(3-fluoro-2-phosphonylmethoxypropyl)adenine
Gancyclovir, GCV; 9-(1,3-dihydroxy-2-propoxymethyl)guanine
GS-7340; 9-[R-2-[[(S)-[[(S)-1-(isopropoxycarbonyl)ethyl]amino]-phenoxyphosphinyl]methoxy]propyl]adenine
HPMPA; (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine
HPMPC; (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (Cidofovir)
Hydroxyurea, Droxia ®
Indinavir, Crixivan ®
Kaletra ® (lopinavir/ritonavir)
Lamivudine,
3TC, Epivir ™; (2R, 5S, cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one
L-d4C; L-3'-deoxy-2',3'-didehydrocytidine
L-ddC; L-2',3'-dideoxycytidine
L-Fd4C; L-3'-deoxy-2',3'-didehydro-5-fluorocytidine
L-FddC; L-2',3'-dideoxy-5-fluorocytidine
Lopinavir
Nelfinavir, Viracept ®
Nevirapine, Viramune ®
Oxetanocin A;
9-(2-deoxy-2-hydroxymethyl-β-D-erythro-oxetanosyl)adenine
Oxetanocin G;
9-(2-deoxy-2-hydroxymethyl-β-D-erythro-oxetanosyl)guanine
Penciclovir
PMEDAP; 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine
PMPA, tenofovir; (R)-9-(2-phosphonylmethoxypropyl)adenine
PPA; phosphonoacetic acid
Ribavirin; 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide
Ritonavir, Norvir ®
Saquinavir, Invirase ®, Fortovase ®
Sorivudine, BvaraU; 1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil
Stavudine, d4T, Zerit ®; 2',3'-didehydro-3'-deoxythymidine
Trifluorothymidine, TFT; Trifluorothymidine
Trizivir ® (abacavir sulfate/lamivudine/zidovudine)
Vidarabine, araA; 9-β-D-arabinofuranosyladenine
Zalcitabine, Hivid ®, ddC; 2',3'-dideoxycytidine
Zidovudine, AZT, Retrovir ®; 3'-azido-2',3'-dideoxythymdine
Zonavir; 5-propynyl-1-arabinosyluracil

Another aspect of the present invention is a three-part combination comprising tenofovir DF, FTC, and 9-[(R)-2-[[(5)-[[(S)-1-(isopropoxycarbonyl)ethyl]amino]phenoxy-

phosphinyl]methoxy]propyl] adenine, also designated herein as GS-7340, which has the structure:



GS-7340 is a prodrug of tenofovir and the subject of commonly owned, pending application, U.S. Ser. No. 09/909,560, filed Jul. 20, 2001 and Becker et al WO 02/08241.

For example, a ternary unitary dosage may contain 1 mg to 1000 mg of tenofovir disoproxil fumarate, 1 mg to 1000 mg of emtricitabine, and 1 mg to 1000 mg of the third active ingredient. As a further feature of the present invention, a unitary dosage form may further comprise tenofovir DF, emtricitabine, the third active ingredient, or physiologically functional derivatives of the three active ingredients thereof, and a pharmaceutically acceptable carrier.

Combinations of the present invention enable patients greater freedom from multiple dosage medication regimens and ease the needed diligence required in remembering and complying with complex daily dosing times and schedules. By combining tenofovir disoproxil fumarate and emtricitabine into a single dosage form, the desired daily regimen may be presented in a single dose or as two or more sub-doses per day. The combination of co-formulated tenofovir DF and emtricitabine may be administered as a single pill, once per day.

A further aspect of the invention is a patient pack comprising at least one active ingredient: tenofovir disoproxil fumarate, emtricitabine, or a physiologically functional derivative of either of the combination and an information package or product insert containing directions on the use of the combination of the invention.

Segregation of active ingredients in pharmaceutical powders and granulations is a widely recognized problem that can result in inconsistent dispersions of the active ingredients in final dosage forms. Some of the main factors contributing to segregation are particle size, shape and density. Segregation is particularly troublesome when attempting to formulate a single homogenous tablet containing multiple active ingredients having different densities and different particle sizes. Glidants are substances that have traditionally been used to improve the flow characteristics of granulations and powders by reducing interparticulate friction. See Lieberman, *Lachman, & Schwartz, Pharmaceutical Dosage Forms: Tablets, Volume* 1, p. 177-178 (1989), incorporated herein by reference. Glidants are typically added to pharmaceutical compositions immediately prior to tablet compression to facilitate the flow of granular material into the die cavities of tablet presses. Glidants include: colloidal silicon dioxide, asbestos free talc, sodium aluminosilicate, calcium silicate, powdered cellulose, microcrystalline cellulose, corn starch, sodium benzoate, calcium carbonate, magnesium carbonate, metallic stearates, calcium stearate, magnesium stearate, zinc stearate, stearowet C, starch, starch 1500, magnesium lauryl sulfate, and magnesium oxide. Exemplary Tablet Formulation A has colloidal silicon dioxide (Examples). Glidants can be used to increase and aid blend composition homogeneity in formulations of anti-HIV drugs (U.S. Pat. No. 6,113,920). The novel compositions of the present invention may contain glidants to effect and maintain homogeneity of active ingredients during handling prior to tablet compression.

The present invention provides pharmaceutical formulations combining the active ingredients tenofovir DF and emtricitabine, or physiologically functional derivatives thereof, in a sufficiently homogenized form, and a method for using this pharmaceutical formulation. An object of the present invention is to utilize glidants to reduce the segregation of active ingredients in pharmaceutical compositions during pre-compression material handling. Another object of the present invention is to provide a pharmaceutical formulation combining the active ingredients tenofovir DF and emtricitabine, or physiologically functional derivatives thereof, with a pharmaceutically acceptable glidant, resulting in a mixture characterized by a pharmaceutically acceptable measure of homogeneity.

Formulations include those suitable for oral, rectal, nasal, topical (including transdermal, buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods represent a further feature of the present invention and include the step of bringing into association the active ingredients with the carrier, which constitutes one or more accessory ingredients, and maintaining chemical stability. In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, caplets, cachets or tablets each containing a predetermined amount of the active ingredients; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. povidone, gelatin, hydroxypropyl methylcellulose), lubricant, inert diluent, preservative, disintegrant (e.g. sodium starch glycollate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose) surface-active or dispersing agent. Molded tablets may be made by molding a mixture of the powdered compound moistened with an inert liquid diluent in a suitable machine. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredients therein using, for example, cellulose ether derivatives (e.g., hydroxypropyl methylcellulose) or methacrylate derivatives in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredients in

a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier. Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylates. Topical administration may also be by means of a transdermal iontophoretic device.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for penile administration for prophylactic or therapeutic use may be presented in condoms, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Pharmaceutical formulations suitable for rectal administration wherein the carrier is a solid are most preferably presented as unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art. The suppositories may be conveniently formed by admixture of the active combination with the softened or melted carrier(s) followed by chilling and shaping in moulds.

Formulations suitable for parenteral administration include aqueous and nonaqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents; and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Exemplary unit dosage formulations are those containing a daily dose or daily subdose of the active ingredients, as hereinbefore recited, or an appropriate fraction thereof. It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include such further agents as sweeteners, thickeners and flavoring agents.

The compounds of the combination of the present invention may be obtained in a conventional manner, known to those skilled in the art. Tenofovir disoproxil fumarate can be prepared, for example, as described in U.S. Pat. No. 5,977,089. Methods for the preparation of FTC are described in WO 92/14743, incorporated herein by reference.

Composition Use

Compositions of the present invention are administered to a human or other mammal in a safe and effective amount as described herein. These safe and effective amounts will vary according to the type and size of mammal being treated and the desired results of the treatment. Any of the various methods known by persons skilled in the art for packaging tablets, caplets, or other solid dosage forms suitable for oral administration, that will not degrade the components of the present invention, are suitable for use in packaging. The combinations may be packaged in glass and plastic bottles. Tablets, caplets, or other solid dosage forms suitable for oral administration may be packaged and contained in various packaging materials optionally including a dessicant, e.g. silica gel. Packaging may be in the form of unit dose blister packaging. For example, a package may contain one blister tray of tenofovir DF and another blister tray of emtricitabine pills, tablets, caplets, or capsule. A patient would take one dose, e.g. a pill, from one tray and one from the other. Alternatively, the package may contain a blister tray of the co-formulated combination of tenofovir DF and emtricitabine in a single pill, tablet, caplet or capsule. As in other combinations and packaging thereof, the combinations of the invention include physiological functional derivatives of tenofovir DF and FTC.

The packaging material may also have labeling and information related to the pharmaceutical composition printed thereon. Additionally, an article of manufacture may contain a brochure, report, notice, pamphlet, or leaflet containing product information. This form of pharmaceutical information is referred to in the pharmaceutical industry as a "package insert." A package insert may be attached to or included with a pharmaceutical article of manufacture. The package insert and any article of manufacture labeling provides information relating to the pharmaceutical composition. The information and labeling provides various forms of information utilized by health-care professionals and patients, describing the composition, its dosage and various other parameters required by regulatory agencies such as the United States Food and Drug Agency.

Assays of the Combinations

The combinations of the inventions may be tested for in vitro activity against HIV and sensitivity, and for cytotoxicity in laboratory adapted cell lines, e.g. MT2 and in peripheral blood mononuclear cells (PBMC) according to standard assays developed for testing anti-HIV compounds, such as WO 02/068058 and U.S. Pat. No. 6,475,491. Combination assays may be performed at varying concentrations of the compounds of the combinations to determine EC_{50} by serial dilutions.

Exemplary Formulations

The following examples further describe and demonstrate particular embodiments within the scope of the present invention. Techniques and formulations generally are found in *Remington's Pharmaceutical Sciences* (Mack Publishing Co., Easton, Pa.). The examples are given solely for illustration and are not to be construed as limitations as many variations are possible without departing from spirit and scope of the Invention. The following examples are intended for illustration only and are not intended to limit the scope of the invention in any way. "Active ingredient" denotes tenofovir disoproxil fumarate, emtricitabine, or a physiologically functional derivative of either thereof.

Tablet Formulation

The following exemplary formulations A, B, C, D, E, and F are prepared by wet granulation of the ingredients with an aqueous solution, addition of extragranular components and then followed by addition of magnesium stearate and compression.

Formulation A:

	mg/tablet
Tenofovir Disoproxil Fumarate	300
emtricitabine	200
Microcrystalline Cellulose	200
Lactose Monohydrate	175
Croscarmellose Sodium	60
Pregelatinized Starch	50
Colloidal silicon dioxide	5
Magnesium Stearate	10
total:	1000

Formulation B:

	mg/tablet
Tenofovir Disoproxil fumarate	300
emtricitabine	100
Microcrystalline Cellulose	200
Lactose Monohydrate	180
Sodium Starch Glycollate	60
Pregelatinized Starch	50
Magnesium Stearate	10
total:	900

Formulation C:

	mg/tablet
Tenofovir Disoproxil fumarate	200
emtricitabine	200
Microcrystalline Cellulose	200
Lactose Monohydrate	180
Sodium Starch Glycollate	60
Pregelatinized Starch	50
Magnesium Stearate	10
total:	900

Formulation D:

	mg/tablet
Tenofovir Disoproxil fumarate	300
emtricitabine	25
Microcrystalline Cellulose	200
Lactose Monohydrate	180
Sodium Starch Glycollate	60
Pregelatinized Starch	50
Magnesium Stearate	10
total:	825

Formulation E:

	mg/tablet
Tenofovir Disoproxil fumarate	200
emtricitabine	25
Microcrystalline Cellulose	200
Lactose Monohydrate	180
Sodium Starch Glycollate	60
Pregelatinized Starch	50
Magnesium Stearate	10
total:	725

Formulation F:

	mg/tablet
Tenofovir Disoproxil fumarate	100
emtricitabine	100
Microcrystalline Cellulose	200
Lactose Monohydrate	180
Sodium Starch Glycollate	60
Pregelatinized Starch	50
Magnesium Stearate	10
total:	700

Formulation G (Controlled Release Formulation):

This formulation is prepared by wet granulation of the ingredients with an aqueous solution, followed by the addition of magnesium stearate and compression.

	mg/tablet
Tenofovir Disoproxil fumarate	300
emtricitabine	200
Hydroxypropyl Methylcellulose	112
Lactose B.P.	53
Pregelatinized Starch B.P.	28
Magnesium Stearate	7
total:	700

Drug release takes place over a period of about 6-8 hours and is complete after 12 hours.

Capsule Formulations

Formulation H:

A capsule formulation is prepared by admixing the ingredients and filling into a two-part hard gelatin or hydroxypropyl methylcellulose capsule.

	mg/capsule
Active Ingredient	500
Microcrystalline Cellulose	143
Sodium Starch Glycollate	25
Magnesium Stearate	2
total:	670

Formulation I (Controlled Release Capsule):

The following controlled release capsule formulation is prepared by extruding ingredients a, b, and c using an extruder, followed by spheronization of the extrudate and drying. The dried pellets are then coated with release-controlling membrane (d) and filled into a two-piece, hard gelatin or hydroxypropyl methylcellulose capsule.

	mg/capsule
(a) Active Ingredient	500
(b) Microcrystalline Cellulose	125
(c) Lactose B.P.	125
(d) Ethyl Cellulose	13
total:	763

Formulation J (Oral Suspension):

The active ingredients are admixed with the ingredients and filling them as dry powder. Purified water is added and mixed well before use.

Active Ingredient	500 mg
Confectioner's Sugar	2000 mg
Simethicone	300 mg
Methylparaben	30 mg
Propylparaben	10 mg
Flavor, Peach	500 mg
Purified Water q.s. to	5.00 ml

Formulation K (Suppository):

One-fifth of the Witepsol H15 is melted in a steam jacketed pan at 45° C. maximum. The active ingredients are sifted through a 200 micron sieve and added to the molten base with mixing, using a Silverson fitted with a cutting head, until a smooth dispersion is achieved. Maintaining the mixture at 45° C., the remaining Witepsol H15 is added to the suspension and stirred to ensure a homogenous mix. The entire suspension is passed through a 250 micron stainless steel screen and, with continuous stirring, is allowed to cool to 40° C. At a temperature of 38° C. to 40° C., 2.02 g of the mixture is filled into suitable, 2 ml plastic molds. The suppositories are allowed to cool to room temperature.

	mg/Suppository
Active Ingredient	500
Hard Fat, B.P. (Witepsol H15 - Dynamit Nobel)	1770
total	2270

Fixed Dose Combination Tablet

A fixed dose combination tablet of tenofovir disoproxil fumarate (TDF) 300 mg/emtricitabine 200 mg was formulated using a wet granulation/fluid-bed drying process using conventional methods. See: U.S. Pat. No. 5,935,946; L. Young (editor). Tableting Specification Manual 5th ed., American Pharmaceutical Association, Washington, D.C., (2001); L. Lachman, H. Lieberman (editors). Pharmaceutical Dosage Forms: Tablets (Vol 2), Marcel Dekker Inc., New York, 185-202 (1981); J. T. Fell and J. M. Newton, J. Pharm. Pharmacol. 20, 657-659 (1968); US Pharmacopeia 24-National Formulary 19, "Tablet Friability", Chapter <1216>, Page 2148 (2000).

The effects of granulation water level (ranging from 40% to 50% w/w) and wet massing time were studied on the physicochemical properties of the final powder blend and its performance with respect to blend uniformity and compressibility (tablet compactibility). In addition, content uniformity, assay, stability and dissolution performance was evaluated for the TDF/emtricitabine fixed dose combination tablets.

Formulation Equipment

Equipment included a high shear mixer equipped with a pressure tank and spray nozzle tip to add the granulating water, a fluid-bed dryer, a mill, a tumble blender, a rotary tablet press, and a tablet deduster.

Formulation Process

The dried, milled powder was blended with the extragranular microcrystalline cellulose and croscarmellose sodium and then blended with magnesium stearate. Powder samples were removed after mixing with the magnesium stearate. The blend samples were evaluated for, bulk density, mesh analysis and compressibility. The powder blend mixed with the magnesium stearate was compressed into tablets on a press setup.

Materials

The following Table 1 lists the quantitative composition of the TDF/emtricitabine tablet formulation.

TABLE 1

Ingredient	% w/w	Unit Formula for tablet cores (mg/tablet)	Quantity per 12 kg Batch (kg)
Tenofovir Disoproxil Fumarate[a]	30.0	300.0	3.60
Emtricitabine[a]	20.0	200.0	2.40
Pregelatinized Starch, NF/EP	5.0	50.0	0.60
Croscarmellose Sodium, NF/EP	6.0	60.0	0.72
Lactose Monohydrate, NF/EP[a]	8.0	80.0	0.96
Microcrystalline Cellulose, NF/EP[c]	30.0	300.0	3.60
Magnesium Stearate, NF/EP	1.0	10.0	0.12
Purified Water, USP/EP	[b]	[b]	[b]
Totals	100.0	1000.0	12.00

[a]Actual weight is adjusted based on the Drug Content Factor (DCF) of tenofovir disoproxil fumarate and emtricitabine.
[b]Water removed during drying.

Characterization Equipment

Moisture content was measured by loss on drying using a heat lamp/balance system. The powder blend was sampled with a sampling thief fitted with chambers to determine powder blend uniformity. Duplicate samples were removed from each of several locations in the blender. Blend uniformity analysis was performed on one sample from each location.

Particle size analysis of the final powder blend was determined by sifting a multi-gram sample through a screen using a sonic sifter. The quantity of final powder blend retained on each sieve and the fines collector was determined by calculating the difference in weight between the sieves and fines collector before and after the test. The geometric mean diameter particle size was calculated by logarithmic weighting of the sieved distribution.

Bulk density was determined by filling a graduated cylinder with the final powder blend and measuring the weight differential between the empty and filled graduate cylinder per unit volume.

Tablets were characterized for friability using a friabilator, a hardness tester, a thickness micrometer equipped with a printer, and a weighing balance.

Compression characteristics were determined using a rotary tablet press equipped with a flat-faced, beveled edged punch to a target weight of 400 mg. The powder blends were compressed using target upper punch pressures ranging from approximately 100 to 250 MPa. The apparent normalized ejection force was determined and normalized for tablet thickness and diameter.

Tablet hardness was determined using a hardness tester. Tablet thickness was determined using a micrometer, and tablet weights were determined using a top loading balance.

Wet Granulation

The powders were blended in a granulator and then granulated using water. The impeller and chopper speeds were kept constant in the blender at a low setting during the granulation and wet massing operations. After water addition, the impeller and chopper were stopped and the granulator bowl was opened to observe the granulation consistency and texture. The lid was closed and the wet massing phase was performed. Acceptable granules had 40% w/w and 60% w/w water, respectively.

Wet Milling

To facilitate a uniform drying process, each wet granulation was deagglomerated with a mill fitted with a screen and an impeller. The milled wet granules were charged into a fluid-bed dryer immediately following wet milling.

Fluid-Bed Drying

Milled wet granules were dried using an inlet air setpoint temperature of about 70° C. and airflow of approximately 100 cfm. The target LOD was about 1.0% with a range of not more than (NMT) 1.5%. The total fluid-bed drying time ranged from 53 to 75 minutes. Final LOD ranged from 0.4% to 0.7% for all of the batches dried. The final exhaust temperatures for all the batches ranged from 47° C. to 50° C.

Dry Milling

All dried granules were milled through a perforated screen. The mill was equipped with a square impeller and operated. The lots were milled and manually transferred to the V-blender.

Blending

Each lot was blended using the V-blender. In one set of three formulations, starting with 12 kg materials, final powder blend yield available for compression after blending ranged from 10.5 kg (87.5%) to 11.1 kg (92.5%). The final powder blend bulk density ranged from 0.48 to 0.58 g/cc and the geometric mean diameter particle size ranged from 112 to 221 μm. Percent water and wet massing time affect final powder blend particle size and bulk density.

The powder blending for both tenofovir DF and emtricitabine gave a mean (n=10) strength value for tenofovir DF ranged from 100.6% to 102.8% of target strength for the lots and the relative standard deviation (RSD) was from 0.5% to 1.7%. The mean (n=10) strength value for emtricitabine ranged from 101.3% to 104.1% of target strength for the lots with the relative standard deviation (RSD) ranged from 0.6% to 1.7%. The final powder blend moisture level ranged from 0.8% to 1.1% LOD.

Tablet Compression

The final blends were compressed using a rotary tablet press and the tablets were film-coated.

Three 300 gm formulations (Table 2) were granulated in a granulator equipped with a 1-L bowl. The quantities of intragranular components were based on a 300 g total batch size. The formulations in lots 1 and 2 differed in the amount of microcrystalline cellulose 30% vs. 20% w/w, respectively. Lots 2 and 3 were identical except for the type of binder. Lot 2 contained 5% w/w of pregelatinized starch and lot 3 contained 5% w/w povidone as binder.

TABLE 2

Ingredient	Lot 1 % w/w	Lot 2 % w/w	Lot 3 % w/w
Tenofovir Disoproxil Fumarate	30.0	30.0	30.0
Emtricitabine	20.0	20.0	20.0
Pregelatinized Starch, NF/EP	5.0	5.0	N/A
Povidone, USP/NF (C-30)	N/A	N/A	5.0
Croscarmellose Sodium, NF/EP	6.0	6.0	6.0
Lactose Monohydrate, NF/EP	8.0	18.0	18.0
Microcrystalline Cellulose, NF/EP[a]	30.0	20.0	20.0
Magnesium Stearate, NF/EP	1.0	1.0	1.0
Purified Water, USP/EP	[a]	[a]	[a]
Total	100.0	100.0	100.0

[a]Water removed during drying.

After water addition, the impeller and chopper were stopped and the granulator bowl was opened to observe the granulation consistency and texture. To achieve similar granulation consistency, lots 1, 2, and 3 were granulated with 45%, 40%, and 30% w/w water, respectively. The lid was closed and the wet massing phase was performed. All lots had a 30 sec wet massing resulting in acceptable granulations. The wet granulations from all batches were hand screened through a sieve to deagglomerate. The resulting granulations were tray dried in a convection oven set at 60° C. for approximately 20 hours to an LOD <1.0%. The dried granulations from all batches were hand screened through a sieve. In order to fit the granulation into the small scale (300 mL) V-blender, the final blend batch size was adjusted to 100 g. A portion, 81 g of the resulting blend from Lot 1 was blended with 15 g microcrystalline cellulose, 3 g croscarmellose sodium and 1 g magnesium stearate. 86 g of the resulting granulation from Lot 2 and Lot 3 were each blended with 10 g microcrystalline cellulose, 3 g croscarmellose sodium and 1 g magnesium stearate.

Purity analysis was conducted by reverse-phase HPLC (high performance liquid chromatography). Impurities related to tenofovir disoproxil fumarate and emtricitabine were characterized and measured in the bulk API (active pharmaceutical ingredient) before formulation in the three lots of Table 2, and again after formulation in the resulting tablets. The impurities include by-products from hydrolysis of the exocyclic amino groups of tenofovir disoproxil fumarate and emtricitabine, and the hydrolysis of the disoproxil (POC) esters of tenofovir disoproxil fumarate. In each lot, the sum total of impurities related to tenofovir disoproxil fumarate and emtricitabine was less than 1% after formulation and tablet manufacture.

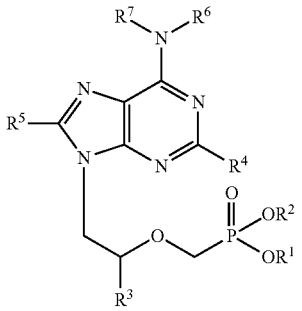
The physicochemical properties of tenofovir disoproxil fumarate and emtricitabine tablets were evaluated by visual appearance, water content, label strength, impurity and degradation product contents, and tablet dissolution. Stability studies were conducted on drug product packaged in container-closure systems that are identical to the intended clinical and commercial container-closure system. There was no sign of discoloration or tablet cracking during the course of the stability study. Film-coated tenofovir disoproxil fumarate and emtricitabine tablets exhibited satisfactory stability at 40° C./75% RH (relative humidity) for up to six months when packaged and stored with silica gel desiccant. No significant loss (defined as 5% degradation) in % label strength of tenofovir DF or emtricitabine was observed after six months at 40° C./75% RH. when packaged and stored with desiccant. The increase in the total degradation products was 1.5% for tenofovir DF and 0.6-0.7% for emtricitabine after six months at 40° C./75% RH when packaged and stored with 3 grams of desiccant.

All publications and patent applications cited herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although certain embodiments are described in detail above, those having ordinary skill in the art will clearly understand that many modifications are possible in the claims without departing from the teachings thereof. All such modifications are intended to be encompassed within the claims of the invention.

Embodiments of the Invention

A1. A pharmaceutical composition comprising an effective amount of a compound of the formula:



(I)

wherein R^1 and R^2 are independently selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_6-C_{20} arylalkyl, C_6-C_{20} substituted arylalkyl, acyloxymethyl esters $—CH_2OC(═O)R^9$ and acyloxymethyl carbonates $—CH_2OC(═O)OR^9$ where R^9 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl and C_6-C_{20} substituted aryl;

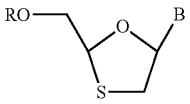
R^3 is selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, or CH_2OR^8 where R^8 is C_1-C_6 alkyl, C_1-C_6 hydroxyalkyl and C_1-C_6 haloalkyl;

R^4 and R^5 are independently selected from H, NH_2, NHR and NR_2 where R is C_1-C_6 alkyl; and

R^6 and R^7 are independently selected from H and C_1-C_6 alkyl;

or a physiologically functional derivative thereof;

in combination with an effective amount of a compound of the formula



(2)

wherein B is selected from adenine, guanine, cytosine, uracil, thymine, 7-deazaadenine, 7-deazaguanine, 7-deaza-8-azaguanine, 7-deaza-8-azaadenine, inosine, nebularine, nitropyrrole, nitroindole, 2-aminopurine, 2-amino-6-chloropurine, 2,6-diaminopurine, hypoxanthine, pseudouridine, 5-fluorocytosine, 5-chlorocytosine, 5-bromocytosine, 5-iodocytosine, pseudocytosine, pseudoisocytosine, 5-propynylcytosine, isocytosine, isoguanine, 7-deazaguanine, 2-thiopyrimidine, 6-thioguanine, 4-thiothymine, 4-thiouracil, O^6-methylguanine, N^6-methyladenine, O^4-methylthymine, 5,6-dihydrothymine, 5,6-dihydrouracil, 4-methylindole, and a pyrazolo[3,4-D]pyrimidine; and

R is selected from H, C_1-C_{18} alkyl, C_1-C_{18} substituted alkyl, C_2-C_{18} alkenyl, C_2-C_{18} substituted alkenyl, C_2-C_{18} alkynyl, C_2-C_{18} substituted alkynyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_2-C_{20} heterocycle, C_2-C_{20} substituted heterocycle, phosphonate, phosphophosphonate, diphosphophosphonate, phosphate, diphosphate, triphosphate, polyethyleneoxy or a physiologically functional derivative thereof; and a pharmaceutically acceptable carrier.

B2. A composition of embodiment A1 wherein, in formula 1, R^1 and R^2 are independently selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_6-C_{20} arylalkyl, C_6-C_{20} substituted arylalkyl, acyloxymethyl esters $—CH_2OC(═O)R^9$ and acyloxymethyl carbonates $—CH_2OC(═O)OR^9$ where R^9 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl and C_6-C_{20} substituted aryl; and R^3, R^4, R^5, R^6 and R^7 are independently H or C_1-C_6 alkyl.

C3. A composition of embodiment A1 wherein, in formula 2, B is cytosine or a 5-halocytosine.

D4. A composition of embodiment A1 wherein, in formula 1, R^1 and R^2 are independently selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_6-C_{20} arylalkyl, C_6-C_{20} substituted arylalkyl, acyloxymethyl esters $—CH_2OC(═O)R^9$ and acyloxymethyl carbonates $—CH_2OC(═O)OR^9$ where R^9 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl and C_6-C_{20} substituted aryl; and R^3, R^4, R^5, R^6 and R^7 are independently H or C_1-C_6 alkyl; and, in formula 2, B is cytosine or a 5-halocytosine.

E5. A composition of embodiment D4 wherein, in formula 1, R^1 and R^2 are independently selected from H, acyloxymethyl esters $—CH_2OC(═O)R^9$ and acyloxymethyl carbonates $—CH_2OC(═O)OR^9$ where R^9 is C_1-C_6 alkyl; and R^3, R^4, R^5, R^6 and R^7 are independently H or C_1-C_6 alkyl; and, in formula 2, B is cytosine or a 5-halocytosine and R is H.

F6. A composition of embodiment E5 wherein, in formula 1, R^1 and R^2 are independently selected from H and $—CH_2OC(═O)OCH(CH_3)_2$; R^3 is $—CH_3$; and R^4, R^5, R^6 and R^7 are H; and, in formula 2, B is 5-fluorocytosine and R is H.

G7. A pharmaceutical composition comprising a pharmaceutically effective amount of [2-(6-amino-purin-9-yl)-1-methyl-ethoxymethyl]-phosphonic acid diisopropoxycarbonyloxymethyl ester fumarate (tenofovir disoproxil fumarate) or a physiologically functional derivative thereof and a pharmaceutically effective amount of (2R, 5S,)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine) or a physiologically functional derivative thereof; and a pharmaceutically acceptable carrier.

H8. A pharmaceutical formulation of embodiment A1 to G7 further comprising a third active ingredient selected from the group consisting of a protease inhibitor, a nucleoside or nucleotide reverse transcriptase inhibitor, a non-nucleoside reverse transcriptase inhibitor, and an integrase inhibitor.

I9. A pharmaceutical formulation of embodiments A1 to H8 in unit dosage form.

J10. A method for the treatment or prevention of the symptoms or effects of an HIV infection in an infected animal which comprises administering to said animal a pharmaceutical composition of embodiments claims A1 to I9.

We claim:

1. A fixed dose combination pharmaceutical dosage form comprising 300 mg tenofovir disoproxil fumarate and 200 mg emtricitabine; a binder selected from the group consisting of povidone, gelatin, hydroxypropyl methylcellulose, cellulose, microcrystalline cellulose, starch, and acacia; a disintegrant selected from sodium starch glycolate, cross-linked-povidone, cross-linked sodium carboxymethylcellulose, and alginic acid; and a lubricant selected from the

group consisting of magnesium stearate, stearic acid, and talc; wherein said pharmaceutical dosage form exhibits equal to or less than 5% degradation of the tenofovir disoproxil fumarate or emtricitabine after 6 months when packaged and stored with silica gel dessicant at 40° C./75% relative humidity; and wherein said pharmaceutical dosage form is a tablet.

2. The pharmaceutical dosage form of claim **1** where there is less than 1% degradation of tenofovir disoproxil fumarate over a 24-hour period.

3. The pharmaceutical dosage form of claim **1** where there is less than 0.1% degradation of tenofovir disoproxil fumarate over a 24-hour period.

4. The pharmaceutical dosage form of claim **1** where there is less than 0.01% degradation of tenofovir disoproxil fumarate over a 24-hour period.

5. The pharmaceutical dosage form of claim **1** comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, pregelatinized starch, croscarmellose sodium, lactose monohydrate, microcrystalline cellulose, and magnesium stearate.

6. The pharmaceutical dosage form of claim **5** comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, 50 mg pregelatinized starch, 60 mg croscarmellose sodium, 80 mg lactose monohydrate, 300 mg microcrystalline cellulose, and 10 mg magnesium stearate.

7. The pharmaceutical dosage form of claim **5** comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, 50 mg pregelatinized starch, 60 mg croscarmellose sodium, lactose monohydrate, 200 mg microcrystalline cellulose, and 10 mg magnesium stearate.

8. The pharmaceutical dosage form of claim **1** comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, pregelatinized starch, croscarmellose sodium, lactose monohydrate, microcrystalline cellulose, magnesium stearate, and colloidal silicon dioxide.

9. The pharmaceutical dosage form of claim **8** comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, 50 mg pregelatinized starch, 60 mg croscarmellose sodium, 175 mg lactose monohydrate, 200 mg microcrystalline cellulose, 10 mg magnesium stearate, and 5 mg colloidal silicon dioxide.

10. The pharmaceutical dosage form of claim **8** comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, hydroxypropyl methylcellulose, lactose, pregelatinized starch, and magnesium stearate.

11. The pharmaceutical dosage form of claim **8** comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, 112 mg hydroxypropyl methylcellulose, lactose, pregelatinized starch, and 7 mg magnesium stearate.

12. The pharmaceutical dosage form of claim **1** comprising less than 1% of impurities related to tenofovir disoproxil fumarate and emtricitabine.

13. A method for the treatment of the symptoms or effects of an HIV infection in an infected animal which comprises administering to said animal the pharmaceutical dosage form of claim **1**.

14. A method for the treatment of the symptoms or effects of an HIV infection in an infected animal which comprises administering to said animal the pharmaceutical dosage form of claim **8**.

15. The pharmaceutical dosage form of claim **1**, wherein the starch is pregelatinized starch.

16. A fixed dose combination pharmaceutical dosage form comprising 300 mg tenofovir disoproxil fumarate and 200 mg emtricitabine; a binder selected from the group consisting of povidone, gelatin, hydroxypropyl methylcellulose, cellulose, microcrystalline cellulose, pregelatinized starch, and acacia; a disintegrant selected from sodium starch glycolate, crosslinked-povidone, cross-linked sodium carboxymethylcellulose, maize starch, and alginic acid; and a lubricant selected from the group consisting of magnesium stearate, stearic acid, and talc; wherein said pharmaceutical dosage form exhibits equal to or less than 5% degradation of the tenofovir disoproxil fumarate or emtricitabine after 6 months when packaged and stored with silica gel dessicant at 40° C./75% relative humidity and wherein said pharmaceutical dosage form is a tablet.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 9,457,036 B2
APPLICATION NO. : 14/523758
DATED : October 4, 2016
INVENTOR(S) : Dahl 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 Specification

Column 6, Line 18, replace "4-IV)-triazolyl," with --4-N)-triazolyl,--.

Column 6, Line 18, replace "3-N)-oxazole," with --4-(1-O, 3-N)-oxazole,--.

Column 10, Line 7, replace "(Farman" with --(Fasman--.

Column 18, Line 67, replace "[[(5)-[[(S)-1-(isopropoxycarbonyl)ethyl]amino]phenoxy" with --[[(S)-[[(S)-1-(isopropoxycarbonyl)ethyl]amino]phenoxy--.

Column 28, Line 57, replace "defined as 5%" with --defined as ≥ 5%--.

Signed and Sealed this
Twenty-fifth Day of April, 2017

Michelle K. Lee

Michelle K. Lee
Director of the United States Patent and Trademark Office

EXHIBIT F

US009744181B2

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

(10) **Patent No.: US 9,744,181 B2**
(45) **Date of Patent: *Aug. 29, 2017**

(54) **COMPOSITIONS AND METHODS FOR COMBINATION ANTIVIRAL THERAPY**

(71) Applicant: **Gilead Sciences, Inc.**, Foster City, CA (US)

(72) Inventors: **Terrence C. Dahl**, Sunnyvale, CA (US); **Mark M. Menning**, San Francisco, CA (US); **Reza Oliyai**, Foster City, CA (US)

(73) Assignee: **Gilead Sciences, Inc.**, Foster City, CA (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.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **14/523,783**

(22) Filed: **Oct. 24, 2014**

(65) **Prior Publication Data**

US 2015/0111856 A1 Apr. 23, 2015

Related U.S. Application Data

(63) Continuation of application No. 14/227,653, filed on Mar. 27, 2014, which is a continuation of application No. 12/204,174, filed on Sep. 4, 2008, now Pat. No. 8,716,264, which is a continuation of application No. 10/540,794, filed as application No. PCT/US2004/000832 on Jan. 13, 2004, now abandoned.

(60) Provisional application No. 60/440,246, filed on Jan. 14, 2003, provisional application No. 60/440,308, filed on Jan. 14, 2003.

(51) **Int. Cl.**

A61K 9/20	(2006.01)
A61K 31/675	(2006.01)
A61K 31/513	(2006.01)
A61K 31/7076	(2006.01)
A61K 45/06	(2006.01)
A61K 31/683	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 31/675*** (2013.01); ***A61K 9/2009*** (2013.01); ***A61K 9/2013*** (2013.01); ***A61K 9/2018*** (2013.01); ***A61K 9/2054*** (2013.01); ***A61K 9/2059*** (2013.01); ***A61K 31/513*** (2013.01); ***A61K 31/683*** (2013.01); ***A61K 31/7076*** (2013.01); ***A61K 45/06*** (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,524,846	A	8/1970	Moffatt et al.
3,622,677	A	11/1971	Short et al.
3,682,930	A	8/1972	Bourquin et al.
3,994,974	A	11/1976	Murakami et al.
4,003,878	A	1/1977	Skaar et al.
4,258,062	A	3/1981	Jonas et al.
4,355,032	A	10/1982	Verheyden et al.
4,384,005	A	5/1983	McSweeney
4,430,343	A	2/1984	Iemura et al.
4,476,248	A	10/1984	Gordon et al.
4,724,233	A	2/1988	De Clercq et al.
4,808,716	A	2/1989	Holy et al.
4,816,570	A	3/1989	Farquhar
4,879,288	A	11/1989	Warawa et al.
4,935,507	A	6/1990	Takaya et al.
4,957,924	A	9/1990	Beauchamp
4,968,788	A	11/1990	Farquhar
5,047,407	A	9/1991	Belleau et al.
5,075,445	A	12/1991	Jarvest et al.
5,142,051	A	8/1992	Holy et al.
5,151,426	A	9/1992	Belleau et al.
5,155,268	A	10/1992	Hester
5,177,064	A	1/1993	Bodor
5,179,104	A	1/1993	Chu et al.
5,204,466	A	4/1993	Liotta et al.

(Continued)

FOREIGN PATENT DOCUMENTS

EP	0 182 024 A2	5/1986
EP	0 206 459 A2	12/1986

(Continued)

OTHER PUBLICATIONS

Decision Denying Petitioner's Request for Rehearing for Case PTAB-IPR2014-00885, dated Aug. 3, 2015, 15 pages.
Decision Denying Petitioner's Request for Rehearing for Case PTAB-IPR2014-00887, dated Aug. 3, 2015, 13 pages.
Decision Denying Petitioner's Request for Rehearing for Case PTAB-IPR-2014-00888, dated Aug. 3, 2015, 15 pages.
Joint Motion to Dismiss and Stipulation by Mylan Inc., Mylan Pharmaceuticals Inc., Case No. 1:14-cv-00099-IMF-JSK (Dated: Oct. 30, 2015), 3 pages.
Order on Stipulation for Dismissal: Ordered, adjudged and decreed that all claims, counterclaims or causes of action asserted in this suit by and between Gilead, Emory and Mylan are dismissed with prejudice. Signed by District Judge Irene M. Keeley on Nov. 2, 2015. (jss), Case No. 1:14-cv-00099-IMF-JSK (Entered: Nov. 2, 2015), 2 pages.

(Continued)

Primary Examiner — Alton Pryor
(74) *Attorney, Agent, or Firm* — Fish & Richardson P.C.

(57) **ABSTRACT**

The present invention relates to therapeutic combinations of [2-(6-amino-purin-9 yl)-1-methyl-ethoxymethyl]-phosphonic acid diisopropoxycarbonyloxymethyl ester (tenofovir disoproxil fumarate, Viread®) and (2R,5S,cis)-4-amino-5-fluoro-1-(2 hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine, Emtriva™, (−)-cis FTC) and their physiologically functional derivatives. The combinations may be useful in the treatment of HIV infections, including infections with HIV mutants bearing resistance to nucleoside and/or non-nucleoside inhibitors. The present invention is also concerned with pharmaceutical compositions and formulations of said combinations of tenofovir disoproxil fumarate and emtricitabine, and their physiologically functional derivatives, as well as therapeutic methods of use of those compositions and formulations.

30 Claims, No Drawings

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,208,221	A	5/1993	Kim et al.	
5,210,085	A	5/1993	Liotta et al.	
5,386,030	A	1/1995	Kim et al.	
5,432,172	A	7/1995	Kashman et al.	
5,453,503	A	9/1995	Aikins et al.	
5,466,806	A	11/1995	Belleau et al.	
5,476,938	A	12/1995	Vemishetti et al.	
5,486,520	A	1/1996	Belleau et al.	
5,506,347	A	4/1996	Erion et al.	
5,512,596	A	4/1996	Kim et al.	
5,514,557	A	5/1996	Moghaddam	
5,514,798	A	5/1996	Bischofberger et al.	
5,519,021	A	5/1996	Payne et al.	
5,538,975	A	7/1996	Dionne	
5,587,480	A	12/1996	Belleau et al.	
5,618,820	A	4/1997	Dionne	
5,618,964	A	4/1997	Cheng et al.	
5,627,186	A	5/1997	Cameron et al.	
5,663,159	A	9/1997	Starrett, Jr. et al.	
5,696,254	A	12/1997	Mansour et al.	
5,733,788	A	3/1998	Bischofberger	
5,744,596	A	4/1998	Mansour et al.	
5,756,706	A	5/1998	Mansour et al.	
5,763,606	A	6/1998	Mansour et al.	
5,792,756	A	8/1998	Starrett, Jr. et al.	
5,798,340	A	8/1998	Bischofberger et al.	
5,814,639	A	9/1998	Liotta et al.	
5,859,021	A	1/1999	Cameron et al.	
5,905,082	A	5/1999	Roberts et al.	
5,914,331	A	6/1999	Liotta et al.	
5,922,695	A	7/1999	Arimilli et al.	
5,935,946	A	8/1999	Munger, Jr. et al.	
5,977,089	A	11/1999	Arimilli et al.	
6,043,230	A	3/2000	Arimilli et al.	
6,057,305	A	5/2000	Holy et al.	
6,069,249	A	5/2000	Arimilli et al.	
6,069,252	A	5/2000	Liotta et al.	
6,113,920	A	9/2000	Maye et al.	
6,114,343	A	9/2000	Liotta et al.	
6,121,315	A	9/2000	Nair et al.	
6,180,639	B1	1/2001	Coates et al.	
6,194,391	B1	2/2001	Schinazi et al.	
6,312,662	B1	11/2001	Erion et al.	
6,417,191	B1	7/2002	Barry et al.	
6,639,071	B2	10/2003	Thompson et al.	
RE38,333	E	11/2003	Arimilli et al.	
6,642,245	B1	11/2003	Liotta et al.	
6,703,396	B1	3/2004	Liotta et al.	
6,812,233	B1	11/2004	Liotta	
6,939,964	B2	9/2005	Thompson et al.	
7,094,413	B2	8/2006	Buelow et al.	
7,795,217	B2	9/2010	Adra	
7,851,165	B2	12/2010	Fuhrmann et al.	
8,067,473	B2	11/2011	Alchanati et al.	
8,592,397	B2 *	11/2013	Dahl	A61K 31/513 514/81
8,598,185	B2 *	12/2013	Dahl	A61K 9/2054 514/221
8,716,264	B2 *	5/2014	Dahl	A61K 31/513 514/81
8,871,271	B2 *	10/2014	Dahl	A61K 9/2077 424/489
9,018,192	B2	4/2015	Dahl et al.	
2001/0012518	A1	8/2001	Makool-Morehead et al.	
2001/0014352	A1	8/2001	Batra et al.	
2003/0203969	A1	10/2003	Bevec et al.	
2004/0180089	A1	9/2004	Plachetka et al.	
2004/0224917	A1	11/2004	Dahl et al.	
2004/0253218	A1	12/2004	Eisenbach-Schwartz et al.	
2005/0197320	A1	9/2005	Chen et al.	
2006/0128692	A1	6/2006	Chen et al.	
2006/0246130	A1	11/2006	Dahl et al.	
2007/0036861	A1	2/2007	Oury et al.	
2007/0077295	A1	4/2007	Dahl et al.	
2007/0099902	A1	5/2007	Dahl et al.	
2009/0036408	A1	2/2009	Dahl et al.	
2009/0143314	A1	6/2009	Dahl et al.	
2014/0037732	A1	2/2014	Dahl et al.	
2014/0213556	A1	7/2014	Dahl et al.	
2014/0370102	A1	12/2014	Dahl et al.	
2015/0111855	A1	4/2015	Dahl et al.	

FOREIGN PATENT DOCUMENTS

EP	0 269 947	A1	6/1988
EP	0 369 409	A1	5/1990
EP	0 382 526	A2	8/1990
EP	0 481 214	A1	4/1992
EP	0 482 657	A2	4/1992
EP	0 595 635		5/1994
EP	0 632 048	A1	1/1995
EP	0 647 649	A1	4/1995
EP	0 694 547	A2	1/1996
EP	1 256 585	A1	11/2002
EP	1 332 757	A1	8/2003
GB	942 152	A	11/1963
GB	1 523 865	A	9/1978
GB	2 111 043	A	6/1983
JP	10-511682		11/1998
JP	2001-502718		2/2001
WO	WO 88/05438		7/1988
WO	WO 91/19721	A1	12/1991
WO	WO 92/01698		2/1992
WO	WO 92/09611	A1	6/1992
WO	WO 92/13869		8/1992
WO	WO 92/14743	A2	9/1992
WO	WO 93/03027	A1	2/1993
WO	WO 94/03466		2/1994
WO	WO 94/03467	A2	2/1994
WO	WO 95/07919		3/1995
WO	WO 95/07920	A1	3/1995
WO	WO 95/32957	A1	12/1995
WO	WO 96/30025		3/1996
WO	WO 96/18605	A1	6/1996
WO	WO 98/04569	A1	2/1998
WO	WO 98/18477		5/1998
WO	WO 99/25352	A1	5/1999
WO	WO 99/61026		12/1999
WO	WO 00/16755		3/2000
WO	WO 00/25797	A1	5/2000
WO	WO 00/64427	A2	11/2000
WO	WO 01/64221	A1	9/2001
WO	WO 02/08241	A2	1/2002
WO	WO 02/062123	A2	8/2002
WO	WO 02/068058	A2	9/2002
WO	WO 02/070518	A1	9/2002
WO	WO 03/045327	A2	6/2003
WO	WO 03/059327		7/2003
WO	WO 2004/052296	A2	6/2004
WO	WO 2004/064845	A1	8/2004
WO	WO 2005/021001	A1	3/2005
WO	WO 2006/135933	A2	12/2006
WO	WO 2006/135993	A2	12/2006
WO	WO 2007/068934	A2	6/2007
WO	WO 2010/017343	A2	2/2010
WO	WO 2012/003413	A1	1/2012
WO	WO 2012/068535		5/2012

OTHER PUBLICATIONS

Report to USPTO on the filing or determination of an action regarding re 232 Order/Stipulation on Motion to Dismiss. (jss) Case No. 1:14-cv-00099-IMF-JSK (Entered: Nov. 5, 2015), 1 page.

"Institute of Human Virology Hosts Prominent AIDS Meeting," Sep. 11, 2014, Business Wire, retrieved from the Internet on Nov. 3, 2015 http://somvweb.som.umaryland.edu/absolutenm/templates/?a=2879&z=41, 2 pages.

The Telegraph, "Inventor of Tamiflu profits from swine flu pandemic" Jul. 27, 2009, 2 pages.

Gallant, "Efficacy and Safety of Tenofovir DF vs stavudine in combination therapy" JAMA vol. 992 No. 2, Jul. 14, 2004.

Gallant "Early Virologic Nonresponse to Tenofovir, Abacavir, and Lamivudine in HIV-Infected Antiretroviral-Naive Subjects," Dec. 1, 2005 JID:192:1921-1930.

(56) **References Cited**

OTHER PUBLICATIONS

"Birth of a Notion: From the Lab to the Marketplace; Technology Transfer at Emory," Oct. 31, 1996, retrieved from the Internet on Nov. 3, 2015 at http://www.whsc.emory.edu/_pubs/em/1996fall/tech_transfer.html, 6 pages.
Medical Review 21-752: tenofovir and emtricitabine, Jul. 26, 2004, 26 pages.
Gilead's Poster 616—10th Conference on Retroviruses and Opportunistic Infections. (CROI) Feb. 10-14, 2003, 1 page.
Stone, "Human Immunodeficiency Virus Type 1 Reverse Transcriptase Mutation Selection during In Vitro Exposure to Tenofovir Alone or Combined with Abacavir or Lamivudine," Antimicrobial Agents and Chemotherapy vol. 48 No. 4, Apr. 2004, p. 1413-1415.
ADIS R&D Profile, Drugs R&D Jan. 2003; 4(1): 42-48.
Theory and Practice of Industrial Pharmacy, 3rd Edition, Lachman et al., Eds., Lea & Febiger, pp. 293-294 (1986).
EMA Scientific Discussion for Viread®.
Rudnic and Schwartz, "Oral Solid Dosage Forms" in "Remington: The science and Practice of Pharmacy", 2000, Eds Gennaro et al., p. 858-871.
Declaration of Dr. Reza Oliyai, dated Sep. 17, 2015, 2 pages.
Order Granting Motion to Withdraw for PTAB-IPR2014-00885, dated Nov. 19, 2015, 3 pages.
Order Granting Motion to Withdraw for PTAB-IPR2014-0086, dated Nov. 19, 2015, 4 pages.
Order Granting Motion to Withdraw for PTAB-IPR2014-0087, dated Nov. 19, 2015, 3 pages.
Order Granting Motion to Withdraw for PTAB-IPR2014-0088, dated Nov. 19, 2015, 4 pages.
Decision Denying Petitioner's Request for Rehearing of Decision Denying Insitution of Inter Partes Review for PTAB-IPR2014-00886, dated Nov. 19, 2015, 12 pages.
Extended Search Report issued by the European Patent Office for Application No. 15154733.8, dated Nov. 9, 2015.
"AIDS," Monthly Index of Medical Specialties, pp. 194-198 (2002).
"Anti-HIV Drug Updates—Three Drugs on the Near Horizon," Project Inform Perspective 35:4-7 (2003).
"Atripla Fact Sheet", www.fda.gov, [Online], Jul. 12, 2006, pp. 1-2 retrieved from the internet www.fda.gov/cder/drug/inforpage/atripla/factsheet.htm [retrieved on Jan. 31, 2007.
"Gilead Buys Triangle in $464M Deal" Pharma Marketletter, 1 page (Dec. 9, 2002).
"Gilead Captures Triangle for $464 Million," Chemical Market Reporter 262(21):1 page (Dec. 9, 2002).
"Gilead set to acquire Triangle for $464m," BT Catalyst 17(1):1 page (Jan. 1, 2003).
"HIV Treatment Information," Project Inform, (on line), Jan. 2006, pp. 1-3, retrieved from http://www.projinf.org/bn/news_013006.html [retrieved on Jan. 31, 2007].
"Rescriptor," Patient Prescribing Information Leaflet, 7 pages (2001).
"Scientific Discussion," EMEA, pp. 1/28-3/28, European Medicines Agency: (Feb. 2005).
Adis Data Information, "Emtricitabine/Tenofovir Disoproxil Fumarate," Drugs in R & D 5(3):160-161 (2004).
Alexander et al., "Investigation of (Oxodioxolenyl)methyl carbamates as nonchiral bioreversible prodrug moieties for chiral amines," J. Med. Chem. 39:480-486, 1996.
Amended Answer and Counterclaim against Gilead Sciences, Inc. filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Jul. 20, 2009).
Anderson, "Antiviral dynamics and sex differences of zidovudine and lamivudine triphosphate concentrations in HIV-infected individuals," AIDS 17:2159-2168 (2003).
Ansel et al., "Pharmaceutical Dosage Forms and Rug Delivery Systems," 7th Edition, Lippincott Williams & Wilkins, pp. 209-213 (1999).
Answer and Counterclaim against Gilead Sciences, Inc. filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Feb. 5, 2009).
Answer filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (May 10, 2010).
Answer filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01851 (May 17, 2010).
Arimilli et al., "Orally bioavailable acylic nucleoside phosphonate prodrugs: Adefovir, Dipivoxil and Bis(POC)PMPA," vol. 3 (accepted for publication), Adv. Antiviral Drug Design, 1998.
Arimilli et al., "Synthesis, in vitro biological evaluation and oral bioavailability of 9-[2-(phosphonomethoxy)propyl]adenine (PMPA) prodrugs," Antiviral Chem. & Chemo. 8(6):557-567, 1997.
Arribas et al., "Tenofovir Disoproxil Fumarate, Entricitabine and Efavirenz Compared with Zidovudine/Lamivudine and Efavirenz in Treatment-Naive Patients 144-Week Analysis," JAIDS 47(1):74-78 (2008).
Balzarini et al, "Differential antiherpesvirus and antiretrovirus effects of the (S) and (R) enantiomers of acyclic nucleoside phosphonates: potent and selective in vitro and in vivo antiretrovirus activities of (R)-9-(2-phosphonomethoxypropyl)-2,6-diaminopurine." Antimicrob Agents Chemother. Feb. 1993; 37(2): 332-338.
Banker, Modern Pharmaceutics, Drugs and the Pharmaceutical Sciences, p. 340, Mercel Dekker, Inc. 1996.
Bartlett et al., "Overview of the effectiveness of triple combination therapy in antiretroviral-naive HIV-1 infected adults," AIDS 15:1369-1377 (2001).
Beauchamp et al., "Amino acid ester prodrugs of acyclovir," Antivir. Chem. and Chemoth. 3(3):157-64, 1992.
Benzaria et al., "New prodrugs of 9-(2-phosphonomethoxyethyl)adenine (PMEA): Synthesis and stability studies," Nucls. & Nuclt. 14(3-5):563-565, 1995.
Benzaria et al., "Synthesis, in Vitro Antiviral Evaluation, and Stability Studies of Bis(S-acyl-2-thioethyl) Ester Derivatives of 9-[2-(Phosphonometyoxy)ethyl]adenine (PMEA) as Potential PMEA Prodrugs with Improved Oral Bioavailability," J. Med. Chem. 39:4958-4965 (1996).
Berge et al., "Pharmaceutical salts," J. Pharm. Sci. 66(1):1-19, 1977.
Blackburn et al., "DNA and RNA structure," pp. 15-81, Nucleic Acids in Chemistry and Biology, 1996.
Bristol Myers Squibb "Sustiva®," http://www.fda.gov/medwaTCH/SAFETY/2005/Sustiva_PI61005.pdf, pp. 3-40 (retrieved Jan. 31, 2007).
Bundgaard et al., "Design and Application of Prodrugs," pp. 113-191, Textbook of Drug Design and Development, 1991, 1 page.
Byrn (editor), Solid State Chemistry of Drugs, 2cd Edition, p. 22, 1999.
Colla et al., "Synthesis and antiviral activity of water-soluble esters of acyclovir [9-[(2-Hydroxyethoxy)methyl]guanine]," J. Med. Chem. 26:602-04, 1983.
Communication about intention to grant a European patent for Patent Publication EP 1583542 (Application No. 04701819.7) issued by the European Patent Office (Nov. 5, 2007).
Communication and Annex for Patent Publication EP 1583542 (Application No. 04701819.7) issued by the European Patent Office (Jul. 17, 2007).
Communication and Annex for Patent Publication EP 1583542 (Application No. 04701819.7) issued by the European Patent Office (Jul. 26, 2006).
Communication and Annex for Patent Publication EP 1583542 (Application No. 04701819.7) issued by the European Patent Office (Oct. 24, 2005).
Communication from the Examining Division of the EPO for EP 1890681 B1 (Application No. 06773194.3) (May 13, 2009).
Communication of further notices of opposition pursuant to Rule 79(2) EPC for EP 1583542 B1 (Application No. 04701819.7) and Request to File Observations (Apr. 23, 2009).
Communication of Intent to Grant EP 1890681 B1 (Application No. 06773194.3) and Druckexemplar issued by the European Patent Office (Jul. 15, 2008).

(56) **References Cited**

OTHER PUBLICATIONS

Communication of notices of opposition pursuant to Rule 79(2) EPC for EP 1890681 B1 (Application No. 06773194.3) and Request to File Observations (Nov. 12, 2009).
Communication pursuant to Article 94(3) EPC for Patent Publication EP 1923063 (Application No. 08152527.1) issued by the European Patent Office (Sep. 4, 2009).
Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc. and Emory University Case No. 10-CV-01798 (Mar. 5, 2010).
Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc. Case No. 08-CV-10838 (Dec. 12, 2008).
Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Mar. 5, 2010).
Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company. Case No. 10-CV-01851 (Mar. 9, 2010).
Conference Call Transcript—Gilead Sciences Conference call to Discuss Triangle Pharmaceuticals Acquisition. Event Date/Time Dec. 4, 2002/ 9:00 AM ET (11 pages).
Correction of an Error in the Decision According to Rule 140 EPC for EP 1890681 B1 (Application No. 06773194.3) (Jul. 5, 2011).
Dando et al., "Emtricitabine/Tenofovir Disoproxil Fumarite," Drugs 64(18):2075-2082 (2004).
Davidsen et al., "N-(Acyloxyalkyl)pyridinium salts as soluble prodrugs of a potent platelet activating factor antagonist," J. Med. Chem. 37(26):4423-4429, 1994.
De Clercq et al., "(S)-9-(2,3-dihydroxypropyl)adenine: An aliphatic nucleoside analog with broad spectrum antiviral activity," Science 200:563-565, 1978.
De Clercq, "Antiviral drugs: current state of the art," J. Clin. Virol. 22:73-89 (2001).
De Clercq et al., "New Developments in Anti-HIV Chemotherapy," Curr. Med. Chem. 8(13):1543-1572 (2001).
De Clercq et al., "New developments in anti-HIV chemotherapy," Farmaco 56(1-2):3-12 (2001).
De Clercq, "Highlights in the Development of New Antiviral Agents," Mini-Rev. Med. Chem. 2(2):163-175 (2002).
De Clercq, "New developments in anti-HIV chemotherapy," Biochem Biophys Acta 1587(2-3):258-275 (2002).
De Lombaert et al., "N-Phosphonomethyl Dipeptides and their Phosphonate Prodrugs, a New Generation of Neutral Endopeptidase (NEP, EC 3.4.24.11) Inhibitor," J. Med. Chem. 37:498-511 (1994).
Decision of Hearing of the Indian Patent Office for Patent Application No. 3383/DELNP/2005 (Mar. 25, 2009).
Decision of Rejection for Application No. 7000806/1999 issued by the Korean Intellectual Property Office (Jan. 20, 2006) (translation).
Decision of Rejection for Patent Application No. 7000636/2000 issued by the Korean Intellectual Property Office (May 10, 2006) (translation).
Decision of Rejection for Patent Application No. 7007002/2008 issued by the Korean Intellectual Property Office (Jan. 7, 2009) (translation).
Decision of Rejection for Patent Application No. 86110757 issued by the Intellectual Property Office of Taiwan (Nov. 11, 1999) (translation).
Decision of Rejection for Patent Application No. 87112168 issued by the Taiwanese Intellectual Property Office (Apr. 6, 2001) (translation).
Decision of the Opposition Division for EP Patent EP 1583542 B1 (Application No. 04701819.7), Claims, Grounds for the Decision and Provision of a Copy of the minutes (Jan. 31 and Feb. 14, 2011).
Decision to Grant Patent Application No. 06773194.3 (EP 1890681B1) issued by the European Patent Office (Dec. 11, 2008).
Declaration of Colleen Tracy in Support of a Motion for Leave to File Amended Complaint Case No. 10-CV-01796 (Apr. 11, 2011).
Declaration of James Galbraith in Opposition of a Motion for Leave to File Amended Complaint Case No. 10-CV-01796 (Apr. 25, 2011).
Delehanty et al. Slides from the oral presentation for "A Randomized Study of Three Doses of FTC Versus 3TC in HIV-Infected Patients," 6th CROI (Jan. 31-Feb. 4, 1999) Chicago.
Delehanty et al., "A Phase I/II Randomized, Controlled Study of FTC Versus 3TC in HIV-Infected Patients," 6th CROI (Jan. 31-Feb. 4, 1999) Chicago, Session 5, Abstract 16.
Drugs and the Pharmaceutical Sciences, vol. 1999, p. 60 (Mark Gibson, ed), 2009.
Engel, R., "Phosphonates as analogues of natural phosphates," Chem. Rev. 77(3):349-367, 1977.
Examination Report for Patent Application No. 540728 issued by the Intellectual Property Office of New Zealand (Apr. 24, 2007).
Examination Report Patent Application No. 564045 issued by the Intellectual Property Office of New Zealand (Oct. 6, 2009).
Examiner's First Report on Patent Application No. 2009200414 issued by the Australian Patent Office (Feb. 24, 2010).
Examiner's First Report on Patent Application No. 85827/98 issued by the Australian Patent Office (Feb. 28, 2001).
Examiner's First Report Patent Application No. 20026257795 issued by the Australian Patent Office (Sep. 29, 2009).
Examiner's Remarks for Patent Application No. a 2008 00493 issued by the Ukrainian Patent Office (2010).
Examiner's Second Report on Patent Application No. 85827/98 issued by the Australian Patent Office (Mar. 27, 2002).
Examiner's First Report on Patent Application No. 2004206821 issued by the Australian Patent office (Aug. 28, 2007).
Examiner's Remarks for Patent Application No. 200501134/28 issued by the Eurasian Patent Office (received Jul. 30, 2009) (translation).
Examiner's Second Report on Patent Application No. 2004206821 issued by the Australian Patent office (Aug. 20, 2008).
Extended European Search Report for Patent Publication EP 1923063 (Application No. 08152527.1) issued by the European Patent Office (Mar. 10, 2009).
Farquhar et al., "Biologically Reversible Phosphate-Protective Groups," J. Pharm. Sci. 72:324-325 (1983).
Farquhar et al., "Synthesis and antitumor evaluation of Bis[(pivaloxy)methyl] 2'-deoxy-5-fluorouridine 5'-monophosphate (FdUMP): a strategy to introduce nucleotides into cells," J. Med. Chem. 37(23):3902-03, 1994.
Fasman et al., pp. 385-394, Practical Handbook of Biochem. and Molec. Biol., 1989.
FDA: "Guidance for industry fixed dose combination and co-packaged drug products for treatment of HIV," www.fda.org. [on line], May 2004, pp. 1-17, (retrieved from http://www.fda.gov/oc/initiatives/hiv/hivguidance.html>[retrieved on Jan. 31, 2007].
Fell et al., "The tensile strength of lactose tablets" J. Pharm. Pharmacol. 20:657-659 (1968).
Feng et al. 2009 "The triple combination of tenofovir, emtricitabine and efavirenz show synergistic anti-HIV-1 activity in vitro: a mechanism of action study," Retrovirology 6:44, http://www.retrovirology.com/content/6/1/44.
Feng, J. et al, "Mechanistic studies show that 9-)-FTC-TP is a better inhibitor of HIV-1 reverse transcriptase than 3TC-TP," FASEB 13:1511-1517 (1999).
Final Office Action for Patent Application No. 86110757 issued by the Intellectual Property Office of Taiwan (Sep. 5, 2000) (translation).
Final Office Action for Patent Application. No. 89123708 issued by the Intellectual Property Office of Taiwan (Apr. 12, 2001) (translation).
First Amended Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Jul. 1, 2009).
First Examination Report for Application No. 564102 Issued by the Intellectual Property Office of New Zealand (Oct. 6, 2009).
First Examination Report for Patent Application No. 3383/DELNP/2005 from the Indian Patent Office (Jul. 31, 2007).
First Examination Report for Patent Application No. 602/DEL/2007 issued by the Indian Patent Office (Nov. 18, 2009).

(56) **References Cited**

OTHER PUBLICATIONS

First Office Action for Patent Application No. 200410046290.X issued by the Chinese Patent Office (Jun. 17, 2005) (translation).
First Office Action for Patent Application No. 200480002190.5 issued by the Patent Office of the People's Republic of China (Aug. 4, 2006).
First Office Action for Patent Application No. 200510099916.8 issued by the Chinese Patent Office (Jun. 15, 2007) (translation).
First Office Action for Patent Application No. 97197460.8 issued by the Chinese Patent Office (Jan. 16, 2004) (translation).
First Official Action for Patent Application No. a 2008 00555 issued by the Ukrainian Patent Office (2011) (translation).
Fiske et al., "Pharmacokinetics, safety and tolerability of single escalating doses of DMP 266, an HIV non-nucleoside reverse transcriptase inhibitor, in healthy volunteers," Pharm. Res. 14(11 Suppl.): S609 (1997).
Flaherty et al., "Synthesis and selective monoamine oxidase B-inhibiting properties of 1-Methyl-1,2,3,6-tetrahydropyrid-4-yl carbamate derivatives: potential prodrugs of (R)- and (S)-Nordeprenyl," J. Med. Chem. 39:4759-4761, 1996.
Folkmann et al., "Acyloxymethyl carbonochloridates. New intermediates in prodrug synthesis," Synthesis, pp. 1159-1166, 1990.
Frampton et al., "Emtricitabine: A Review of Its Use in the Management of HIV Inspection," Drugs 65(10):1427-1448 (2005).
Freeman et al.., "3 Prodrug Design for Phosphate and Phosphonates," Progress in Medicinal Chemistry 34:112-147 (1997).
Fridland, "Tenofovir," Curr. Opin. Anti-Infect. Invest. Drugs 2(3):295-301 (2000).
Fung et al., "Tenofovir Disoproxil Fumarate: A Nucleotide Reverse Transcriptase Inhibitor for the Treatment of HIV Infection," Clin. Therapeutics 24(10):1515-1548 (2002).
Further Examination Report for Patent Application No. 540728 issued by the Intellectual Property Office of New Zealand (Feb. 11, 2008).
Further Examination Report for Patent Application No. 540728 issued by the Intellectual Property Office of New Zealand (Jun. 18, 2008).
Generics [UK] Limited, Notice of Opposition of EP Patent EP 1583542B1 (Application No. 04701819.7) (Mar. 18, 2009).
Generics [UK] Limited, Written Submission in preparation for Oral Proceedings for EP Patent EP 1583542 B1 (Application No. 04701819.7) (Sep. 17, 2010).
Gerhartz (editor) Ullmann's Encyclopedia of Industrial Chemistry vol. B2, Unit Operations I, 5th Edition, p. 3-7.
Gilead "Truvada®," http://www.fda.gov/medwatch/SAFETY/2005/Oct_PI/Truvada_PI.pdf, pp. 1-29 (retrieved Jan. 31, 2007).
Gilead Sciences Inc., Appeal Grounds against the Decision of the Opposition Division for EP 1890681 B1 (Application No. 06773194.3) (Oct. 17, 2011).
Gilead Sciences Inc., Notice of Appeal against the Decision of the Opposition Division for EP 1890681 B1 (Application No. 06773194.3) (Aug. 16, 2011).
Gilead Sciences Inc., Written Submission in preparation for Oral Proceedings for the Opposition of EP 1890681 B1 (Application No. 06773194.3) (Feb. 4, 2011).
Gilead Sciences Inc., Written Submission in preparation for Oral Proceedings for the Opposition of EP 1890681 B1 (Application No. 06773194.3) (Mar. 2 & 4, 2011).
Gilead Sciences Inc., Written Submission in preparation to/during Oral Proceedings for EP Patent EP 1583542 B1 (Application No. 04701819.7) (Sep. 17, 2010).
Gilead Sciences, Inc., "Data Comparing Viread (R) and Emtriva (R) to Combivir (R) as Part of Combination HIV Therapy Published in New England Journal of Medicine," p. 1-5, Press Release, 2006.
Gilead Sciences, Inc., "Gilead Sciences to Acquire Triangle Pharmaceuticals for $464 Million; Gilead to Launch Coviracil in 2003; Will Develop Co-Formulation of Viread and Coviracil," 2 pages, Press Release (2002).
Gilead Sciences, Inc., "U.S. FDA Approves Gilead Sciences' Emtriva A one-capsule, Once-Daily Medication for the Treatment of HIV," pp. 3-7, Press Release, 2003.
Gilead Sciences, Inc., Physician Insert for Truvada, pp. 1-30 (2007).
Gilead, Bristol-Myers Squibb "Atripla™," http://www.fda.gov/cder/foi/;abe;/2006/0219371b1.pdf, pp. 4-53 (retrieved Jan. 31, 2007).
Gilead: "Bristol-Myers Squibb and Gilead announce data supporting bioequivalence for single PIII fixed dose regimen of Sustiva® (efavirenz) and Truvada® (emtricitabine and tenofovir fumarate)" Gilead Press Release (online Jan. 9, 2006), pp. 1-5, retrieved from http://www.gilead.com/press.
Gilead: "Gilead provides update on development of fixed-dose regimen of Truvada (emtricitabine and tenofovir disoproxil fumarate) and Sustiva (efavirenz)," Gilead Press Release, [on line], Apr. 26, 2005, pp. 1-3, retrieved from http://www.gilead.com/press.
Hammer et al., "Ether, carbonate and urethane deoxynucleoside derivatives as prodrugs," Acta Chemica Scandinavia 50:609-622, 1996.
Harris et al., "Genotypic Analysis of HIV-1 Infected ART Naive Patients Receiving Emtricitabine (FTC) or Lamivudine (3TC) in a Double Blind Equivalence Trial," 5th International Workshop on Drug Resistance and Treatment Strategies, Jun. 4-8, No. 104 (2001).
Havlir et al., "In Vivo Antagonism with Zidovudine plus Stavudine Combination Therapy," J. infect. Disease 182:321-325 (2000).
Hazen et al., "Relative Anti-HIV-1 Efficacy of Lamivudine and Emtricitabine In Vitro Is Dependent on Cell Type," J. AIDS 32:255-258 (2003).
Hostetler et al., "Greatly Enhanced Inhibition of Human Immunodeficiency Virus Type 1 Replication in CEM and HT 4-6C Cells by 3'-Deoxythymidine Diphosphate Dimyristoylglycerol, a Lipid Prodrug of 3'-Deoxythymidine," Antimicro. Agent Chemo. 36(9):2025-2029 (1992).
Hostetler et al.., "Synthesis and Antiretroviral Activity of Phospholipid Analogs of Azidothymidine and Other Antiviral Nucleosides," J. Biol. Chem. 265(11):6112-6117 (1990).
Ibbotson et al., Drugs 2003, 63(11), 1089-1096.
Ikeda et al., "Studies of prodrugs III. A convenient and practical preparation of Amphicillin prodrugs," Chem. Pharm. Bull. 32:4316-4322, 1984.
Information about the Results of Oral Proceedings for EP Patent EP 1583542 B1 (Application No. 04701819.7), Claims, Amended Claims and Minutes of the Oral Proceeding (Nov. 19, 2010).
International Preliminary Report on Patentability for PCT/US2004/000832 (Dec. 29, 2004).
International Preliminary Report on Patentability for PCT/US2006/023222 (Oct. 8, 2007).
International Preliminary Report on Patentability for PCT/US2006/023223 (Oct. 8, 2007).
International Search Report and Written Opinion mailed Jul. 12, 2004 for PCT/US2004/000832, Int'l. Filing Date Jan. 13, 2004.
International Search Report for PCT/US1997/013244 (Oct. 20, 1997).
International Search Report for PCT/US1998/015254 (Nov. 25, 1998).
International Search Report for PCT/US2006/023222 (Feb. 23, 2007).
International Search Report for PCT/US2006/023223 (Feb. 23, 2007).
Ishida and Asao, "Self-association and unique DNA binding properties of the anti-cancer agent TAS-103, a dual inhibitor of topoisomerases I and II," Biochem. Biophys. Acta 1587(2-3):155-163 (2002).
Iyer et al., "Synthesis of acyloxyalkyl acylphosphonates as potential prodrugs of the antiviral Trisodium phosphonoformate (Foscarnet sodium)," Tet. Lett. 30(51): 7141-7144, 1989.
Jones et al.., "Minireview: nucleotide prodrugs," Antiviral Res. 27:1-17 (1995).
Kearney et al., "Effect of Demographic Variables on the Pharmacokinetics of Tenofovir DF in HIV-Infected Patients and Healthy Subjects," 41st ICAAC Abstracts, Chicago, IL, Sep. 22-25, 2001, Abstract A-504.

(56) **References Cited**

OTHER PUBLICATIONS

Khamnei et al., "Neighboring Group Catalysis in the Design of Nucleotide Prodrugs," J. Med. Chem. 39:4109-4115 (1996).
King et al. "Potency of Nonnucleoside Reverse Transcriptase Inhibitors (NNRTIs) Used in Combination with Other Human Immunodeficiency Virus NNRTIs, NRTIs, or Protease Inhibitors," Antimicrobial Aqents and Chemotherapy 46(6):1640-1646 (2002).
Kleinebudde et al. European journal of Pharmaceutics and Biopharmaceutics, 2004, 58, 317-326.
Krise et al., "Prodrugs of phosphates, phosphonates, and phosphinates," Advanced Drug Delivery Reviews 19:287-310, 1996.
Kucera et al., "Novel Membrane-Interactive Ether Lipid Analogs That Inhibit Infectious HIV-1 Production and Induce Defective Virus Formation," AIDS Res. & Hum. Retro. 6:491-501 (1990).
Lachman, et al. (1987) "The Theory and Practice of Industrial Pharmacy", Varghese Publishing House, Dadar Bombay, pp. 330-331.
Landgrebe, J. A., "Crystallization and filtration," Theory and Practice in the Organic Laboratory, 3rd Edition, pp. 65-77, 1982.
Letter Regarding the Opposition Procedure for EP Patent EP 1583542 B1 (Application No. 04701819.7) (Mar. 11, 2010).
Lieberman et al., "," Pharmaceutical Dosage Forms 1:177-178 (1989).
Lindahl et al., "Synthesis of an acyloxymethyl prodrug of the Inositol phosphate alpha-Trinositol," J. Carbohydrate Chemistry 15(5):549-554, 1996.
Liu et al., "Thymidylate synthase as a translational regulator of cellular gene expression," Biochem. Biophys. Acta 1587(2-3):174-182 (2002).
Loveday, "Nucleoside reverse transcriptase inhibitor resistance," JAIDS 26:S10-S24 (2001).
Maillard et al., "Adenosine receptor prodrugs: Synthesis and biological activity of derivatives of potent A1-selective agonists," J. Pharm. Sci. 83(1):46-53, 1994.
Margot et al., "Development of HIV-1 Drug Resistance Through 144 Weeks in Antiretroviral-Naive Subjects on Emtricitabine, Tenofovir Disoproxil Fumarate, and Efavirenz Compared with Lamivudine/Zidovudine and Efavirenz in Study GS-01-934," JAIDS 52(2):209-221 (2009).
Margot et al., "Genotypic and phenotypic analyses of HIV-1 in antiretroviral-experienced patients treated with tenofovir DF," AIDS 16:1227-1235 (2002).
Margot et al., "Resistance development over 144 weeks in treatment-naive patients receiving tenofovir disoproxil fumarate or stavudine with lamivudine and efavirenz in Study 903," HIV Medicine 7:442-450 (2006).
Masho et al., "Review of Tenofovir-Emtricitabine," Ther. Clin. Risk Manag. 3(6):1097-1104 (2007).
McColl et al., "Pooled Analysis of Recent Emtricitabine and Lamivudine Clinical Trials Reveals Differences in Rates of Development of the M184V/I Mutation," Poster No. PE7.3/17, 10th European AIDS Conference (EACS) Nov. 17-20, 2005, Dublin Ireland.
McIntee et al., "Probing the mechanism of action and decomposition of amino acid phosphomonoester amidates of antiviral nucleoside prodrugs," J. Med. Chem. 40(2):3323-31, 1997.
Memorandum of Law in Opposition of a Motion for Leave to File Amended Complaint Case No. 10-CV-01796 (Apr. 25, 2011).
Miller et al., Sixth International Congress on Drug Therapy in HIV Infection, Nov. 17-21, 2002 (1 page).
Mills et al., "ARTEMIS: Efficacy and Safety of Darunavir/ritonavir (DRV/r) 800/100mg Once-daily vs Lopinavir/ritonavir (LPV/r) in Treatment-naive, HIV-1-infected Patients at 96 wks," 48th Annual ICAAC/IDSA, 46th Annual Meeting, Washington, D.C. Oct. 25-28, 2008, Presentation No. H-1250c.
Minutes of the Oral Proceedings before the Opposition Division for EP 1890681 B1 (Application No. 06773194.3) and Appendices (Apr. 5, 2011).
Molina et al., "A Lopinavir/Ritonavir-Based Once-Daily Regimen Results in Better Compliance and Is Non-inferior to a Twice-Daily Regimen Through 96 Weeks," AIDS Research and Human Retroviruses 23(12):1505-1514 (2007).
Molina et al., "Once-Daily Combination Therapy with Emtricitabine, Didanosine, and Efavirenz in Human Immunodeficiency Virus-Infected Patients," J. Infect. Dis. 182:599-602 (2000).
Mulato et al., "Anti-HIV activity of adefovir (PMEA) and PMPA in combination with antiretroviral compounds: in vitro analyses," Antiviral Res. 36(2):91-97 (1997).
Murry et al., "Reversion of the M184V Mutation in Simian Immunodeficiency Virus Reverse Transcriptase is Selected by Tenofovir, Even in the Presence of Lamivudine," J. Virol. 77(2):1120-1130 (2003).
Naesens et al., "Antiretroviral activity and pharmacokinetics in mice of oral Bis(Pivaloyloxymethyl)-9-(2-phosphonylmethoxyethyl)adenine, the Bis(Pivaloyloxymethyl)ester prodrug of 9-(2-Phosphonylmethoxyethyl)adenine," Antimicro AG & Chemo. 40(1)22-28, 1996.
Newman and Byrn, "Solid-state analysis of the active pharmaceutical ingredient in drug products" Drug Discovery Today, 8(19) 898-905 (2003).
Notari, "Prodrug Design," Pharmaceutical Therapy, 14:25-53, 1981.
Notice of Appeal of the Decision of the Opposition Division for EP Patent EP 1583542 B1 (Application No. 04701819.7) (Mar. 29, 2011).
Office Action for Korean Patent Application No. 7013069/2005 issued by the Korean Intellectual Property Office (May 22, 2007).
Office Action for Patent Application No. 10-2000-7000636 issued by the Korean Intellectual Property Office (Aug. 19, 2005) (translation).
Office Action for U.S. Appl. No. 08/900,752 issued by the United States Patent and Trademark Office (Apr. 16, 1998).
Office Action for Patent Application No. 11-510067 issued by the Japanese Patent Office (Dec. 11, 2007) (translation).
Office Action for Patent Application No. 2,261,619 issued by the Canadian Patent Office (Dec. 22, 2004).
Office Action for Patent Application No. 2,298,059 issued by the Canadian Intellectual Property Office (Apr. 25, 2007).
Office Action for Patent Application No. 2,512,475 issued by the Canadian Patent Office (Jan. 10, 2008).
Office Action for Patent Application No. 2,611,520 issued by the Canadian Patent Office (Jun. 7, 2010).
Office Action for Patent Application No. 2006-500939 issued by the Japanese Patent Office (Mar. 25, 2010).
Office Action for Patent Application No. 2006-500939 issued by the Japanese Patent Office (Nov. 9, 2009).
Office Action for Patent Application No. 7007002/2008 issued by the Korean Intellectual Property Office (Jun. 11, 2008) (translation).
Office Action for Patent Application No. 7009376/2009 issued by the Korean Intellectual Property Office (Oct. 9, 2009) (translation).
Office Action for Patent Application No. 87112168 issued by the Taiwanese Intellectual Property Office (Mar. 1, 2004) (translation).
Office Action for Patent Application No. 87112168 issued by the Taiwanese Intellectual Property Office (May 6, 2002) (translation).
Office Action for Patent Application No. 87112168 issued by the Taiwanese Intellectual Property Office (Oct. 20, 2000) (translation).
Office Action for Patent Application No. 93112403 issued by the Taiwanese Intellectual Property Office (Apr. 27, 2005) (translation).
Official Action for Application No. 095120445 issued by the Taiwanese Intellectual Property Office (Nov. 29, 2011) (translation).
Official Action for Application No. 2008-517083 issued by the Japanese Patent Office (Aug. 2, 2011) (translation).
Official Action for Ex Parte Reexamination of U.S. Pat. No. 5,922,695 (Dec. 11, 2007).
Official Action for Ex Parte Reexamination of U.S. Pat. No. 5,935,946 (Jan. 16, 2008).
Official Action for Ex Parte Reexamination of U.S. Pat. No. 5,977,089 (Jan. 16, 2008).
Official Action for Ex Parte Reexamination of U.S. Pat. No. 6,043,230 (Dec. 11, 2007).

(56) **References Cited**

OTHER PUBLICATIONS

Official Action for Patent Application No. 10-1999-7000806 issued by the Korean Intellectual Property Office (Apr. 28, 2005) (translation).
Official Action for Patent Application No. 10-508318 issued by the Japanese Patent Office (Apr. 10, 2007) (translation).
Official Action for Patent Application No. 200501134/28 issued by the Eurasian Patent Office (Dec. 25, 2008).
Official Action for Patent Application No. 200501134/28 issued by the Eurasian Patent Office (Oct. 15, 2006).
Official Action for Patent Application No. 333687 issued by the Intellectual Property Office of New Zealand (Mar. 2, 1999).
Official Action for Patent Application No. 7001077/2008 issued by the Korean Intellectual Property Office (Sep. 13, 2010).
Official Action for Patent Application No. 93100813 issued by the Taiwanese Intellectual Property Office (Jun. 22, 2005) (translation).
Official Action for Patent Application. No. 200800033/27 issued by the Eurasian Patent Office (2010) (translation).
Opinion on Patent Application No. 1-2005-00812 issued by the Vietnamese Patent Office (Jul. 27, 2008).
Opponents Comments to the Reply Statement by the Applicant relating to Patent Application No. 3383/DELNP/2005 (Aug. 14, 2008).
Order Granting Request for Ex Parte Reexamination of U.S. Pat. No. 5,922,695 (Jul. 13, 2007).
Order Granting Request for Ex Parte Reexamination of U.S. Pat. No. 5,935,946 (Jul. 13, 2007).
Order Granting Request for Ex Parte Reexamination of U.S. Pat. No. 5,977,089 (Jul. 13, 2007).
Order Granting Request for Ex Parte Reexamination of U.S. Pat. No. 6,043,230 (Jul. 13, 2007).
Osol et al., Editor, Remington's Pharmaceutical Sciences, Sixteenth Edition, pp. 1554-1557, 1980.
Pallella et al., J. Med. Chem. 338:853-860 (1998).
Parikh, Handbook of Pharmaceutical Granulation Tech., NY, Marcel Dekker Inc., 1996.
Pharmaceutical Dosage Forms and Drug Delivery Systems, 6th Ed. pp. 171-174 (1995).
Pharmaceutical Technology (2005), Big Pharma Companies Team Up to Develop Once-Daily Triple-Combination HIV Drug,vol. 29, No. 4.
Piantadosi et al., "Synthesis and Evaluation of Novel Ether Lipd Nucleoside Conjugates for Anti-HIV-1 Activity," J. Med. Chem. 34:1408-1414 (1991).
Plaintiff's Reply to Teva USA's Amended Counterclaim, filed by Gilead Sciences, Emory University, Case No. 08-CV-10838 (Aug. 10, 2009).
Plaintiff's Reply to Teva USA's Second Amended Counterclaim, filed by Gilead Sciences, Emory University,Case No. 08-CV-10838 (Oct. 15, 2009).
Plaintiff's Reply to Teva's Counterclaim, filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Feb. 25, 2009).
Pozniak et al., "Tenofovir Disoproxil Fumarate, Emtricitabine, and Efavirenz Versus Fixed-Dose Zidovudine/Lamivudine and Efavirenz in Antiretroviral-Naive Patients," JAIDS 43(5):535-540 (2006).
Pre-Grant Opposition Petition against Brazilian Patent Application PI 0406760-6 (Aug. 20, 2010).
Puech et al., "Intracellular delivery of nucleoside monophosphates through a reductase-mediated activation process," Antiviral Res. 22:155-174 (1993).
Pujari et al., "Safety and long term effectiveness of generic fixed-dose formulations of nevirpine-based HAART amongst antiretroviral-naïve HIV-infected patients in India," World Health Organization, [on line], Dec. 16, 2003, pp. 99-116; (Retrieved from: http://libdoc.who.int/publications/2003/a86263.pdf.
Rejection Decision for Patent Application No. 200480002190.5 issued by the Patent Office of the People's Republic of China (Jan. 15, 2010).
Rejection of Patent Application No. 93100813 issued by the Taiwanese Intellectual Property Office (Feb. 22, 2006) (translation).
Reply of the Patent Proprietor to the Notice of Opposition of EP 1890681 B1 (Application No. 06773194.3) (Jun. 22, 2010).
Reply of the Patent Proprietor to the Notices of Opposition of EP Patent EP 1583542 B1 (Application No. 04701819.7) (Jan. 4, 2010).
Request for Ex Parte Reexamination of U.S. Pat. No. 5,922,695 (submitted Apr. 30, 2007).
Request for Ex Parte Reexamination of U.S. Pat. No. 5,935,946 (submitted Apr. 30, 2007).
Request for Ex Parte Reexamination of U.S. Pat. No. 5,977,089 (submitted Apr. 30, 2007).
Request for Ex Parte Reexamination of U.S. Pat. No. 6,043,230 (submitted Apr. 30, 2007).
Response to the Written Opinion of the ISA for PCT/US2006/023222 (May 10, 2007).
Response to the Written Opinion of the ISA for PCT/US2006/023223 (May 10, 2007).
Revised International Search Report for PCT/US2004/000832 (Aug. 5, 2004).
Revocation of European Patent EP 1890681 B1 (Application No. 06773194.3) (Jun. 8, 2011).
Richman, "Antiretroviral activity of emtricitabine, a potent nucleoside reverse transcriptase inhibitor," Antivir. Ther. 6(2):83-88 (2001).
Richman, "HIV Chemotherapy," Nature 410:995-1001 (2001).
Ristig et al., "Tenofovir Disoproxil Fumarate Therapy for Chronic Hepatitis B in Human Immunodeficiency Virus/Hepatitis B Virus-Coinfected Individuals for Whom Interferon-α and Lamivudine Therapy Have Failed," J. Infect. Dis.186:1844-1847 (2002).
Robinson et al., "Discovery of the Hemifumarate and (alpha-L-Alanyloxy)methylester as prodrugs of an antirheumatic oxindole: Prodrugs for enolic OH group," J. Med. Chem. 39:10-18, 1996.
Rousseau et al., "Prototype trial design for rapid dose selection of antiretroviral drugs: an example using emtricitabine (Coviracil)," Journal of Antimicrobial Chemotherapy 48:507-513 (2001).
Safadi et al., "Phosphoryloxymethyl carbamates and carbonates—Novel water soluble prodrugs for amines and hindered alcohols," Pharm. Res. 10(9):1350-1355, 1993.
Sakamoto et al., "Studies on prodrugs II. Preparation and characterization of (5-substituted 2-oxo-1,3-dioxolen-4-yl)methyl esters of Ampicillin," Chem. Pharm. Bull. 32(6):2241-2248, 1983.
Samara et al., "Pharmacokinetic analysis of Diethylcarbonate prodrugs of Ibuprofen and Naproxen," Biopharmaceutics & Drug Disposition 16:201-210, 1995.
Sanne et al., "Genotypic Analysis of HIV-1 Infected ART-Naive Patients Receiving Emtricitabine (FTC) or Lamivudine (3TC) in a Double Blind Equivalence Trial," Poster No. 4433, presented at the XIV International AIDS Conference Jul. 7-12, 2002, Barcelona, Spain.
Sanne et al., "Two Randomized, Controlled, Equivalence Trials of Emtricitabine (FTC) to Lamivudine (3TC)," Poster 4432 presented at the XIV International AIDS Conference, Jul. 7-12, 2002, Barcelona, Spain.
Schinazi et al., "Characterization of Human Immunodeficiency Viruses Resistant to Oxathiolane-Cytosine Nucleosides," Antimicrobial Agents and Chemotherapy 374:875-881 (1993).
Schinazi et al., "Selective Inhibition of Human Immunodeficiency Viruses by Racemates and Enantiomers of cis-5-Fluoro-1-[2-(Hydroxymethyl)-1,3-Oxathiolan-5-yl]Cytosine," Antimricobial Agents and Chemotherapy 36(11):2423-2431(1992).
Schinazi et al., Letter to the Editor "Assessment of the Relative Potency of Emtricitabine and Lamivudine," J. AIDS 34(2)243-245 (2003).
Search and Examination Report for Appln No. AP/P/2005/003348 issued by the African Regional Intellectual Property Organization, 5 pages (Apr. 10, 2008).
Second Amended Answer and Counterclaim against Gilead Sciences, Inc. filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Filed Oct. 9, 2009).

(56) **References Cited**

OTHER PUBLICATIONS

Second Amended Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Sep. 25, 2009).
Second Office Action for Application No. 200680026180.4 issued by the State Intellectual Property Office of the People's Republic of China (Oct. 20, 2011) (translation).
Second Office Action for Patent Application No. 200510099916.8 issued by the Chinese Patent Office (Nov. 16, 2007) (translation).
Second Office Action for Patent Application No. 97197460.8 issued by the Chinese Patent Office (Aug. 5, 2005) (translation).
Second Official Action for Patent Application No. a 2005 07947 issued by the Ukrainian Patent Office (2006) (translation).
Serafinowska et al., "Synthesis and in vivo Evaluation of prodrug of 9-{2-(Phosphonomethoxy)ethoxy} adenine," J. Med. Chem. 38:1372-1379, 1995.
Shaw et al., "Metabolism and pharmacokinetics of novel oral prodrugs of 9-[(R)-2-(phosphonomethoxy)propyl]adenine (PMPA) in dogs," Pharm. Res. 14(12):1824-1829, 1997.
Siddiqui et al., "Design and Synthesis of Lipophilic Phosphoramidate D4T-MP Prodrugs Expressing High Potency Against HIV in Cell Culture: Structural Determinants for In Vitro Activity and QSAR," J. Med. Chem. 42(20):4122-4128 (1999).
Smith et al., "Randomized, double-blind, placebo-matched, multicenter trial of abavacir/lamivudine or tenofovir/emtricitabine with lopinavir/ritonavir for inital HIV treatment," AIDS 23:1547-1556 (2009).
Srinivas et al., "Metabolism and in vitro antiretroviral activities of Bis(Pivaloyloxymethyl) prodrugs of acyclic nucleoside phosphonates," Antimicro AG & Chemo. 37(10):2247-2250, 1993.
Srivastva et al., "Bioreversible phosphate protective groups: Synthesis and stability of model acyloxymethyl phosphates," Bioorg. Chem. 12:118-129, 1984.
Starrett et al., "Synthesis and in vitro evaluation of a phosphonate prodrug:bis(pivaloyloxymethyl) 9-(2-phophonylmethoxyethyl)adenine," Antiviral Res. 19:267-273, 1992.
Starrett et al., "Synthesis, oral bioavailability determination, and in vitro evaluation of prodrugs of the Antiviral agent 9-[2-(phosphonomethoxy)ethyl)adenine (PMEA)," J. Med. Chem. 37:1857-1864, 1994.
Substantive Examination Report for Patent Application No. W00 2005 02145 from the Indonesian Patent Office (2010).
Sueoka et al., "Pharmacokinetics of Alkoxycarbonyloxy ester prodrugs of PMPA in dogs," Abstract, American Association of Pharmaceutical Science, Western Regional Meeting, Apr. 24-25, 1997.
Summons to Attend Oral Proceedings and Annex to the Communication for EP Patent EP1583542B1 (Application No. 04701819.7) (May 21, 2010).
Summons to Attend Oral Proceedings and Annex to the Communication for the Opposition of EP 1890681 B1 (Application No. 06773194.3) (Oct. 14, 2010).
Tamari, "A Decade in HIV Treatment: What Is the State of the Art and How Did We Arrive," Clinical Excellence for Nurse Practitioners 5(1):4-12 (2001).
Teva Pharmaceutical Industries Ltd., Notice of Opposition of EP Patent EP1583542B1 (Application No. 04701819.7) (Mar. 13, 2009).
Teva Pharmaceutical Industries Ltd., Written Submission in preparation for Oral Proceedings for EP Patent EP1583542B1 (Application No. 04701819.7) (Sep. 16, 2010).
Teva Pharmaceuticals Industries Ltd., Notice of Opposition of EP 1890681B1 (Application No. 06773194.3) (Oct. 7, 2009).
Teva Pharmaceuticals Industries Ltd., Written Submission in preparation for Oral Proceedings for the Opposition of EP 1890681B1 (Application No. 06773194.3) (Mar. 17, 2011).
Third Amended Answer filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Filed Aug. 6, 2010).
Third Amended Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Jul. 6, 2010).
Third Office Action for Patent Application No. 97197460.8 issued by the Chinese Patent Office (Jun. 9, 2006) (translation).
Third Official Action for Patent Application No. a 2005 07947 issued by the Ukrainian Patent Office (2007) (translation).
Thornber "Isosterism and Molecular Modification in Drug Design" Chem. Soc. Reviews 18: 563-580, 1979.
Tisdale et al., "Rapid in vitro selection of human immunodeficiency virus type 1 resistant to 3'-thiacytidine inhibitors due to a mutation in the YMDD region of reverse transcriptase," Proc. Natl. Acad. Sci. USA 90:5653-5656 (1993).
Tsai et al., "Effects of (R)-9-(2-phophonylmethoxypropyl)adenine monotherapy on chronic SIV infection in macaques," Aids Res. & Hum. Retro. 13(8):707-712, 1997.
Tsai et al., "Prevention of SIV infection in macaques by (R)-9-(2-phosphonylmethoxypropyl)adenine," Science 270:1197-1199, 1995.
U.S. Department of Health and Human Services (2004) "Guidance for Industry Fixed Dose Combination and Co-Packaged Drug Products for Treatment of HIV—Draft Guidance" pp. 1-21.
Ueda et al., "Vinyl compounds of nucleic acid bases I. Synthesis of N-vinyluracil, N-vinylthymine, and N-vinyladenine," Die Makromolekulare Chemie 120:13-20, 1968.
USP 24 The United States Pharmacopeia (2000).
Wainberg et al. "In vitro selection and characterization of HIV-1 with reduced susceptability to PMPA," Antiviral Therapy 4:87-94 (1999).
Walmsley et al., "Gemini: A Noninferiority Study of Saquinavir/Ritonavir Versus Lopinavir/Ritonavir as Initial HIV-1 Therapy in Adults," J. Acquir. Immune Defic. Syndr. 50(4):367-374 (2009).
Ait-Khaled, et al., "Zidovudine appears to prevent selection of K65R and L74V mutations normally selected by abacavir mono- or combination therapies not containing zidovudine" Antiviral Therapy, 2002, 7:S107 (Abstract).
Arimilli et al., "Nucleotide Analogues," U.S. Appl. No. 60/022,708, 40 pages (filed Jul. 26, 1996).
Arzneiformenlehre. Ein Lehrbuch für Pharmazeuten, List et al., Eds., Wissenschaftliche Verlagsgesellschaft mbH, pp. 79 and 477 (1985). An English translation is not readily available. Applicants believe this reference discloses that preparations with lactose can undergo the Maillard reaction with amines. Such preparations result in discolorations. Amines and reducing sugars may also form N-glycosylamines which may react further in a Maillard reaction.
BioWorld Today, "About BioWorld," 1 page, http://www.bioworld.com/servlet/com.accumedia.web.Dispatcher?next=aboutUs (2010).
Borroto-Esoda et al., "In vitro evaluation of the anti-HIV activitiy and metaboloc interactions of tenofovir and emtricitabine," Antiviral Therapy 11:377-384 (2006).
Brogan et al., "Cost-Effectiveness of Nucleoside Revers Transcriptase Inhibitor Pairs in Efavirenz-Based Regimens for Treatment-Naïve Adults with HIV Infection in the United States," Value in Health 14:657-664 (2011).
Communication concerning Correction of the EP Specification for Patent Publication EP 1583542 (Application No. 04701819.7) issued by the European Patent Office (Jul. 22, 2008).
Communication of Notice of Opposition of EP 1583542 B1 (Application No. 04701819.7)—first information to the patent proprietor (Mar. 24, 2009).
Communication of Notice of Opposition of EP 1583542 B1 (Application No. 04701819.7)—first information to the patent proprietor (Mar. 26, 2009).
Communication pursuant to Article 94(3) EPC for Patent Publication EP 1923063 (Application No. 08152527.1) issued by the European Patent Office (Mar. 26, 2012).
Crowley, "Drug-Excipient Interactions," Pharm. Tech., 6 pages (2001).

(56) **References Cited**

OTHER PUBLICATIONS

Dahl et al., Amended Transmittal of U.S. Appl. No. 10/540,794, Compositions and methods for combination antiviral therapy, filed Mar. 20, 2006.
De Clercq, "New Anti-HIV Agents and Targets," Medicinal Research Reviews 22(6):531-565 (2002).
Decision to Grant a European patent for EP Appln No. 04701819.7 and Druckexemplar (May 23, 2008).
Department of Health and Human Services, "Guidelines for the Use of Antiretroviral Agents in HIV-1-infected Adults and Adolescents," pp. 1, 33-49 (Nov. 3, 2008).
Examiner's Second Report on Patent Application No. 2009200414 issued by the Australian Patent Office (Aug. 29, 2011).
European Search Report, EP 2386294 (Application No. 11167101.2), 15 pages (Dec. 29, 2011).
Eyjolfsson, "Lisinopril-Lactose Incompatibility," Drug Devel. Indust. Pharm. 24(8):797-798 (1998).
First Examination Report for Patent Application No. 6665/DELNP/2008 issued by the Indian Patent Office (Jun. 30, 2011).
FTC 101 Virology analysis, TPI Document No. 14022 (2002).
Gallant, et al., "Early Non-Response to Tenofovir DF (TDF) + Abacavir (ABC) and Lamivudine (3TC) in a Randomized Trial Compared to Efavirenz (EFV) + ABC and 3TC: ESS30009 Unplanned Interim Analysis," Abstr Intersci Conf Antimicrob Agents Chemother Intersci Conf Antimicrob Agents Chemother, Abstract No. H-1722a (2003).
Giron, "Applications of thermal analysis in the pharmaceutical industry," J. Pharm. Biomed. Anal. 4(6):755-770 (1986).
Glaxo Marketing Material, Epivir + Ziagen, 6 pages (2003).
Huff, "Five New Drugs Enter the Homestretch," The Body: The Complete HIV/AIDS Resource, 3 pages (2002).
Information About the Results of the Oral Proceedings for EP 1890681 B1 (Application No. 06773194.3) (Apr. 5, 2011).
Jamsek, et al. "Poor Virological Responses and Early Emergence of Resistance in Treatment Naïve, HIV-infected Patients Receiving a Once Daily Triple Nucleoside Regimen of Didanosine, Lamivudine, and Tenofovir DF" 11th Conf Retrovir Oppor Infect, Abstract No. 51 (2004).
Kusmierek et al., "Kinetics and Mechanisms of Hydrolytic Reactions of Methylated Cytidines under Acidic and Neutral Conditions," Acta Chem. Scand. 43:196-202 (1989).
Lanier, et al. "Prediction of NRTI Optins by Linking Reverse Transcriptase Genotype to Phenotypic Breakpoints" 10th Conf Retrovir Oppor Infect, Abstract No. 586 (2003).
Lindahl, "Instability and decay of the primary structure of DNA," Nature 362:709-715 (1993).
Lu, et al., "Determination of Clinical Cut-Offs for Reduced Response to Tenofovir DF therapy in Antiretroviral-Experienced Patients," Antiviral Therapy 7(1):S104, Abstract No. 125 (2002).
Marcelin et al., "Resistance profiles of emtricitabine and lamivudine in tenofovir-containing regimens," J. Antimicrob. Chemother. 67:1475-1478 (2012).
Margot et al., "In Vitro Human Immunodeficiency Virus Type 1 Resistance Selections with Combinations of Tenofovir and Emtricitabine or Abacavir and Lamivudine," Antimicrobial Agents and Chemotherapy 50(12):4087-4095 (2006).
Merck Index, 13th Edition, p. ONR-65 (2001).
Molina et al., "CASTLE: Atazanavir-Ritonavir vs Lopinavir-Ritonavir in Antiretroviral-Naïve HIV-1 Infected Patients: 96 Week Efficacy & Safety," 48th Annual ICAAC/IDSA 46th Annual Meeting, Washington , D.C., Presentation No. H-1250d (Oct. 25-28, 2008).
National Institutes of Health, "Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents," 9 pages (2011/2012).
Notice of Allegation and Detailed Statement in respect of Tenofovir Disoproxil Fumarate and Emtricitabine (Truvada®) and Canadian Patent Nos. 2,261,619 and 2,298,059, 56 pages (Nov. 22, 2011).
Notice of Allegation and Detailed Statement in respect of TRUVADA and Canadian Patent No. 2,512,475, 37 pages (Nov. 22, 2011).
Office Action for Korean Patent Application No. 7013069/2005 issued by the Korean Intellectual Property Office (Jan. 23, 2008).
Office Action, 10 pages, from U.S. Appl. No. 12/195,161 (mailed May 7, 2010).
Office Action, 10 pages, from U.S. Appl. No. 12/204,174 (mailed Oct. 1, 2009).
Office Action, 7 pages, from U.S. Appl. No. 10/540,794 (mailed Sep. 21, 2006).
Office Action, 8 pages, from U.S. Appl. No. 10/540,794 (mailed Oct. 31, 2007).
Office Action, 8 pages, from U.S. Appl. No. 12/204,174 (mailed Jun. 4, 2010).
Office Action, 9 pages, from U.S. Appl. No. 10/540,794 (mailed May 16, 2007).
Official Action and Preliminary Notice of Allowance from The Eurasian Patent Office for Application No. 200501134/28 (May 14, 2010).
Official Action for Application No. 2008-517083 issued by the Japanese Patent Office (Mar. 23, 2012) (translation).
Official Action for Patent Application No. 93100813 issued by the Taiwanese Intellectual Property Office (Mar. 16, 2011) (translation).
Official Action, 2 pages, from JP appl. No. 2010-175808 (mailed Nov. 6, 2012) translation attached.
Official Communication for Patent Application No. 2100/DEL/2007 issued by the Indian Patent Office (Jan. 16, 2013).
Opposition filed in Indian Patent Appl. No. 9661/DELNP/2007 by Cipla Limited (Jun. 30, 2009).
Osborne, "Gilead plans $300M notes sale to recoup some merger costs.(Gilead Sciences Inc.)," Bioworld Today, 13(239), 1 page (Dec. 16, 2002).
Osborne, "Gilead, Triangle Plan Merger: $464 Deal Pairs HIV Drugs," Bioworld Today 13(233):1,6 (2002).
Pharmaceutical Dosage Forms. Tablets. 2nd Ed., revised and expanded, Lieberman et al., eds., pp. 93 and 98 (1990).
Project Inform, "Perspective," pp. 1-28 (2003).
Quan et al., "Endogenous Reverse Transcriptase Assays Reveal Synergy between Combinations of the M184V and other Drug-Resistance-conferring Mutations in Interactions with Nucleoside Analog Triphosphates," J. Mol. Biol. 277:237-247 (1998).
Reply to the statement of appeal grounds, EP 1583542 B1 (Application No. 04701819.7), 50 pages (Oct. 18, 2011).
Request for Correction of EP Appln No. 04701819.7 (Jul. 8, 2008).
Request for Correction of EP Appln No. 04701819.7 (Jun. 27, 2008).
Response to the Noting of Loss of Rights pursuant to Rule 112(1) EPC dated Sep. 3, 2012, Patent Publication EP 2386294 (Application No. 11167101.2) (Nov. 13, 2012).
Response to the Noting of Loss of Rights pursuant to Rule 112(1)EPC dated Nov. 14, 2012, Patent Publication EP 1923063 (Application No. 08152527.1) (Jan. 24, 2013).
Response to the reply letter of Teva Pharmaceutical Industries Ltd. dated Oct. 18, 2011, EP 1583542 B1 (Application No. 04701819.7), 35 pages (Aug. 9, 2012).
Reversal of Rejection Decision for Application No. 200480002190.5 by the Patent Rexamination Board (Patent Office of the People's Republic of China) (Jun. 10, 2010).
Riaz and Ami, "Stability of Aminophylline," Pak. J. Pharm. Sci. 6(1):35-44 (1993).
Scrip, "Gilead Acquires Triangle for $464 Million," 2 pages (Dec. 6, 2002).
Second Office Action for Patent Application No. 200480002190.5 issued by the Patent Office of the People's Republic of China (May 11, 2011).
Smirnov et al., "A Comparative Study of the Kinetics of Cytarabine Hydrolytic Deamination in Aqueous Solutions," Pharm. Chem. J. 34(8):451-454 (2000).
Staszewski et al., NEJM, 1999, 341 5,1865-1873.
Teva Pharmaceutical Industries, Ltd, (Opponent), Opposition Brief against Israel Patent No. 169243 (dated Jul. 26, 2009).

(56) **References Cited**

OTHER PUBLICATIONS

Teva's Response to Patentee's Appeal, 5 pages, EP Pat. No. 1890681, EP Appl. No. 06773194.3 (mailed May 3, 2012).
Theory and Practice of Industrial Pharmacy, 3rd Edition, Lachman et al., Eds., Lea & Febiger, pp. 324-329 (1986).
Thoithi et al., "Investigation of the Kinetics of Degradation of Hexopyranosylated Cytosine Nucleosides Using Liquid Chromatography," Nucleosides, Nucleotides & Nucleic Acids 19(1 &2):189-203 (2000).
Truvada Patient Information Leaflet, 33 pages (2008).
Viread (Tenofovir Disoproxil Fumarate Tablets) Summary of Product Characteristics, EMEA, SmPC, 37 pages (Feb. 5, 2002).
Viread Label pp. 1-44 (2001).
Viread Patient Information Leaflet, 21 pages (2002).
Virji-Jeganathan, "BVV Stock Table Highlights," Bioventure View, 17(25): pp. 6-7 (Dec. 10, 2002).
Wang "FTC: A Potent and Selective Anti-HIV and Anti-HBV Agent Demonstrating Desirable Pharmacokinetic (PK Characteristics," Abstracts of the IDSA, 36th Annual Meeting, Session 58, Poster 415, Hepatits A, B, and C in HIV-Infected Persons Friday, 4-6 pm (1998).
Wirth et al., "Maillard Reaction of Lactose and Fluoxetine Hydrochloride, a Secondary Amine," J. Pharm. Sci. 87(1):31-39 (1998).
Zalac et al., "Paracetamol-Propyphenazone Interaction and Formulation Difficulties Associated with Eutectic Formation in Combination Solid Dosage Forms," Chem. Pharm. Bull. 47(3):302-307 (1999).
Affirmation of Andrew W. Williams in Support of Plaintiffs Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co's Opening Claim Construction Brief and Exhibits Case No. 10-CV-01851 (Dec. 2, 2011).
Answer and Counterclaim filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 09-CV-04463 (Aug. 10, 2009).
Answer to Amended Complaint filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jun. 29, 2011).
Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. filed by Gilead Sciences, Inc. Case No. 09-CV-04463 (May 8, 2009).
Declaration of Paul A. Bartlett in Support of Plaintiffs' Claim Constructions and Exhibits filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Nov. 4, 2011).
Declaration of Michael J. Freno in Support of Teva's Opening Claim Construction Brief and Exhibits filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Nov. 4, 2011).
Declaration of Adam C. LaRock in Support of Plaintiffs' Claim Constructions and Exhibits filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Nov. 4, 2011).
Declaration of Adam C. LaRock in Support of Plaintiffs' Rebuttal Claim Constructions and Exhibits filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Dec. 16, 2011).
Declaration of Natalie Lieber and Exhibits filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Dec. 2, 2011).
Declaration of Natalie Lieber in Support of Plaintiffs' Rebuttal Claim Constructions and Exhibits filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Jan. 13, 2012).
Declaration of Daniel P. Margolis in Support of Teva's Opening Claim Construction Brief and Exhibits filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Dec. 2, 2011).
Declaration of Daniel P. Margolis in Support of Teva's Rebuttal Claim Construction Brief and Exhibits filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Dec. 16, 2011).
Declaration of Daniel P. Margolis in Support of Teva's Rebuttal Claim Construction Brief and Exhibits filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jan. 13, 2012).
Declaration of Allan S. Myerson in Support of Plaintiffs' Claim Constructions and Exhibits filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Dec. 2, 2011).
Declaration of Robin D. Rogers, Ph.D. in Support of Teva's Claim Constructions and Exhibits Case No. 10-CV-01851 (Jan. 13, 2012).
Declaration of Karen C. Shen in Support of Teva's Opening Claim Construction Brief and Exhibits Case No. 10-CV-01851 (Dec. 2, 2011).
Declaration of Slaven Jesic in Support of Teva's Rebuttal Claim Construction Brief and Exhibits Case No. 10-CV-01851 (Jan. 13, 2012).
Declaration of Andrew W. Williams in Support of Plaintiffs Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co's Rebuttal Claim Construction Brief and Exhibits Case No. 10-CV-01851 (Jan. 13, 2012).
Defendants' Memorandum in Opposition to Plaintiff's Pretrial Memorandum filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jan. 28, 2013).
Defendants' Memorandum in Opposition to Plaintiffs' Pretrial Memorandum filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01851 (May 24, 2013).
Defendants' Notice Pursuant to 35 U.S.C. § 282 filed by Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co. Case No. 10-CV-01851 (May 22, 2013).
Defendants' Pretrial Memorandum filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jan. 14, 2013).
Defendants' Pretrial Memorandum filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01851 (May 10, 2013).
Defendants' Proposed Findings of Fact and Conclusions of Law filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jan. 14, 2013).
Defendants' Proposed Findings of Fact and Conclusions of Law filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01851 (May 10, 2013).
Letter to Judge Richard J. Sullivan Case No. 10-CV-01796 (Jan. 13, 2012).
Fourth Amended Answer filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Oct. 17, 2011).
Fourth Amended Complaint for Patent Infringement against Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. and Exhibits filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Oct. 3, 2011).
First Amended Complaint for Patent Infringement filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Jun. 15, 2011).
Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co's Opening Claim Construction Brief Case No. 10-CV-01851 (Dec. 2, 2011).
Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co's Rebuttal Claim Construction Brief Case No. 10-CV-01851 (Jan. 13, 2012).
Order signed by Judge Richard J. Sullivan Case No. 08-CV-10838 (May 26, 2010).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01798 (May 26, 2010).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01796 (Dec. 5, 2011).
Order signed by Judge Richard J. Sullivan Case No. 08-CV-10838 (Dec. 19, 2011).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01796 (Apr. 26, 2012).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01796 (Jan. 18, 2013).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01796 (Jan. 30, 2013).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01851 (May 14, 2013).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01851 (May 24, 2013).

(56) **References Cited**

OTHER PUBLICATIONS

Order signed by Judge Richard J. Sullivan Case No. 10-CV-01851 (Jun. 5, 2013).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01851 (Jul. 19, 2013).
Plaintiff's Opposition to Defendants' Pretrial Memorandum by Gilead Sciences, Inc. Case No. 10-CV-01796 (Jan. 28, 2013).
Plaintiffs' Opening Claim Construction Brief filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Nov. 4, 2011).
Plaintiff's Opening Claim Construction Brief filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Dec. 2, 2011).
Plaintiffs' Opening Pretrial Brief filed by Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co. Case No. 10-CV-01851 (May 10, 2013).
Plaintiff's Pretrial Memorandum filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Jan. 14, 2013).
Plaintiff's Proposed Findings of Fact and Conclusions of Law filed by Gilead Sciences, Inc. Case No. 10-CV-01796 (Jan. 14, 2013).
Plaintiffs' Proposed Findings of Fact and Conclusions of Law filed by Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co. Case No. 10-CV-01851 (May 10, 2013).
Plaintiffs' Rebuttal Claim Construction Brief filed by Gilead Sciences, Inc., Emory University Case No. 08-CV-10838 (Dec. 16, 2011).
Plaintiffs' Rebuttal Claim Construction Brief filed by Gilead Sciences, Inc., Emory University Case No. 10-CV-01796 (Jan. 13, 2012).
Plaintiffs' Reply to Teva USA's Counterclaim filed by Gilead Sciences, Inc., Emory University Case No. 09-CV-04463 (Aug. 31, 2009).
Plaintiffs' Response to Defendants' Pretrial Memorandum filed by Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co. Case No. 10-CV-01851 (May 24, 2013).
Request for Leave to Submit Supplemental Expert Witness Affidavit of Jerry L. Atwood, Ph.D. on behalf of Plaintiffs filed by Merck, Sharp & Dohme Corp and Bristol-Myers Squib Company Co. Case No. 10-CV-01851 (May 24, 2013).
Stipulation and Agreement Regarding U.S. Pat. No. 5,922,965, U.S. Pat. No. 5,935,946, U.S. Pat. No. 5,977,089, and U.S. Pat. No. 6,043,230 Case No. 10-CV-01796 (Oct. 9, 2012).
Teva's Opening Claim Construction Brief filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Nov. 4, 2011).
Teva's Opening Claim Construction Brief filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Dec. 2, 2011).
Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd.'s Opening Claim Construction Brief Case No. 10-CV-01851 (Dec. 2, 2011).
Teva's Rebuttal Claim Construction Brief filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 08-CV-10838 (Dec. 16, 2011).
Teva's Rebuttal Claim Construction Brief filed by Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd. Case No. 10-CV-01796 (Jan. 13, 2012).
Teva Pharmaceuticals USA, Inc and Teva Pharmaceuticals Industries, Ltd.'s Rebuttal Claim Construction Brief Case No. 10-CV-01851 (Jan. 13, 2012).
Transcript of Proceedings held on Apr. 26, 2012 Case No. 10-CV-01796.
Transcript of Proceedings held on Oct. 3, 3012, 2012 Case No. 10-CV-01796.
Bloor S., et al, Patterns of HIV drug resistance in routine clinical practice; a survey of almost 12000 samples from the USA in 1999, Antiviral Therapy 5(3):132.
Carpenter, C.C.J., et al., "Antiretroviral Therapy for HIV Infection in 1997: Updated Recommendations of the International AIDS Society—USA Panel," The Journal of the American Medical Association 277(24):1962-1969 (Jun. 25, 1999).
Cherry, et al., "Mutations at codon 184 in simian immunodeficiency virus reverse transcriptase confer resistance to the (-) enantiomer of 2,,3'-dideoxy-3,-thiacytidine," Antimicrob., Agents Chemother. 41:2763-2765 (1997) http://aac.asm.org/content/41/12/2763.full.pdf+html (retrieved on Jan. 29, 2014).
Delehanty J., et al., "Selection of FTC dose based on viral kinetics and pharmacokinetics in an accelerated clinical trial design," 12th World AIDS Conference, Geneva, Switzerland, Abstract No. 12208 (Jul. 1998) http://www.aegis.org/DisplavContent/print.aspx?SectionlD=341007 (retrieved on Jan. 30, 2014).
Flexner, C., " HIV-Protease Inhibitors," The New England Journal of Medicine 338(18):1281-1292 (Apr. 30, 1998).
Gosselin, et al., "Anti-human immunodeficiency virus activities of the (3-L enantiomer of 2',3'-dideoxycytidine and its 5-fluoro derivative in vitr," Antimicrob Agents Chemother 38: 1292-1297 (1994) http://aac.asm.org/content/38/6/1292.full.pdf (retrieved on Jan. 30, 2014).
Harrigan, P.R., et al., "Phenotypic susceptibilities to tenofovir in a large panel of clinically derived human immunodeficiency virus type 1 isolates," Antimicrob Agents Chemother. 46:1067-1072 (2002) http://aac.asm.Org/content/46/4/1067.full.pdf+html (retrieved on Jan. 30, 2014).
Miller, Margot N.A., et al., "Antiviral activity of tenofovir (PMPA) against nucleoside-resistant clinical HIV samples," Nucleosides Nucleotides Nucleic Acids,; 20:1025-1028 (2001).
Miller M.D., et al., Human Immunodeficiency Virus Type 1 Expressing the Lamivudine-Associated M184V Mutation in Reverse Transcriptase Shows Increased Susceptibility to Adefovir and Decreased Replication Capability In Vitro, The Journal of Infectious Diseases 179:92-100 (1999) http://jid.oxfordjournals.org/content/179/1/92.full.pdf (retrieved on Jan. 30, 2014).
Robinson B.S., et al. BMS-232632, a Highly Potent Human Immunodeficiency Virus Protease Inhibitor That Can Be Used in Combination with Other Available Antiretroviral Agents.—Antimicrobial Agents and Chemotherapy 44(8):2093-2099 (Aug. 2000) http://www.ncbi.nlm.nih.gov/pmc/articles/PMC90019/pdf/acQ02093.pdf (retrieved on Jan. 30, 2014).
Srinivas R.V. et al., "Antiviral Activities of 9-R-2-Phosphonomethoxypropyl Adenine (PMPA) and Bis(isopropyloxymethylcarbonyl)PMPA against Various Drug-Resistant Human Immunodeficiency Virus Strains," Antimicrob. Agents Chemother 42(6):1484-1487 (Jun. 1988) http://aac.asm.org/content/42/6/1484.full.pdf+html (retrieved on Jan. 30, 2014).
Staszewski S., et al., "Efficacy and safety of tenofovir disoproxil fumarate (TDF) versus stavudine (d4T) when used in combination with lamivudine (3TC) and efavirenz (EFV) in HIV-1 infected patients naive to antiretroviral therapy (ART): 48-week interim results," XIV International AIDS Conference, Barcelona, Spain, Abstracts No. 9804:7-12 (Jul. 2002) http://www.iasocietv.org/Default.aspx?pageld=12&abstractld=9804 (retrieved on Jan. 30, 2014).
Van Rompay, K. K., et al., "Virulence and reduced fitness of simian immunodeficiency virus with the M184V mutation in reverse transcriptase," J. Virology, 76(12):6083-6092 (2002) http://www.ncbi.nlm.nih.gov/pmc/articles/PMC136201/pdf/0196.pdf (retrieved on Jan. 30, 2014).
Van Rompay, et al., "9-[2-(Phosphonomethoxy)propyl]adenine therapy of established simian immunodeficiency virus infection in infant rhesus macaques," Antimicrob. Agents Chemother. 40:2586-2591 (1996) http://www.ncbi.nlm.nih.gov/pmc/articles/PMC163581/pdf/402586.pdf (retrieved on Jan. 31, 2014).
Zao, Biokad Objection against the application of Eurasian Patent No. 015145 in the Russian Federation dated Jul. 10, 2013.
Patent Examination Report No. 1, Australia patent appl. No. 2011253996, 2 pages (Nov. 15, 2012).
Office Action for Patent Application No. 200680026180.4 issued by the Chinese Patent Office (May 29, 2013) (translation).
Summons to attend Oral Proceedings EPO for Application No. 067731595.0 dated Jul. 9, 2013.
Communication and Annex from PO for Application No. 067731595.0 dated Jul. 9, 2013.
Response to Summons and Annex rom EPO for Application No. 067731595.0 dated Sep. 25, 2013.

(56) **References Cited**

OTHER PUBLICATIONS

Brief Communication from EPO for Application No. 067731595.0 dated Nov. 10, 2013.
Communication from EPO for Application No. 067731595.0 dated Nov. 15, 2013.
Minutes of Oral Proceedings from EPO for Application Application No. 067731595.0 dated Nov. 25, 2013.
Communication from EPO for Application No. 067731595.0 dated Dec. 4, 2013.
Excerpt of The Merck Veterinary Manual (Clarence M. Fraser et al. eds., 7th ed., 1991).
Excerpts from Daniel S. Kemp & Frank Vellaccio, Organic Chemisty (Worth Publishers, Inc. 1980).
Excerpts from K. Peter C. Vollhardt, Organic Chemistry (W.H. Freeman and Company 1987).
Excerpts from the Reexamination of U.S. Pat. No. 6,043,230.
Excerpts of Teva Pharmaceuticals USA, Inc.'s Detailed Statement of the Factual and Legal Bases for Its Opinion That U.S. Pat. No. 5,814,639, U.S. Pat. No. 5,914,331, U.S. Pat. No. 5,922,695, U.S. Pat. No. 5,935,946, U.S. Pat. No. 5,977,089. and U.S. Pat. No. 6,043,230 are Invalid, Unenforceable or Not Infringed by the Manufacture, Use or Sale of lts Emtricitabine & Tenofovir Disoproxil Fumarate Tablets, 200 mg/300 mg dated Jan. 28, 2010 ("2010 Detailed Statement").
Excerpts of Panel on Antiretroviral Guidelines for Adults and Adolescents, Department of Health and Human Services, Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents 1-167 (20 11).
Excerpts of Teva Pharmaceuticals USA, Inc.'s Detailed Statement of the Factual and Legal Bases for Its Opinion That U.S. Pat. No. 6,642,245 and U.S. Pat. No. 6,703,396 are Invalid, Unenforceable or Not Infringed by the Manufacture, Use or Sale of Its Emtricitabine & Tenofovir Disoproxil Fumarate Tablets, 200 mg/300 mg dated Nov. 3, 2008 ("2008 Detailed Statement"); "Teva Pharmaceuticals USA, Inc.'s Detailed Statement of the Factual and Legal Bases for Its Opinion That U.S. Pat. No. 6,642,245 and U.S. Pat. No. 6,703,396 Are Invalid, Unenforceable or Not Infringed by the Manufacture, Use or Sale of Its Efavirenz/Emtricitabine/Tenofovir Disoproxil Fumarate Tablets, 600 mg/200 mg/300 mg" dated Mar. 30, 2009 ("2009 Detailed Statement"); and Teva Pharmaceuticals USA, Inc.'s Detailed Statement of the Factual and Legal Bases for Its Opinion That U.S. Pat. No. 5,814,639, U.S. Pat. No. 5,914,331, U.S. Pat. No. 5,922,695, U.S. Pat. No. 5,935,946, U.S. Pat. No. 5,977,089 and U.S. Pat. No. 6,043,230 are Invalid, Unenforceable or Not Infringed by the Manufacture, Use or Sale of Its Emtricitabine & Tenofovir Disoproxil Fumarate Tablets, 200 mg/300 mg dated Jan. 28, 2010 ("2010 Detailed Statement").
Excerpts of the Gilead Sciences, Inc., Q2, 2011 Earnings Results Conference Call and Webcast presentation, pp. 16 & 19 (Jul. 26, 2011).
Excerpts of the Gilead Sciences, Inc., Q2, 2011 Earnings Results Conference Call and Webcast presentation, p. 16-18 (Jul. 26, 2011).
Fischl, Margaret A. et al., Prolonged Zidovudine Therapy in Patients with AIDS and Advanced AIDS-Related Complex, 262 J.A.M.A. 2405 (1989).
Harada, Shinji et al., *Infection of HTLV-IIIILAV in HTLV-I-Carrying Cells MT-2 and MT-4 and Application in a Plaque Assay*, 229 Science 563 (1985).
Hoong, Lee K. et al., Enzyme-Mediated Enantioselective Preparation of Pure Enantiomers of the Antiviral Agent 2',3'-Dideoxy-5-.fluoro-3'-thiacytidine (FTC) and Related Compounds, 57 J. Organic Chemistry 5563 (1992).
Larder, Brendan A. et al., HIVwith Reduced Sensitivity to Zidovudine (AZT) Isolated During Prolonged Therapy, 243 Science 1731 (1989).
Norton, Terry M. et al., Efficacy of Acyclovir Against Herpesvirus Infection in Quaker Parakeets, 52(12) Am. J. Vet. Res. Sep. 2007 (Dec. 1991).
Order signed by Judge Richard J. Sullivan Case No. 10-CV-01796 (Jan. 17, 2012).
Stella, Valentino J. et al., *Prodrugs and Site-Specific Drug Delivery*, 23 Journal of Medicinal Chemistry 1275 (1980).
Teva Pharmaceuticals USA, Inc.'s Detailed Statement of the Factual and Legal Bases for Its Opinion That U.S. Pat. No. 5,922,695, U.S. Pat. No. 5,935,946, U.S. Pat. No. 5,977,089, and U.S. Pat. No. 6,043230 are Invalid, Unenforceable or Not Infringed by the Manufacture, Use or Sale of Tenofovir Disoproxil Fumarate Tablets, 300 mg dated Jan. 25, 2010 ("2010 Detailed Statement").
Castello and Mattocks, "Discoloration of Tablets Containing Amines and Lactose," J. Pharm. Sci. 51(92):106-108 (Feb. 1962).
"Pill" Encarta Dictionary, 2 pages (2009), retrieved from encarta.msn.com on Mar. 16, 2009.
"Time-Release," Compact Oxford English Dictionary, 1 page (2009), retrieved from www.askoxford.com/concise_oed on Mar. 12, 2009.
Petition for Inter Partes Review of U.S. Pat. No. 5,922,695, for Case No. IPR2014-00885, filed Jun. 4, 2014, 75 pages.
Corrected Petition for Inter Partes Review of U.S. Pat. No. 5,922,695, for Case No. IPR2014-00885, filed Jun. 24, 2014, 74 pages.
Related Matters for Case No. IPR2014-00885, filed Jun. 25, 2014, 6 pages.
Patent Owner Preliminary Response for Case No. IPR2014-00885, filed Sep. 18, 2014, 69 pages.
Moyle, Graeme and Gazzard,Brian, "Current Knowledge and Future Prospects for the Use of HIV Protease Inhibitors," 51:701-712, Drugs, May 1996.
Danner, et al., "A Short-Term Study of the Safety, Pharmacokinetics, and Efficacy of Ritonavir, and Inhibitor of HIV-1 Protease," 333:1528-1533, The New England Journal of Medicine, Dec. 1995.
Link, Derek; "The Collapse of Early Intervention at the Ninth International AIDS Conference," 7(6) Treatment Issues, 1993.
Bechtel-Boenning,Christine;"State of the Art Antiviral Treatment of HIV Infection," 31:1-13, NIH Nursing Clinics of North America, Mar. 1996.
Leary, Warren E.; "F.D.A. Panel Urges Fast Action on Approving a New AIDS Drug," N.Y. Times, Nov. 8, 1995.
HIV/AIDS Historical Time Line 1995-1999, FDA, 6 pages.
Vistide® (cidofovir injection) Package Insert, 2 pages.
Lacy et al., "Evaluation of the Subchronic Toxicity of an Oral Prodrug of the Anti-HIV Nucleotide Analog (9-2-Phosphonylmethoxyethyl)Adenine (PMEA)," 15(1) at 179 Abstract 958, Abstracts of the 34th Annual Meeting of the Society of Toxicology, 1995.
Lalezari et al., "(S)-1-[3-Hydroxy-2-(Phosphonylmethoxy)propyl] cytosine (Cidofovir): Results of Phase I/II Study of a Novel Antiviral Nucleotide Analogue," 171:788-796, The Journal of Infectious Diseases, 1995.
Foscavir® (foscarnet sodium) Package Insert, 2 pages.
Lee, William A. and Martin, John C., "Perspectives on the development of acyclic nucleotide analogs as antiviral drugs", 71:254-259, Antiviral Research, 2006.
Park et al., "Acyclovir Permeation Enhancement Across Intestinal and Nasal Mucosae by Bile Salt-Acylcarnitine Mixed Micelles," 9:1262-1267, Pharmaceutical Research, 1992.
Fix et al., "Acylcarnitines: drug absorption-enhancing agents in the gastrointestinal tract," 251:G332-G340, Am. J. Physiol., 1986.
Fleisher et al., "Improved oral drug delivery: solubility limitations overcome by the use of prodrugs," 19:115-130, Advanced Drug Delivery Reviews, 1996.
Negi et al., "Studies on Orally Active Cephalosporins: Synthesis and Structure-Activity Relationships of New 3-Substituted Carbamoyloxymethyl Cephalosporins," 47:1507-1525, The Journal of Antibiotics, 1994.
Safadi et al., "Phosphoryloxymethyl Carbamates and Carbonates—Novel Water-Soluble Prodrugs for Amines and Hindered Alcohols," 10:1350-55, Pharmaceutical Research, 1993.
Samara et al., "Pharmacokinetic Analysis of Diethylcarbonate Prodrugs of Ibuprofen and Naproxen," 16:201-210, Biopharmaceutics & Drug Disposition, 1995.
World Health Organization Model List of Essential Medicines, 18th Ed. (2013), 47 pages.

(56) **References Cited**

OTHER PUBLICATIONS

Institution Decision Case No. IPR2014-00885, filed Dec. 9, 2014, 23 pages.
Mylan's Updated Exhibit List, Case No. IPR2014-00885, dated Jan. 7, 2015, 9 pages.
Rehearing Request, Case No. IPR2014-00885, dated Jan. 8, 2015, 17 pages.
U.S. Pat. No. 5,922,695C1 (Ex Parte Reexamination Certificate) to Murty N. Arimilli, Kenneth C. Cundy, Joseph P. Dougherty, Choung U. Kim, Reza Oliyai and Valentino J. Stella, issued on Oct. 14, 2008 (Mylan's Updated Exhibit List Jan. 7, 2015.
U.S. Pat. No. 5,922,695 Prosecution File History (U.S. Appl. No. 08/900,746, filed Jul. 25, 1997).
U.S. Pat. No. 5,922,695 Reexamination File History (Reexamination Request No. 90/008,555, filed Apr. 30, 2007).
U.S. Pat. No. 5,977,089C1 (Ex Parte Reexamination Certificate) to Murty N. Arimilli, Kenneth C. Cundy, Joseph P. Dougherty, Choung U. Kim, Reza Oliyai and Valentino J. Stella, issued on Nov. 25, 2008.
U.S. Pat. No. 5,977,089 Reexamination File History (Reexamination Request No. 90/008,550, filed Apr. 30, 2007).
U.S. Pat. No. 6,043,230 (Ex Parte Reexamination Certificate) to Murty N. Arimilli, Kenneth C. Cundy, Joseph P. Dougherty, Choung U. Kim, Reza Oliyai and Valentino J. Stella, issued on Oct. 14, 2008.
U.S. Pat. No. 6,043,230 Prosecution File History (U.S. Appl. No. 09/314,606, filed May 19, 1999).
U.S. Pat. No. 6,043,230 Reexamination File History (Reexamination Request No. 90/008,549, filed Apr. 30, 2007).
U.S. Pat. No. 5,935,946C1 (Ex Parte Reexamination Certificate) to John D. Munger, Jr., John C. Rohloff and Lisa M. Schultze, issued on Oct. 14, 2008.
U.S. Pat. No. 5,935,946 Prosecution File History (U.S. Appl. No. 08/900,752), filed Jul. 25, 1997.
U.S. Pat. No. 5,935,946 Reexamination File History (Reexamination Request No. 90/008,556, filed Apr. 30, 2007).
Gilead Sciences, Inc. v. *Teva Pharmaceuticals USA, Inc. et al.*, 10cv-07196-RJS (S. Dist. NY (Foley Square)) (consolidation of 08-cv-10838 ; 12-cv-06351; 12-cv-06294; 12-cv-07910), dated Feb. 4, 2014, 1618 pages.
Fischl, M.A., et al., "The Efficacy of Azidothymidine (AZT) in the Treatment of Patients with AIDS and AIDS Related Complex," New England J. Med. 317:185 (1987).
Richman, D. et al., "The Toxicity of Azidothymidine (AZT) in the Treatment of Patients with AIDS and AIDS Related Complex," New England J. Med. 317:192 (1987).
Larder, B.A., Darby, G. and Richman, D., "HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy," Science 243:1731 (1989).
Kieburtz, KD, et al "Extended follow-up of peripheral neuropathy in patients with AIDS and AIDS related complex treated with dideoxyinosine," J. Acquir. Immune Defic. Syndr. 5:60 (1992).
Allan, J.D., et al. "Long term follow-up of didanosine administered orally twice daily to patients with advanced human immunodeficiency virus infection and hematologic intolerance of zidovudine," Clin. Infect. Dis. 16(S1):S46 (1993).
Simpson, D.M. and Tagliati, M., "Nucleoside Analogue Associated Peripheral Neuropathy in Human Immunodeficiency Virus Infection," J. Acquir. Immune Defic. Syndr. 9:153 (1995).
Van Leeuwen, R., et al., "Evaluation of Safety & Efficacy of 3TC (Lamivudine) in Patients with Asymptomatic or Mildly Symptomatic Human Immunodeficiency Virus Infection: A Phase I/II Study," J. Infect. Dis. 171:1166 (1995).
Beck, EJ., et al. "Survival and the use and costs of hospital services for London AIDS patients treated with AZT," Int. J. STD AIDS 7:507 (1996).
Boucher, C., et al., "High level Resistance to (-) Enantiomeric 2',Deoxy3'-Thiacytidine In Vitro is Due to One Amino Acid Substitution in the Catalytic Site of Human Immunodeficiency Virus Type 1 Reverse Transcriptase," Antimicro. Agents Chemother. 37:2231 (1993).
Mayers, D., "Rational Approaches to Resistance: Nucleoside Analogues," AIDS 10(S1):S913 (1996).
Cleland, A. Watson, HG, Robertson, P., Ludlum, CA and Brown, HA, "Evolution of zidovudine resistance genotype in Humalmmunodeficiency Virus I in infected patients," AIDS 12:618 (1996).
Anderson, BD, Shirasaka, T, Kojima E, Yarchoan, R and Mitsuya H, "Identification of drug related genotypic changes in HIV1 from serum using the selective polymerase chain reaction," Antiviral Res. 25:24558 (1994).
Volberding, P., "The Need for Additional Options in the Treatment of Human Immunodeficiency Virus Infection," J. Infect. Diseases 171(S2):150 (1995).
Holy, A., Rosenberg, I., Dvorakova, H. and DeClercq, E., "Synthesis and Evaluation of Acyclic Nucleotide Analogs," Nucleosides & Nucleotides 7:667 (1988).
Srinivas, R., Robbins, B., Connelly,M, Gong, Y., Bischofberger, B., Fridland, A., "Metabolism and In Vitro Antiretroviral Activities of Bis(Pivaloyloxymethyl) Prodrugs of Acyclic Nucleoside Phosphonates," Antimicro. Agents Chemother. 37:2247 (1993).
Heljtink, R.A., et al., "Inhibitory effects of acyclic nucleoside phosphonates on human hepatitis B virus and duck hepatitis B virus infections in tissue culturQ," Antimicrobial. Agents Chemotherap. 38:2180 (1994).
Tsai, C., et al., "Prevention of SIV Infection in Macaques by (R)9(2Phosphonylmethoxypropyl)adenine," Science 270:1197 (1995).
Cohen, J., "New Drug Shows Promise in Monkeys," Science 270:1121-1122 (1995).
Gong, Y., Marshall, D., Srinivas, R., Fridland, A., "Susceptibilities of zidovudineresistant variants of human immunodeficiency virus type 1 to inhibitor by acyclic nucleoside phosphonates," Antimicrobial Agents Chemother. 38:1683 (1994).
Examiner's Amendment and Notice of Allowability (dated Apr. 14, 1998), 5 pages.
Kubo, et al. "Nonpeptide angiotensin II receptor antagonists. Synthesis and biological activity of benzimidazolecarboxylic acids." J Med Chem. 1993 vol. 36, Issue No. 15, pp. 2343-2349.
Balzarini, et al. "Differential Antiherpesvirus and Antiretrovirus Effects of the (S) and (R) Enantiomers of Acyclic Nucleoside Phosphonates: Potent and Selective In Vitro and In Vivo Antiretrovirus Activities of (R)-9-(2-Phosphonomethoxypropyl)-2,6-Diaminopurine" Antimicrobial Agents and Chemotherapy, 1993, vol. 37, No. 2, p. 332-338.
James, John S. "AIDS Treatment News, PMPA in Perspective" AIDS Treatment News Issue No. 236, Dec. 1, 1995.
Editorials, "Carnitine Deficiency" Lancet 335:631-33 (1990).
Islam, I., Hinshaw, R., Chong, K-T., Kato,A., Borchardt, R. and Fisher, J.F. "Synthesis and Antiviral Activity of [2-[[4-[3-[(1-Methylethyl)amino]-2-Pyridyl]-Piperazinyl]Carbonyl]-1H-Indol-5-yl] (BHAP) Acylsphingosine HIV Reverse Transcriptase Inhibitors," Bioorg. Chem. 23:499-511 (1995).
De Clercq, E., "Antiviral Therapy for Human Immunodeficiency Virus Infections," Clin. Microbiol. Rev. 8:200-239 (1995).
Bundgaard, H., "Design of prodrugs," pp. 4-5, (1985) 1 page.
Holme, E. et al., "Carnitine deficiency induced by pivampicillin and pivmecillinam therapy" Lancet 2(8661):469-73 (1989).
"FDA's Policy Statement for the Development of New Stereoisomeric Drugs", Chirality, vol. 4, Issue 5, pp. 338-340, 1992.
Bighley, L.D., et al. "Salt forms of drugs and absorption" in Encyclopedia of Pharm. Tech., Eds. J. Swarbrick and J.C. Boylan, vol. 13 (Marcel Dekker, Inc., New York) (1996), 49 pages.
Gould, Phillip L. "Salt selection for basic drugs", International Journal for Pharmaceutics, (1986) vol. 33, pp. 201-217.
Bisoprolol Fumarate Label (Final Printed Labeling, May 1, 1992).
Bischofberger, N, et al. "Bis(POC)PMPA, an orally bioavailable prodrug of the antiretroviral agent PMPA" Conf. on Retroviruses and Opportunistic Infections 4th:104 (abstract No. 214) (Jan. 22-26, 1997).
Expert Declaration of J. Allen McCutchan, M.D., M.Sc. (Dated May 29, 2014).

(56) **References Cited**

OTHER PUBLICATIONS

Expert Declaration of Jed F. Fisher Ph.D. (Dated Jun. 3, 2014).
Bordwell, F.G. "Equilibrium Acidities in Dimethyl Sulfoxide Solution" Acc. Chem. Res. (1988) vol. 21, pp. 456-463.
Miyazaki, T., Yanagida, S., Itoh, A., and Okahara, M., "Synthesis and Alkali-cation Complexing Properties of 12-Crown-4 Derivatives", The Chemical Society of Japan, Bull. Chem. Soc. Jpn., (1982) vol. 55, pp. 2005-2009.
Petition for Inter Partes Review of U.S. Pat. No. 5,935,946 for Case No. IPR2014-00886, filed Jun. 4, 2014, 75 pages.
Corrected Petition for Inter Partes Review of U.S. Pat. No. 5,922,695, for Case No. IPR2014-00886, filed Jun. 24, 2014, 74 pages.
Related Matters for Case No. IPR2014-00886, filed Jun. 25, 2014, 6 pages.
Patent Owner Preliminary Response for Case No. IPR2014-00886, filed Sep. 18, 2014, 70 pages.
Institution Decision Case No. IPR2014-00886, filed Dec. 9, 2014, 21 pages.
Mylan's Updated Exhibit List, Case No. IPR2014-00886, dated Jan. 7, 2015, 9 pages.
Request for Rehearing IPR2014-00886, dated Jan. 16, 2014, 15 pages.
Physician's Desk Reference (51 ed. 1997), 28 pages.
Famvir® Package Insert, 16 pages.
Hepsera® Package Insert, 29 pages.
Bastin et al., Organic Process Research and Development, 4:427-435, 2000.
Van Westrenen et al., "The synthesis of polyhydroxycarboxylates. Part 6. N-Alkylation of amino compounds by a Michael-type addition with maleate," Recl. Tray. Chim. Pays-Bas, 109:474-478, 1990.
Stahl and Basel, Characterization and Improvement of the Stability Behaviour of Drug Substances in Stability Testing in the EC, Japan and the USA 56 (Dr. Wolfgang Grimm and Dr. Kurt Krummen, eds., 1993.
Petition for Inter Partes Review of U.S. Pat. No. 5,977,089, for Case No. IPR2014-00887, filed Jun. 4, 2014, 64 pages.
Corrected Petition for Inter Partes Review of U.S. Pat. No. 5,977,089, for Case No. IPR2014-00887, filed Jun. 24, 2014, 63 pages.
Related Matters for Case No. IPR2014-00887, filed Jun. 25, 2014, 6 pages.
Patent Owner Preliminary Response for Case No. IPR2014-00887, filed Sep. 18, 2014, 69 pages.
Institution Decision Case No. IPR2014-00887, filed Dec. 9, 2014, 17 pages.
Mylan's Updated Exhibit List, Case No. IPR2014-00887, dated Jan. 7, 2015, 9 pages.
Rehearing Request, Case No. IPR2014-00887, dated Jan. 8, 2015, 17 pages.
Petition for Inter Partes Review of U.S. Pat. No. 6,043,230, for Case No. IPR2014-00888, filed Jun. 4, 2014, 65 pages.
Corrected Petition for Inter Partes Review of U.S. Pat. No. 6,043,230, for Case No. IPR2014-00888, filed Jun. 24, 2014, 64 pages.
Related Matters for Case No. IPR2014-00888, filed Jun. 25, 2014, 6 pages.
Patent Owner Preliminary Response for Case No. IPR2014-00888, filed Sep. 28, 2014, 67 pages.
Institution Decision Case No. IPR2014-00888, filed Dec. 9, 2014, 20 pages.
Mylan's Updated Exhibit List, Case No. IPR2014-00888, dated Jan. 7, 2015, 9 pages.
Rehearing Request, Case No. IPR2014-00888, dated Jan. 8, 2015, 17 pages.
Complaint for Patent Infringement. Document filed by Emory University, Gilead Sciences, Inc, Case No. 1:12-cv-06293-RJS (Filed: Aug. 16, 2012), 14 pages.
Lupins Limited's Answer, Separate Defenses, and Counterclaims to Plaintiffs' Complaint. Document filed by Lupin Limited, Case No. 1:12-cv-06293-RJS (Filed Oct. 22, 2012), 21 pages.
Plaintiffs' Reply to Defendant's Counterclaims. Document filed by Emory University, Gilead Sciences, Inc., Case No. 1:12-cv-06293-RJS (Filed: Nov. 9, 2012), 10 pages.
Letter to Judge Richard J. Sullivan from Peter J. Curtin dated Sep. 3, 2013 Case No. 1:12-cv-06293-RJS (Filed: Sep. 4, 2013), 1 page.
Stipulation and Agreement Regarding U.S. Pat. No. 6,703,396, U.S. Pat. No. 6,642,245, U.S. Pat. No. 5,814,639 and U.S. Pat. No. 5,914,331: Case No. 1:12-cv-06293-RJS (Dated: Sep. 4, 2013), 6 pages.
Letter to Judge Richard J. Sullivan from David B. Bassett dated Sep. 16, 2014 re: Settlement. Document filed by Emory University, Gilead Sciences, Inc., Case No. 1:12-cv-06293-RJS (Filed: Sep. 16, 2014), 1 page.
Exhibit A to Letter addressed to Judge Richard J. Sullivan from David B. Bassett dated Sep. 16, 2014 re: Settlement. Document filed by Emory University, Gilead Sciences, Inc., Case No. 1:12-cv06293-RJS (Filed: Sep. 16, 2014).
Order on Stipulation for Dismissal of Plaintiffs, Gilead Sciences, Inc. ("Gilead") and Emory University ("Emory"), and Defendant, Lupin Limited ("Lupin"), Case No. 1:12-cv-06293-RJS (Dated: Sep. 17, 2014), 2 pages.
Complaint for Patent Infringment. Document filed by Gilead Sciences, Inc., Case No. 1:12-cv-07910-RJS (Filed: Oct. 24, 2012), 9 pages.
Lupins Limited's Answer, Separate Defenses, and Counterclaims to Plaintiff's Complaint. Document filed by Lupin Limited, Case No. 1:12-cv-07910-RJS (Filed: Dec. 20, 2012), 23 pages.
Plaintiff's Reply to Defendant's Counterclaims. Document filed by Gilead Sciences, Inc., Case No. 1:12-cv-07910-RJS (Filed: Jan. 9, 2013), 9 pages.
Order Case No. 1:12-cv-07910-RJS (Dated: Jul. 24, 2013), 1 page.
Complaint for Patent Infringment. Document filed by Gilead Sciences, Inc. Case No. 2:14-cv-00796-JRG-RSP (Filed: Jul. 24, 2014), 14 pages.
Joint Motion for Order on Stipulation for Dismissal. Case No. 2:14-cv-00796-JRG-RSP (Filed: Sep. 16, 2014), 3 pages.
Order on Stipulation for Dismissal. Signed by Magistrate Judge Roy S. Payne on Sep. 23, 2014. (nkl,) Case No. 2:14-cv-00796-JRG-RSP (Dated: Sep. 23, 2014).
Complaint for Patent Infringment. Document filed by Gilead Sciences, Inc. Case No. 1:12-cv-06351-RJS. (Filed: Aug. 20, 2012), 23 pages.
First Amended Complaint for Patent Infringment.Document filed by Gilead Sciences, Inc., Case No. 1:12-cv-06351-RJS (Filed: Sep. 11, 2012), 47 pages.
Cipla Limited's Answer and Separate Defenses to Plaintiff's First Amended Complaint. Document filed by Cipla Limited, Case No. 1:12-cv-06351-RJS) (Filed: Dec. 10, 2012), 30 pages.
Second Amended Complaint for Patent Infringement. Document filed by Gilead Sciences, Inc., Case No. 1:12-cv-06351-RJS (Filed: Oct. 17, 2013), 24 pages.
Defendant Cipla's Unneccesary Opening Claim Construction Brief. Document filed by Cipla Limited, Case No. 1:12-cv-06351-RJS) (Filed: Oct. 25, 2013), 25 pages.
Declaration of Aaron M. Johnson in Support of Defendant Cipla's Unneccesary Opening Claim Construction Brief. Case No. 1:12-cv-06351-RJS (Filed: Oct. 25, 2013), 3 pages.
Stella, et al., "Prodrugs—Do They Have Advantages in Clinical Practice?" *Drugs*, 29:455-473 (1985).
Exhibit 10 to Declaration of Aaron M. Johnson in Support re: 46 Claim Construction Statement. Document filed by CIPLA Limited, Case No. 1:12-cv-06351-RJS (Filed: Oct. 25, 2013), 3 pages.
"Prophylaxis" *Stedman's Medical Dictionary* (1995), 3 pages.
Plaintiff's Opening Claim Construction Brief. Document filed by Gilead Sciences, Inc., (Filed: Oct. 25, 2013), 18 pages.
Declaration of Jason Johnson. Document filed by Gilead Sciences, Inc., Case No. 1:12-cv-06351-RJS (Filed: Oct. 25, 2013), 3 pages.

(56) **References Cited**

OTHER PUBLICATIONS

Exhibit A to Declaration of Jason Johnson in Support of Plaintiff's Opening Claim Construction Brief. Document filed by Gilead Sciences, Inc., Case No. 1:12-cv-06351-RJS (Filed: Oct. 25, 2013), 25 pages.
Exhibit B to Declaration of Jason Johnson in Support of Plaintiff's Opening Claim Construction Brief. Document filed by Gilead Sciences, Inc., Case No. 1:12-cv-06351-RJS (Filed: Oct. 25, 2013), 9 pages.
Exhibit C to Declaration of Jason Johnson in Support of Plaintiff's Opening Claim Construction Brief. Document filed by Gilead Sciences, Inc., Case No. 1:12-cv-06351-RJS (Filed: Oct. 25, 2013), 3 pages.
Gilead Scienes, Inc. Q4 2012 Earnings Results Conference call and Webcast presentation (Feb. 4, 2013), 3 pages.
Gilead Scienes, Inc. Q2 2011 Earnings Results Conference call and Webcast presentation (Jul. 26, 2011), 4 pages.
Exhibit G to of Jason Johnson in Support of Plaintiff's Opening Claim Construction Brief. Document filed by Gilead Sciences, Inc., Case No. 1:12-cv-06351-RJS (Filed: Oct. 25, 2013), 2 pages.
Declaration of Amy Patick in Support of Plaintiff's Opening Claim Construction Document filed by Gilead Sciences, Inc.,. Case No. 1:12-cv-06351-RJS (Filed:Oct. 25, 2013), 13 pages.
Exhibit 1 to Declaration of Amy Patick. Document filed by Gilead Sciences, Inc.,. Case No. 1:12-cv-06351-RJS (Filed:Oct. 25, 2013), 15 pages.
Defendant Cipla Limited's Answer and Separate Defenses to Plaintiff'S Second Amended Complaint. Case No. 1:12-cv-06351-RJS (Filed: Nov. 1, 2013), 16 pages.
Defendant CIPLA's Reply to Plaintiff's Opening Claim Construction Brief. Document filed by CIPLA Limited. Case No. 1:12-cv-06351-RJS (Filed: Nov. 22, 2013), 15 pages.
Second Declaration of Aaron M. Johnson in Support of Defendant CIPLA's Reply to Plaintiff's Opening Claim Construction Brief. Document filed by CIPLA Limited, Case No. 1:12-cv-06351-RJS (Filed: Nov. 22, 2013) , 2 pages.
Honess, R.W. and Watson, D. H.; "Unity and Diversity in the Herpesviruses," *J. Gen. Virol.* (1977), 37:15-37.
Chang, C., et al, "Expression of the precore region of an avian hepatitis B virus is not required for viral replication," *J. Virol.* 1987, 61(10): 3322.
Plantiff's Rebuttal Claim Construction Brief. Document filed by Gilead Sciences, Inc., Case No. 1:12-cv-06351-RJS (Filed: Nov. 22, 2013), 10 pages.
Declaration of Jason Johnson in Support of Plantiff's Rebuttal Claim Construction Brief. Document filed by Gilead Sciences, Inc., (Case No. 1:12-cv-06351-RJS (Filed: Nov. 22, 2013), 2 pages.
Exhibit A to Declaration of Jason Johnson in Support of Plantiff's Rebuttal Claim Construction Brief. Document filed by Gilead Sciences, Inc., Case No. 1:12-cv-06351-RJS (Filed: Nov. 22, 2013), 5 pages.
Letter addressed to Judge Richard J. Sullivan from Christopher P. Borello dated Jul. 28, 2014, Document filed by Gilead Sciences, Inc., Case No. 1:12-cv-06351-RJS (Filed: Jul. 28, 2014), 1 page.
Order on Stipulation for Dismissal Case No. 1:12-cv-06351-RJS—(Dated: Jul. 29, 2014).
Complaint for Patent Infringment. Filed by Gilead Sciences, Inc., Case No. 1:12-CV-06294-RJS (Filed: Aug. 16, 2012), 19 pages.
Lupin Limited's Answer, Separate Defenses, and Counterclaims to Plaintiff's Complaint. Filed by Lupin Limited Case No. 1:12-CV-06294-RJS (Filed: Oct. 22, 2012), 22 pages.
Plaintiff's Reply to Defendant's Counterclaims. Filed by Gilead Sciences, Inc., Case No. 1:12-CV-06294-RJS (Filed: Nov. 9, 2012), 9 pages.
Order: Filed in Associated Cases:1:12-cv-06294-RJS, 1:12-cv-07910-RJS (Dated: Jul. 23, 2013), 1 page.
Stipulation and Agreement Regarding U.S. Pat. No. 5,922,695, U.S. Pat. No. 5,935,946, U.S. Pat. No. 5,977,089 and U.S. Pat. No. 6,043,230: 1. (Dated: Aug. 27, 2013), 4 pages.
Order (Dated: May 30, 2014), 1 page.
Order (Dated May 30, 2014), 4 pages.
Complaint for Patent Infringement. Filed by Gilead Sciences, Inc., Emory University, Case No. 1:14-cv-05352-RJS (Filed Jul. 18, 2014), 12 pages.
Lupin Limited's Answer, Separate Defenses, and Counterclaims to Plaintiff's Complaint. Filed by Lupin Limited.for Case No. 1:14-cv-05352-RJS (Dated: Aug. 22, 2014), 16 pages.
Letter addressed to Judge Richard J. Sullivan from David B. Bassett dated Sep. 16, 2014. Document filed by Emory University, Gilead Sciences, Inc.,Case No. 1:14-cv-05352-RJS (Filed Sep. 16, 2014), 1 page.
Order on Stipulation for Dismissal: Case No. 1:12-cv-06293-RJS (Dated: Sep. 16, 2014), 2 pages.
Complaint for Patent Infringement. Filed by Emory University, Gilead Sciences, Inc., Case No. 1:14-cv-03928-RJS (Filed: Jun. 2, 2014), 18 pages.
Letter addressed to Judge Richard J. Sullivan from Colleen Tracy dated Jun. 27, 2014. Document filed by Emory University, Gilead Sciences, Inc., Case No. 1:14-cv-03928-RJS (Filed: Jun. 27, 2014), 2 pages.
Notice of Voluntary Dismissal Pursuant to F.R.C.P. 41(a)(1)(A)(i) Case No. 1:14-cv-03928-RJS (Dated: Jun. 27, 2014), 1 page.
Complaint for Patent Infringement. Filed by Emory University, Gilead Sciences, Inc., Case No. 1:12-CV-06350 (Filed: Aug. 20, 2012), 14 pages.
Cipla Limited's Answer and Separate Defences to Plaitiffs' Complaint. Filed by Cipla Limited, Case No. 1:12-CV-06350-RJS (Filed: Dec. 10, 2012), 10 pages.
Letter addressed to Judge Richard J. Sullivan from Christopher P. Borello dated Jul. 28, 2014. Document filed by Emory University, Gilead Sciences, Inc., 1:12-cv-06350-RJS (Dated: Jul. 28, 2014), 1 page.
Order on Stipulation for Dismissal. Case No. 1:12-cv-06350-RJS (Dated: Jul. 29, 2014), 2 pages.
Complaint for Patent Infringement. Filed by Gilead Sciences, Inc. and Emory University, Case No. 1:14-cv-00099-IMK (Filed: Jun. 10, 2014), 19 pages.
Appendix 1 to Complaint Filed by Gilead Sciences, Inc. and Emory University, Case No. 1:14-cv-00099-IMK (Filed: Jun. 10, 2014), 19 pages.
Amended Complaint for Patent Infringement. Filed by Gilead Sciences, Inc. and Emory University, Case No. 1:14-cv-00099-IMK (Filed: Jun. 27, 2014), 18 pages.
Answer, Defenses, and Counterclaims of Mylan Pharmaceuticals Inc and Mylan Inc., Filed by Mylan Inc., Mylan Pharmaceuticals Inc., Case No. 1:14-cv-00099-IMK (Filed Aug. 12, 2014), 36 pages.
Plaintiff's Answer to Defendants' Counterclaims. Filed by Gilead Sciences, Inc. and Emory University, Case No. 1:14-cv-00099-IMK (Filed: Sep. 2, 2014), 10 pages.
Amended Answer, Defenses, and Counterclaims of Mylan Pharmaceuticals Inc and Mylan Inc., Filed by Mylan Inc., Mylan Pharmaceuticals Inc., Case No. 1:14-cv-00099-IMK (Filed: Sep. 2, 2014), 36 pages.
Stipulation Concerning Defendants Amended Answer, Defenses and Counterclaims, Case No. 1:14-cv-00099-IMK (Filed: Oct. 2, 2014), 2 pages.
Stipulation Regarding Mylan Inc., Filed by Mylan Inc., Case No. 1:14-cv-00099-IMK (Filed: Dec. 15, 2014), 3 pages.
Second Amended Complaint for Patent Infringement, Filed by Gilead Sciences, Inc. and Emory University,Case No. 1:14-cv-00099-IMK (Filed: Jan. 21, 2015), 21 pages.
Answer, Defenses, and Counterclaims of Mylan Pharmaceuticals Inc and Mylan Inc. to Plaintiffs' Second Amended Complaint, Filed by Mylan Inc., Mylan Pharmaceuticals Inc., Case No. 1:14-cv-00099-IMK (Filed: Feb. 9, 2015), 51 pages.
Joint Stipulation Regarding Claim Construction. Case No. 1:14-cv-00099-IMK (Filed: Feb. 19, 2015), 4 pages.
Gilead's Opening Brief in Support of Its Proposed Claim Constructions for U.S. Pat. No. 8,592,397 and U.S. Pat. No. 8,716,264. Case No. 1:14-cv-00099-IMK (Filed: Feb. 19, 2015), 27 pages.
Declaration of David B. Bassett in Support of Gilead's Opening Brief in Support of Its Proposed Claim Construction. Case No. 1:14-cv-00099-IMK (Filed: Feb. 19, 2015), 134 pages.

(56) **References Cited**

OTHER PUBLICATIONS

Declaration of Patrick J. Sinko, Ph.D., R.Ph., in Support of Gilead's Opening Brief in Support of Its Proposed Claim Construction. Case No. 1:14-cv-00099-IMK (Filed: Feb. 19, 2015), 171 pages.
Declaration of Carlo-Federico Perno, M.D. Ph.D. in Support of Gilead's Claim Construction., Case No. 1:14-cv-00099-IMK (Filed: Feb. 19, 2015), 14 pages.
Exhibit 1 Declaration of Carlo-Federico Perno, M.D. Ph.D. in Support of Gilead's Claim Construction. Case No. 1:14-cv-00099-IMK (Filed: Feb. 19, 2015), 42 pages.
Exhibit 2 Declaration of Carlo-Federico Perno, M.D. Ph.D. in Support of Gilead's Claim Construction.Case No. 1:14-cv-00099-IMK (Filed: Feb. 19, 2015), 2 pages.
Exhibit 3 Declaration of Carlo-Federico Perno, M.D. Ph.D. in Support of Gilead's Claim Construction. Case No. 1:14-cv-00099-IMK (Filed: Feb. 19, 2015), 3 pages.
Muma, R. D., et al, "Zidovudine adherence among individuals with HIV infection." *AIDS Care*. 1995;7(4):439-47.
Richman, D.D., "Antiretroviral drug resistance: mechanisms, pathogenesis, clinical significance." *Antiviral Chemotherapy 4*, 383-395 J. Mills, Ed., Plenum Press, New York, 1996.
Johnson, V., "Combination therapy for HIV-1 infection-overview: preclinical and clinical analysis of antiretroviral combinations" *Antiviral Research* 29 (1996) 35-39.
Akanbi, M.O., et al, "Combination nucleoside/nucleotide reverse transcriptase inhibitors for treatment of HIV infection" *Expert Opin Pharmacother*. Jan. 2012;13(1):65-79.
De Clercq, E., "Anti-HIV drugs: 25 compounds approved within 25 years after the discovery of HIV" *Int J Antimicrob Agents*. Apr. 2009;33(4):307-20.
Heffernan, J.M. and Wahl, L. M., "Natural variation in HIV infection: Monte Carlo estimates that include CD8 effector cells" *Journal of Theoretical Biology* 243 (2006) 191-204.
Ho, D. D., "Viral Counts Count in HIV Infection" vol. 272, 1124-1125, May 24, 1996.
Mellors, J. W., et al, "Prognosis in HIV-1 Infection Predicted by the Quantity of Virus in Plasma" *Science* ,vol. 272, 1167-1170, May 24, 1996.
Plaintiffs' Motion to Strike Mylan's Third Separate Defense. Document filed by Gilead Sciences, Inc. and Emory University, Case No. 1:14-cv-00099-IMK (Filed: Mar. 2, 2015), 3 pages.
Declaration of David Bassett in Support of Plaintiffs' Motion to Strike Mylan's Third Separate Defense. Document filed by Gilead Sciences, Inc. and Emory University, Case No. 1:14-cv-00099-IMK (Filed: Mar. 2, 2015), 12 pages.
Memorandum in Support of Plaintiffs' Motion to Strike Mylan's Third Separate Defense. Document filed by Gilead Sciences, Inc. and Emory University, Case No. 1:14-cv-00099-IMK (Filed: Mar. 2, 2015), 15 pages.
Plaintiffs' Answer to Defentant Mylan Pharmaceuticals Inc.'s Amended Counter Claims. Document filed by Gilead Sciences, Inc. and Emory University, Case No. 1:14-cv-00099-IMK (Filed: Mar. 2, 2015), 12 pages.
Defendants Mylan Inc. and Mylan Pharmaceuticals Inc.'s Answering Claim Construction Brief in Support of Their Proposed Claim Constructions for U.S. Pat. No. 8,592,397 and U.S. Pat. No. 8,716,264. Document filed by Mylan Inc., Mylan Pharmaceuticals Inc., Case No. 1:14-cv-00099-IMK (Filed: Mar. 12, 2015), 28 pags.
Declaration of Karen M. Cassidy in Support of Defendants Mylan Inc. and Mylan Pharmaceuticals Inc.'s Answering Claim Construction Brief in Support of Their Proposed Claim Constructions for U.S. Pat. No. 8,592,397 and U.S. Pat. No. 8,716,264. Document filed by Mylan Inc., Mylan Pharmaceuticals Inc., Case No. 1:14-cv-00099-IMK (Filed: Mar. 12, 2015), 199 pages.
Declaration of Chloe Lynne Thio, M.D. in Support of Defendants Mylan Inc. and Mylan Pharmaceuticals Inc.'s Answering Claim Construction Brief in Support of Their Proposed Claim Constructions for U.S. Pat. No. 8,592,397 and U.S. Pat. No. 8,716,264. Document filed by Mylan Inc., Mylan Pharmaceuticals Inc., Case No. 1:14-cv-00099-IMK (Filed: Mar. 12, 2015), 184 pages.
Declaration of Frank Chrzanowski, Ph.D. in Support of Defendants Mylan Inc. and Mylan Pharmaceuticals Inc.'s Answering Claim Construction Brief in Support of Their Proposed Claim Constructions for U.S. Pat. No. 8,592,397 and 8,716,264. Document filed by Mylan Inc., Mylan Pharmaceuticals Inc., Case No. 1:14-cv-00099-IMK (Filed: Mar. 12, 2015), 32 pages.
Gilead's Motion to Defer Consideration of Mylan's Indefiniteness Argument or, Alternatively, for Leave to File a Reply. Case No. 1:14-cv-00099-IMK (Filed: Mar. 19, 2015), 8 pages.
Declaration of David B. Bassett in Support of Gilead's Motion to Defer Consideration of Mylan's Indefiniteness Argument or, Alternatively, for Leave to File a Reply., Case No. 1:14-cv-00099-IMK (Filed: Mar. 19, 2015), 30 pages.
Mylan Inc.'s and Mylan Pharmaceuticals Inc.'s Response to Plaintiffs' Motion to Strike, Case No. 1:14-cv-00099-IMK (Filed: Mar. 19, 2015), 3 pages.
Motion Requesting Expedited Briefing for Gilead's Motion to Defer Consideration of Mylan's Indefiniteness Argument or, Alternatively, for Leave to File a Reply., Case No. 1:14-cv-00099-IMK (Filed: Mar. 19, 2015), 3 pages.
Defendants Mylan Inc. and Mylan Pharmaceuticals Inc.'s Response to Gilead's Motion to Defer Consideration of Mylan's Indefiniteness Argument or, Alternatively, for Leave to File a Reply. Case No. 1:14-cv-00099-IMK (Filed: Mar. 26, 2015), 9 pages.
Declaration of William O. Adams in Support of Defendants Mylan Inc. and Mylan Pharmaceuticals Inc.'s Response to Gilead's Motion to Defer Consideration of Mylan's Indefiniteness Argument or, Alternatively, for Leave to File a Reply. Document filed by Mylan Inc., Mylan Pharmaceuticals Inc., Case No. 1:14-cv-00099-IMK (Filed: Mar. 26, 2015), 20 pages.
Reply Memorandum in Support of Gilead's Motion to Defer Consideration of Mylan's Indefiniteness Argument. Document filed by Emory University and Gilead Sciences, Inc., Case No. 1:14-cv-00099-IMK (Filed: Mar. 30, 2015), 6 pages.
Joint Stipulation Regarding Claim Construction. Signed by District Judge Irene M. Keeley on Mar. 31, 2015. (jss), Case No. 1:14-cv-00099-IMK (Filed: Mar. 31, 2015), 4 pages.
Notice of Opposition filed by Alphapharm Pty Ltd in the Australian Patent Office for Application No. 2011253996, dated Nov. 28, 2014, 1 page.
Coffin JM, Hughes SH, Varmus HE, editors, "Retroviruses," Cold Spring Harbor (NY) : Cold Spring Harbor Laboratory Press 1997.
White et al., "Molecular Mechanisms of Resistance to Human Immunodeficiency Virus Type 1 with Reverse Transcriptase Mutations K65R and K65R M184V and Their Effects on Enzyme Function and Viral Replication Capacity," Antimicrobial Agents and Chemotherapy, Nov. 2002, p. 2437-3446.
Viread insert, 2001, DNA 21-356, 20 pages.
Epivir Approval Package Mar. 23, 1999, 22 pages.
Whitney, "The M184V Mutation in Reverse Transcriptase Can Delay Reversion of Attenuated Variants of Simian Immunodrficiency Virus," Journal of Vrology vol. 76 (17) p. 8958-8962.
Retrovir Product Information (2001), 23 pages.
Jemesk, Poor Virologic Responses and Early Emergence of Resistance in Treatment Naive, HIV-infected Patients Receiving a Once Daily Triple Nucleoside Regimen of Didanosine, Lamivudine, and Tenefoir DF, Program Abstr. Conf, Ertrovir, Oppor Infect 11th 2004 San Francisco Calf, Feb. 8-11, 2004. Abstract 51.
Farthing, "Early Virologic Failure in a Pilot Study Evaluating the Efficacy of Abacavir, Lamivudine and Tenofovir in the Treatment Naive HIV-Infected Patients," The 2nd IAS Conference on HIV Pathogenesis and Treatment Abstract No. 43, Jul. 2003.
Kagan, "Increasing prevalence of HIV-1 reverse transcriptase mutation K65R correlates with tenofovir utilization," 2004, AntiViral Therapy vol. 9 pp. 827-828.
Fisher , "The safety and efficacy of adefovir dipivoxil in patients with advanced HIV disease: a randomized, placebocontrolled trial," Aids vol. 15 p. 1695-1700.
Cihiar, "Human Renal Organic Anion Transporter 1 (Hoat1) and Its Role in the Nephrotoxicity of Antlvlral Nucleotide Analogs," Nucleosides ,Nucleotides & nucleic Acids, 2001 vol. 20(4-7) p. 641-648.

(56) **References Cited**

OTHER PUBLICATIONS

Charpentier, "Evolution of the K65R, K103N and M184V/I reverse transcriptase mutations prevalence in HIV-1 infected patients experiencing virologic failure between 2005 and 2010," Abstract CROI 2012.
Grant and Hackh's "Chemical Dictionary" 5th ed. R. Grant (editors) 1987, 7 pages.
Foye's Principles of Medicinal Chemistry 5th ed. 2002, 4 pages.
Center for drug evaluation and research 21-356 Chemistry Review (Viread)—12 pages.
Thoithi et. al, "Investigation of the kinetics of degradation of hexopyranosylated cytosine nucleosides using liquid chromatography" Nucleosides ,Nucleotides & Nucleic Acids (2003).
Calculating the pH of an aquesou solution of TDF, 1 page.
Handbook of Pharmaceutical Excipients, Fourth Edition, edited by Rowe, Sheskey, and Weller, "Lactose and Water," 17 pages (Nov. 2002).
Kashuba, "Antiretroviral-Drug Concentrations in Semen: Implications for Sexual Transmission of Human Immunodeficiency Virus Type 1," Antimicrobial Agents and Chemotherapy 43(8):1817-1826 (1999).
The Emtriva Label, 19 pages.
Chemistry Reviews for NDA 21-752 Truvada, 27 pages.
Clinical pharmacology section of the ANCOBON, 6 pages (Mar. 2003).
PROLOPRIM, 3 pages.
ICH Stability Testing of New Drug Substances and Products 2003, 98 pages.
Reynolds et al. "Available Guidance and Best Practices for Conducting Forced Degradation Studies" Pharm Technol 2002, 9 pages.
Carstensen, J.T., *Advanced Pharmaceutical Solids*, 2001, p. 273, 3 pages.
Novak, "Prevalence of Antiretroviral Drug Resistance Mutations in Chronically HIV-Infected, Treatment-Naïve Patients: Implications for Routine Resistance Screening before Initiation of Antiretroviral Therapy," Clin. Infect Dis. Feb. 1, 2005;40(3) 486-74.
Grant et al., "Preexposure Chemoprophylaxis for HIV Prevention in Men Who Have Sex with Men," New England Journal of Medicine 363:27, 2587 (2010).
Time Magazine, Dec. 9, 2010, "AIDS Drugs Lower the Risk of HIV Infection," by Alice Park.
EMEA (European Medicine Agency) Scientific Discussion on Truvada, 2005, 28 pages.
EMEA (European Medicine Agency) Scientific Discussion on Emtriva, 2005,—30 pages.
Pischetsrieder, "Formation of an Aminoreductone during the Maillard Reaction of Lactose with Nr-Acetyllysine or Proteins," J. Agric. Food Chem. 1998, 46, 928-931.
Bharate, "Interactions and incompatibilities of pharmaceutical excipients with active pharmaceutical ingredients: a comprehensive review" J. Excipients and Food Chem. 1 (3) (2010).
Expert Opinion Prof. Richard M. Novak relating to opposition proceedings being conducted in Israel against Gilead Sciences, Inc. ("the Applicant"), concerning Israel patent application No. IL 169243, Sep. 22, 2012, 40 pages.
Second Expert Opinion Prof. Richard M.Novak relating to opposition proceedings being conducted in Israel against Gilead Sciences, Inc. ("the Applicant"), concerning Israel patent application No. IL 169243, Apr. 9, 2014, 50 pages.
Expert Opinion Prof. Joseph Marian Fortunak relating to opposition proceedings being conducted in Israel against Gilead Sciences, Inc. ("the Applicant"), concerning Israel patent application No. IL 169243, Sep. 20, 2012, 30 pages.
Expert Opinion of Dr. G. Patrick Stahly filed in the Israeli Patent Office in opposition proceedings initiated by Teva Pharmaceutical Industries Ltd. against Israeli Patent Application No. 169,243, filed by GileadSciences Inc., 27 pages.
Truvada Summary of Product Characteristics, 35 pages.
Shapiro and Klein "The Deamination of Cytidine and Cytosine by Acidic Buffer Solutions. Mutagenic Implications," *Biochemistry*, vol. 5, No. 7, Jul. 1966, 2358.
Notari, "A Mechanism for the Hydrolytic Deamination of Cytosine Arabinoside in Aqueous Buffer," *Journal of Pharmaceutical Science*, vol. 56, No. 7, Jul. 1967, 804.
Notari, "Intermolecular and Intramolecular Catalysis in Deamination of Cytosine Nucleosides," *Journal of Pharmaceutical Science*, vol. 59, No. 1, Jan. 1970, 28.
Lonnberg, "Competition between the hydrolysis and deandntion of cytidine and its 5-substituted deirivatives in aqueous acid," *Nucleic Acid Research*, vol. 13, Nov. 1985, 2451.
Dong, "Modeling of Autocatalytic Hydrolysis of Adefovir Dipivoxil in Solid Formulations," *Journal of the Pharmaceutical Society of Japan*, 131(4), 643.
Wong, "Major Degradation Product Identified in Several Pharmaceutical Formulations against the Common Cold," *Anal. Chem.*, 2006, 78, 7891.
Aulton, *Pharmaceutics The Science of Dosage Form Design* (1988), 618.
Augsburger, "Tablet Formulation," *Encyclopedia of Pharmaceutical Technology* (3rd. ed.), 3646, 13 pages (2007).
Armstrong, "Tablet Manufacture by Direct Compression," Encyclopedia of Pharmaceutical Technology (3rd. ed.), 3673-3683 (2007).
Chan, "Excipients: Powders and Solid Dosage Forms," Encyclopedia of Pharmaceutical Technology (3rd. ed.), 1646-1655 (2007).
Airaksinen, "Role of Water in the Physical Stability of Solid Dosage Formulations," *Journal of Pharmaceutical Sciences*, vol. 94, No. 10, 19 pages.
Ahlneck and Zografi, "The molecular basis of moisture effects on the physical and chemical stability of drugs in the solid state," *International Journal of Pharmaceutics*, 62 (1990) 87-95.
Kaupp, "Solid-state reactions, dynamics in molecular crystals," *Current Opinion in Solid State & Material Science*, 6 (2002) 131-138.
Byrn, "Chemical reactivity in solid-state pharmaceuticals: formulation implications," *Advanced Drug Deliveries Reviews* 48 (2001), 115-136.
Li, "The Solid State Michael Addition of Indole to 4-Arylidene-3-methyl-5 pyrazolone," *J Heterocyclic. Chem.*, 36, 697 (1999).
Frantz, "The trouble with making combination drugs," News & Analysis section of *Nature Review Drug Discovery*, vol. 5, 881-882 (Nov. 2006).
Summary of Preformulation Report for GS-4331 fumarate salt,1 page, Mar. 4, 1997.
Flemming, "Compaction of lactose drug mixtures: Quantification of the extent of incompatibility by FT-Raman spectroscopy," *European Journal of Pharmaceutics and Biopharmaceutics* 68, 802-810 (2008).
Desai, "Preformulation Compatibility Studies of tamsylate and Fluconazole Drugs with Lactose by DSC," *Journal of Thermal Analysis*, vol. 71, 651-658 (2003).
Gokhale, "Glycosylation of Aromatic Amines I: Characterization of Reaction Products and Kinetic Scheme," *AAPS PharmaSciTech*, vol. 10, No. 2, 317 (Jun. 2009).
Gallo, "Frequent Detection and Isolation of Cytopathic Retroviruses (HTL V-III) from Patients with AIDS and at Risk for AIDS," *Science, New Series*, vol. 224, No. 4648 (May 4, 1984), 500-503.
Expert Opinion of Prof. Robert R. Redfield in opposition proceedings initiated by Teva Pharmaceutical Industries Ltd. against Israeli Patent Application No. 169,243, filed by Gilead Sciences Inc., 38 pages.
Redfield, "Frequent Transmission of HTLV-III Among Spouses of Patients with AIDS-Related Complex and AIDS", *JAMA* 1985; 253(11):1571-1573.
Redfield, "The Walter Reed Staging Classification for HTLV III, LAV Infection", *New England Journal of Medicine* 1986; 314: 131-132.
Baeten, "Antiretroviral Prophylaxis for HIV Prevention in Heterosexual Men and Women," New England Journal of Medicine 367:5, 399 (2012).

(56) **References Cited**

OTHER PUBLICATIONS

Thigpen, "Antiretroviral Preexposure Prophylaxis for Heterosexual HIV Transmission in Botsw," New England Journal of Medicine 367:5, 423 (2012).
Highlights of Prescribing Information for TRUVADA, 45 pages, Jul. 2012.
Grossman, et al., "Drug-Resistant HIV Infection among Drug-Naïve Patients in Israel," Journal of Clinical Infectious Disease 40, 294 (2005).
Bazmi, "In Vitro Selection of Mutations in the Human Immunodeficiency Virus Type 1 Reverse Transcriptase That Decrease Susceptibility to (2)-b-D-Dioxolane-Guanosine and Suppress Resistance to 39-Azido-39-Deoxythymidine," Antimicrobial Agents and Chemotherapy, 44, 1783 (2000).
Roge, "Genotypic and phenotypic changes in antiretroviral-naive patients experiencing failure on randomised treatment with abacavir, didanosine and stavudine," Antiviral Therapy, 7, S125 (2002).
Dart Virology Group and Trial Team "Virological response to a triple nucleoside/nucleotide analogue regimen over 48 weeks in HIV-1-infected adults in Africa," AIDS 20(10), 1391-1399 (2006).
Rey, "Virologic Response of Zidovudine, Lamivudine, and Tenofovir Disoproxil Fumarate Combination in Antiretroviral-Naive HIV-1-Infected Patients," *J. AIDS* 43(5), 530 (2006).
Kirkland, "Response to Lamivudine-Zidovudine plus Abacavir Twice Daily in Antiretroviral-Naive, Incarcerated Patients with HIV Infection Taking Directly Observed Treatment," Clinical Infectious Diseases, 34, 511 (2002).
Little, "Antiretroviral-Drug Resistance Among Patients Recently Infected With HIV," New England Journal of Medicine, 347(6), 385 (2002).
Rubio,"Increase in the frequency of mutation at codon 215 associated with zidovudine resistance in HIV-1-infected antiviral-naive patients from 1989 to 1996," AIDS, 11(9), 1184 (1997).
Winston, "The prevalence and determinants of the K65R mutation in HIV-1 reverse transcriptase in tenofovir-naive patients," AIDS, 16(15), 2087, Oct. 18, 2002.
Pillay, "HIV Type 1 Subtype C Drug Resistance among Pediatric and Adult South African Patients Failing Antiretroviral Therapy," AIDS Research Human Retroviruses, 24, 1449 (2008).
Loemba, "Genetic Divergence of Human Immunodeficiency Virus Type 1 Ethiopian Clade C Reverse Transcriptase (RT) and Rapid Development of Resistance agains," *Antimicrobial Agents and Chemotherapy*, 46(7), 2087, Jul. 2002.
Lambert, "Prevalence of pre-existing resistance-associated mutations to rilpivirine, emtricitabine and tenofovir in antiretroviral-naiv," *Journal of Antimicrobial Chemotherapy*, Jan. 29, 2013.
Arion, "The K65R Mutation Confers Increased DNA Polymerase Processivity to HIV-1 Reverse Transcriptase ," *J. Biological Chemistry*, 37, 15908, Aug. 16, 1996.
Miller, "Human Immunodeficiency Virus Type 1 Reverse Transcriptase Expressing the K70E Mutation Exhibits a Decrease in Specific Activity and Processivity," Molecular Pharmacology, 54, 291 (1998).
The FDA's press release dated Jul. 2, 2002 reporting the new approved dosing for EPIVIR (3TC), titled "New Dosing approved for Epivir (lamivudine)," 1 page.
Raune, "Pharmacodynamic effects of zidovudine 600 mg once daily versus zidovudine 300 mg twice daily in therapy-naïve HIVinfected patients (COD20002)," Late Breaker Poster Exhibition: *The XIV International AIDS Conference* (2002), 1 page.
FDA Press Release Zerit XR, Dec. 31, 2002, titled "Approval of ZERIT® XR, a new formulation that allows once-a-day dosing Dec. 31," 1 page.
FDA Videx EC Approval Letter dated Oct. 31, 2000, 3 pages.
Callendar, "Pharmacokinetics of Oral Zidovudine Entrapped in Biodegradable Nanospheres in Rabbits," *Antimicrobial Agents and Chemotherapy* 43(4), 972, Apr. 1999.
Cohen, *45th Interscience Conference on Antimicrobial Agents and Chemotherapy* Poster H-521, 1 page.
Elion, "Once-Daily Abacavir/Lamivudine/Zidovudine plus Tenofovir for the Treatment of HIV-1 Infection in Antiretroviral-Naïve Subjects: A 48-Week Pilot Study," *HIV Clinical Trials*, 7(6), 324, 2006.
Meier, "Cidofovir-induced End-Stage Renal Failure," Nephrology Dialysis Transplantation, 17, 148 (2002).
Verhelst, "Fanconi Syndrome and Renal Failure Induced by Tenofovir: A First Case Report," American Journal of Kidney Diseases, 40(6), 1331, Dec. 2002.
Coca, "Rapid Communication: Acute Renal Failure Associated with Tenofovir: Evidence of Drug-Induced Nephrotoxicity," American Journal of Medical Science 324(6), 342 Dec. 2002.
Bowonwatanuwong, "A Randomised, Open Label Study to Investigate Abacavir (Abc) and Lamivudine (3TC) as Once Daily (QD) Components of a Triple Combination Regimen (EPV40001).," 1st IAS Conference, Buenos Aires, Argentina, Jul. 8-11, 2001, 1 page.
Statement in Response by Applicant in opposition proceedings initiated by Teva Pharmaceutical Industries Ltd. against Israeli Patent Application No. 169,243, filed by Gilead Sciences Inc., 27 pages.
Memorandum Opinion and Order Granting Plaintiffs' Motion to Defer Consideration of Mylan's Indefiniteness Argument [DKT. No. 119], CV No. 1:14CV99, signed Apr. 6, 2015.
Statement concerning Request for Intervention for Case No. 3k:26-27, Istanbul Patent No. TR 2008/067, dated Apr. 13, 2015, 25 pages.
İlko İlaç San. ve Tic. A.Ş .'s Invalidation Petition for Turkish Patent No. 2008/06970 filed Sep. 10, 2014, 41 pages
Gilead Sciences, Inc.'s Response to İlko İlaç San. ve Tic. A.Ş .'s Invalidation Petition for Turkish Patent No. 2008/06970 filed Dec. 31, 2014, 36 pages.
İlko İlaç San. ve Tic. A.Ş .'s Second Invalidation Petition for Turkish Patent No. 2008/06970 field Feb. 26, 2015 (Translation), 8 pages.
Plaintiffs' Response to Defendants' Pretrial Memrorandum filed by Bristol-Meyers, Squibb Company, Merck, Sharp &Dohme Corp., Case No. 1:10-cv-01851-RJS, (Filed May 28, 2013), 19 pages.
Defendants' Memorandum of Law in Oppositon to Plaintiffs' Opening Pretrial Brief ffiled by Teva Pharmaceutical Industries, Ltd., Teva Pharmaceuticals USA, Inc., Case No. 1:10-cv-01851-RJS, (Filed May 28, 2013), 18 pages.
Stipulation and Order of Dismissal, Case No. 1:10-cv-01851-RJS (Dated Aug. 16, 2013), 2 pages.
Affidavit of Calvin J. Cohen, M.D. filed by Emory University, Gilead Sciences, Inc., Case No. Case: 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 41 pages.
Affidavit of Paul A. Bartlett, Ph.D. filed by Emory University, Gilead Sciences, Inc.,Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 39 pages.
Affidavit of Carlo-Federico Perno, M.D., Ph.D. filed by Emory University, Gilead Sciences, Inc.,Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 50 pages.
Affidavit of Dennis C. Liotta, Ph.D. filed by Emory University, Gilead Sciences, Inc.,Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 31 pages.
Affidavit of Judi Weissinger, Ph.D. filed by Emory University, Gilead Sciences, Inc.,Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 13 pages.
Affidavit of Stephen G. Davies, Ph.D. filed by Emory University, Gilead Sciences, Inc.,Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 42 pages.
Affidavit of Daniel C. Smith, Ph.D. filed by Emory University, Gilead Sciences, Inc.,Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 31.
Affidavit of James Meyers filed by Emory University, Gilead Sciences, Inc.,Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 15.
Plaintiffs' Pretrial Memorandum filed by Emory University, Gilead Sciences, Inc.,Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 60 pages.
Plaintiffs' Proposed Findings of Fact and Conclusions of Law, filed by Emory University, Gilead Sciences, Inc.,Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 85 pages.

(56) **References Cited**

OTHER PUBLICATIONS

Declaration of Harry Boghigian filed by Teva Pharmaceutical Industries Limited, Teva Pharmaceuticals USA, Inc., Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 17 pages.
Declaration of Stanley Roberts, Ph.D., filed by Teva Pharmaceutical Industries Limited, Teva Pharmaceuticals USA, Inc., Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 78 pages.
Defendants' Pretiral Memorandum filed by Teva Pharmaceutical Industries Limited, Teva Pharmaceuticals USA, Inc., Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 47 pages.
Defendants' Proposed Findings of Fact and Conclusions of Law filed by Teva Pharmaceutical Industries Limited, Teva Pharmaceuticals USA, Inc., Case No. 1:08-cv-10838-RJS (Filed Sep. 9, 2013), 68 pages.
Affidavit of Dennis C. Liotta,Ph.D. filed by Emory University, Gilead Sciences, Inc. Case No. 1:08-cv-10838-RJS (Filed Sep. 19, 2013), 31 pages.
Affidavit of Stephen G. Davies, Ph.D. filed by Emory University, Gilead Sciences, Inc. Case No. 1:08-cv-10838-RJS (Filed Sep. 19, 2013), 42.
Plaintiffs' Pretrial Memorandum filed by Emory University, Gilead Sciences, Inc. Case No. 1:08-cv-10838-RJS (Filed Sep. 19, 2013), 60 pages.
Plaintiffs' Proposed Findings of Fact and Conculsions of Law filed by Emory University, Gilead Sciences, Inc. Case No. 1:08-cv-10838-RJS (Filed Sep. 19, 2013), 85 pages.
Defendants' Memorandum of Law in Opposition to Plantiffs' Pretrial Memorandum filed by Teva Pharmaceutical Industries Limited, Teva Pharmaceuticals USA, Inc., Case No. 1:08-cv-10838-RJS (Filed Sep. 23, 2013), 31 pages.
Plaintiffs' Opposition to Teva's Pretrial Memorandum, filed by Emory University, Gilead Sciences, Inc. Case No. 1:08-cv-10838-RJS (Filed Sep. 23, 2013), 41 pages.
Amendend Declaration of Stanley Roberts, Ph.D. filed by Teva Pharmaceutical Industries Limited, Teva Pharmaceuticals USA, Inc., Case No. 1:08-cv-10838-RJS (Filed Oct. 4, 2013), 77 pages.
Transcript of Proceedings regarding Trial held on Oct. 8, 2013 before Judge Richard J. Sullivan, Case No. 1:08-cv-10838-RJS (Dated Oct. 21, 2013), 222 pages.
Transcript of Proceedings regarding Trial held on Oct. 9, 2013 before Judge Richard J. Sullivan, Case No. 1:08-cv-10838-RJS (Dated Oct. 21, 2013), 220 pages.
Transcript of Proceedings regarding Trial held on Oct. 10, 2013 before Judge Richard J. Sullivan, Case No. 1:08-cv-10838-RJS (Dated Oct. 21, 2013), 38 pages.
Transcript of Proceedings regarding Trial held on Oct. 28, 2013 before Judge Richard J. Sullivan, Case No. 1:08-cv-10838-RJS (Dated Nov. 7, 2013), 67 pages.
Stipulated Statement of Corrections to Trial Transcript, Oct. 8-10, 28, 2013 filed by Emory University, Gilead Sciences, Inc. Case No. 1:08-cv-10838-RJS (Filed Dec. 4, 2013), 42 pages.
Plaintiffs' Post-Trial Brief filed by Emory University, Gilead Sciences, Inc. Case No. 1:08-cv-10838-RJS (Filed Dec. 4, 2013), 43 pages.
Defendants' Post-Trial Memorandum filed by Teva Pharmaceutical Industries Limited, Teva Pharmaceuticals USA, Inc., Case No. 1:08-cv-10838-RJS (Filed Dec. 4, 2013), 43 pages.
Defendants' Revised Proposed Findings of Fact and Conclusions of Law filed by Teva Pharmaceutical Industries Limited, Teva Pharmaceuticals USA, Inc., Case No. 1:08-cv-10838-RJS (Filed Dec. 4, 2013), 102 pages.
Plaintiffs' Proposed Findings of Fact and Conclusions of Law filed by Emory University, Gilead Sciences, Inc. Case No. 1:08-cv-10838-RJS (Dec. 5, 2013), 111 Pages.
Plaintiffs' Notice of PTX, A, B, and C (exhibits) in Support of Post-trial Memorandum regarding Post Trial Memorandum filed by Emory University, Gilead Sciences, Inc. Case No. 1:08-cv-10838-RJS, (Filed Dec. 5, 2013), 81 pages.
Plaintiffs' Opposition to Defendants' Post-Trial Memorandum filed by Emory University, Gilead Sciences, Inc. Case No. 1:08-cv-10838-RJS (Filed Dec. 18, 2013), 22 pages.
Defendants' Post-Trial Reply Memorandum filed by Teva Pharmaceutical Industries Limited, Teva Pharmaceuticals USA, Inc., Case No. 1:08-cv-10838-RJS (Filed Dec. 18, 2013), 20 pages.
Stipulation of Dismissal, Case No. 1:08-cv-10838-RJS (Dated Jan. 29, 2014), 3 pages.
Order, Case No. 1:08-cv-10838-RJS (Dated Feb. 13, 2014), 1 page.
Order on Stipulation for Dismissal, Case No. 1:08-cv-10838_RJS (Dated Apr. 30, 2014), 2 pages.
Staszewski, S., J. Gallant, A. Pozniak, J. M. A. H. Suleiman, E. DeJesus, E. Koenig, S. Coleman et al. "Efficacy and safety of tenofovir disoproxil fumarate (TDF) versus stavudine (d4T) when used in combination with lamivudine (3TC) and efavirenz (EFV) in HIV-1 infected patients naive to antiretroviral therapy (ART): 48-week interim results." Oral 17, 14th International AIDS Conference, Jul. 7-12, 2002 (poster).
Staszewski, S., J. Gallant, A. Pozniak, J. M. A. H. Suleiman, E. DeJesus, E. Koenig, S. Coleman et al. "Efficacy and safety of tenofovir disoproxil fumarate (TDF) versus stavudine (d4T) when used in combination with lamivudine (3TC) and efavirenz (EFV) in HIV-1 infected patients naive to antiretroviral therapy (Art): 48-week interim results of Study GS-99-903" Oral 17, 14th International AIDS Conference, Jul. 7-12, 2002 (presentation).
Declaration of Dr. Jonathan A V Coates in the matter Australian patent application 2011253996 in the name of Gilead Sciences, Inc. and in the matter of Opposition by Alphapharm, Jun. 2, 2015.
Declaration of Andrew David Carr in the matter Australian patent application 2011253996 in the name of Gilead Sciences, Inc. and in the matter of Opposition by Alphapharm, Jun. 2, 2015.
Declaration of Helen Grimes in the matter Australian patent application 2011253996 in the name of Gilead Sciences, Inc. and in the matter of Opposition by Alphapharm, Jun. 2, 2015.
Statement of Grounds and Particulars filed by Alphapharm Pty Ltd in the Australian Patent Office for Application No. 2011253996, dated Mar. 17, 2015, 12 pages.
Australian Product Information for EMTRIVA (emtricitabine) Capsules, Apr. 2014, 22 pages.
Australian Product Information for Viread® (tenofovir disoproxil fumarate) 300 mg Tablets, Oct. 2013, 44 pages.
Australian Product Information for 3TC® Tablets and Oral Solution , Feb. 2015, 26 pages.
Australian Product Information for REYATAZ® (atazanavir sulfate), Dec. 2014, 52 pages.
Australian Product Information for STOCRIN® (efavirenz), Mar. 2015, 29 pages.
Australian Product Information for KALETRA, Jan. 30, 2015, 39 pages.
Abstracts of the 10th Conference on Retroviruses and Opportunistic Infections Feb. 10-14, 2003, p. 10, Abstract 564b (A-564b), 5 pages.
Braunwald et al, Harrison's Principles of Internal Medicine (McGraw-Hill, 15th ed, 2001),pp. 1852-1913 (Harrison's).
Cadman et al., "Looking Down the Drugline Pipeline," GMHC Treat Issues 12(3):5-9 (1998).
Rudnic, E., Remington 20th Edition, Chapter 45, pp. 996-1035, (2000).
Memorandum Opinion and Order Construing Patent Claims, signed by Judge Irene M. Keeley, Case No. 1:14-cv-00099-IMK (Dated: May 12, 2015), 44 pages.
Plaintiffs' Motion to Compel Defendants to Produce Documents, Case No. 1:14-cv-00099-IMK (Filed: May 28, 2015), 9 pages.
Wang et al. "Lack of Significant Pharmacokinetic Interactions between Emtricitabine and Other Nucleoside Antivirals in Healthy Volunteers," 41st ICAAC Abstracts, Chicago, IL, Sep. 22-25, 2001, Abstract A-505.
Wang et al. "Pharmacokinetic and pharmacodynamic characteristics of emtrictabine support its once daily dosing," Int. Conf. AIDS, Jul. 7-12 14:abstract TUPeB4546 (2002).
Weller et al., "Orally active Fibrinogen receptor antagonists. 2. Amidoximes as prodrugs of amidines," J. Med. Chem. 39:3139-3147, 1995.

(56) **References Cited**

OTHER PUBLICATIONS

Written Opinion issued by the ISA for PCT/US2006/023223 (dated Feb. 23, 2007).
Written Opinion of the ISA for PCT/US2006/023222 (dated Feb. 23, 2007).
Yeni et al., "Antiretroviral Treatment for Adult HIV Infection in 2002," JAMA 288(2):222-235 (2002).
Yuan et al., "Degradation Kinetics of Oxycarbonyloxymethyl Prodrugs of Phosphonates in Solution," Pharm. Res. 18(2):234-237 (2001).
Zhang et al. "Phase transformation considerations during process development and manufacture of solid oral dosage forms" Adv Drug Del Reviews 56(30), 371-390 (2004).
"Lactose Anhydrous," p. 373 in Analytical Profiles of Drug Substances, vol. 20, Ed. Klaus Florey, Academic Press, Inc. (1990).
"Lactose," Handbook of Pharmaceutical Excipients, 3rd ed. Ed. A.H. Kibbe, American Pharmaceutical Association, pp. 276-285 (2000) pp. 276-285 (2000).
"New Uses for Tenofovir; More Questions about d4T," Project Inform Perspective 35:15-16 (2003).
U.S. Appl. No. 10/757,141, filed Jan. 13, 2004.
U.S. Appl. No. 10/540,794, filed Jan. 13, 2004.
U.S. Appl. No. 11/452,472, filed Jun. 13, 2006.
U.S. Appl. No. 14/472,511, filed Aug. 29, 2014.
U.S. Appl. No. 14/523,758, filed Oct. 24, 2015.
U.S. Appl. No. 14/640,825, filed Mar. 6, 2015.
Communication Pursuant to Rule 114(2) EPC—Third Party Observation—issued by the European Patent Office for Application No. 15154733.8, dated Nov. 25, 2016, 77 pages.
Pre-grant opposition submitted by Blanver for Brazilian Application No. P133048, dated Jan. 9, 2017, 13 pages includes English translation.
Office Action—Rejection Decision—issued by the Brazilian Patent and Trademark Office for Application No. PI0406760-6, dated Jan. 16, 2017, received, 11 pages—Non-English and 14 pages English translation.
Invalidation appeal against Japanese Patent No. 4996241, filed by Kyowa Pharmaceutical Industry Co, Ltd, dated Dec. 27, 2016, 75 pages Non-English and 113 pages English translation.
Vandamme, et al., "Anti-Human Immunodeficiency Virus Drug Combination Strategies," Antiviral Chemistry & Chemotherapy 9:3:187-203.
Rando and Nguyen-Ba, Development of Novel Nucleoside Analogues for Use Against Drug Resistant Strains of HIV-1, DDT 5(10):465-476 (2000).
Rosenbach, et al., "Daily Dosing of Highly Active Antiretroviral Therapy," HIV/AIDS*CID 2002-34:686-692 (2002).
"Chiryou no tebiki [Guide for Therapy]", 6th edition, Nov. 2002 , 36 pages ,Japanese language document.
Seizaigaku text [Pharmaceutics Text]—Hirowaka Publishing Company, 216-217, Nov. 1, 1988, 4 pages Japanese language document.
Material Life, vol. 3, No. 2, pp. 104-109 (1991)—Japanese language document.
Kokei seizai no seizou gijutsu [Manufacturing Technique of Solid Formulation], 152-153, Jan. 27, 2003 Japanese language document.
"Truvada® Combination Tablet" Medicament Interview Form: Jan. 2013, 65 pages, Japanese language document.
Guidelines for Using Antiretroviral Agents Among HIV-Infected Adults and Adolescents, MMWR May 17, 2002, vol. 51, No. RR-7, 64 pages.
Gerhartz (editor) Ullmann's Encyclopedia of Industrial Chemistry vol. B2, Unit Operations I, 5th Edition, p. 3-7 (1988).
"Annex I. Summary of Product Characteristics," for Epivir, 9 pages (1997).
"Annex I. Summary of Product Characteristics," for Truvada film-coated tablets, 14 pages (cited in Statement of Appeal Grounds in the Opposition of European Patent EP1 583 542, filed by Gilead Sciences, Inc, on Jun. 24, 2011).
"graph," and "convolute of graphs,", cited as documents D54 and D55 in opposition proeedings of EP 04701819.7, Statement of Appeal Grounds dated Jun. 24, 2011, 5 pages.
Excerpts from the Reexamination of U.S. Pat. No. 6,043,230, 6 pages, filed Jan. 13, 2012.
Vistide® (cidofovir injection) Package Insert, 8 pages (Sep. 2000).
Foscavir® (foscarnet sodium) Package Insert, 21 pages (Feb. 2012).
Famvir® Package Insert, 32 pages (Apr. 2013).
Hepsera® Package Insert, 58 pages (Nov. 2012).
Whitney, "The M184V Mutation in Reverse Transcriptase Can Delay Reversion of Attenuated Variants of Simian Immunodeficiency Virus," Journal of Virology vol. 76 (17) p. 8958-8962 (Sep. 2002).
Fisher, "The safety and efficacy of adefovir dipivoxil in patients with advanced HIV disease: a randomized, placebo-controlled trial," Aids vol. 15 p. 1695-1700 (Sep. 7, 2001).
Center for drug evaluation and research 21-356 Chemistry Review (Viread)—12 pages (2001).
Calculating the pH of an aqueous solution of TDF, 1 page, cited in Expert Opinion Prof. Joseph Marian Fortunak relating to opposition proceedings being conducted in Israel against Gilead Sciences, Inc. ("the Applicant"), concerning Israel patent application No. IL 169243, Sep. 20, 2012.
The Emtriva Label, 38 pages (Jul. 2003).
Chemistry Reviews for NDA 21-752 Truvada, 27 pages (2004).
Proloprim, 3 pages (2002).
Bharate, "Interactions and incompatibilities of pharmaceutical excipients with active pharmaceutical ingredients: a comprehensive review" J. Excipients and Food Chem. 1(3):3-26 (Dec. 2010).
Expert Opinion of Dr. G. Patrick Stahly filed in the Israeli Patent Office in opposition proceedings initiated by Teva Pharmaceutical Industries Ltd. against Israeli Patent Application No. 169,243, filed by Gilead Sciences Inc. filed on Jul. 24, 2013, 27 pages.
Truvada Summary of Product Characteristics, filed in Israeli Patent Office in opposition proceedings initiated by Teva Pharmaceutical Industries Ltd. against Israeli Patent Application No. 169,243 dated Jul. 24, 2013, 35 pages.
Dong, "Modeling of Autocatalytic Hydrolysis of Adefovir Adefovir Dipivoxil in Solid Formulations," *Journal of the Pharmaceutical Society of Japan*, 131(4), 643-654 (Apr. 2011).
Airaksinen, "Role of Water in the Physical Stability of Solid Dosage Formulations," *Journal of Pharmaceutical Sciences*, vol. 94, No. 10, 19 pages (Oct. 2005).
Expert Opinion of Prof. Robert R. Redfield in opposition proceedings initiated by Teva Pharmaceutical Industries Ltd. against Israeli Patent Application No. 169,243, filed by Gilead Sciences Inc., dated Jul. 24, 2013, 38 pages.
Cohen, 45_{th} *Interscience Conference on Antimicrobial Agents and Chemotherapy*Poster H-521, 1 page (2005).
Memorandum Opinion and Order Granting Plaintiffs' Motion to Defer Consideration of Mylan's Indefiniteness Argument [DKT. No. 119], CV No. 1:14CV99, signed Apr. 6, 2015, 6 pages.
Fortunak evidence in reply relating to opposition proceedings being conducted in Israel against Gilead Sciences, Inc. ("the Applicant"), concerning Israel patent application No. IL 169243 ("the Patent Application"), dated Apr. 9, 2014, 29 pages.
Decision of the Technical Board of Appeals to dismiss appeal of opposition decision in EP 1583542 B1 dated Jun. 26, 2017.
Reply by Blanver Farmoquimica LTDA to counterarguments filed by Gilead Sciences in appeal of BR App. No. PI0406760-6, Inc. dated May 8, 2017 (English Translation).

* cited by examiner

COMPOSITIONS AND METHODS FOR COMBINATION ANTIVIRAL THERAPY

This application is a Continuation of U.S. application Ser. No. 14/227,653 filed Mar. 27, 2014, which is a continuation of U.S. patent application Ser. No. 12/204,174 now U.S. Pat. No. 8,716,264, filed Sep. 4, 2008, which is a continuation of U.S. patent application Ser. No. 10/540,794, filed Mar. 20, 2006, which is a national stage entry of PCT/US04/00832, filed Jan. 13, 2004 which claims the benefit of Provisional Application Nos. 60/440,246 and 60/440,308, both filed Jan. 14, 2003, the disclosures of each of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The invention relates generally to combinations of compounds with antiviral activity and more specifically with anti-HIV properties. In particular, it relates to chemically stable combinations of structurally diverse anti-viral agents.

BACKGROUND OF THE INVENTION

Human immunodeficiency virus (HIV) infection and related diseases are a major public health problem worldwide. Human immunodeficiency virus type 1 (HIV-1) encodes at least three enzymes which are required for viral replication: reverse transcriptase (RT), protease (Prt), and integrase (Int). Although drugs targeting reverse transcriptase and protease are in wide use and have shown effectiveness, particularly when employed in combination, toxicity and development of resistant strains have limited their usefulness (Palella, et al *N. Engl. J. Med.* (1998) 338:853-860; Richman, D. D. *Nature* (2001) 410:995-1001). Human immunodeficiency virus type 1 (HIV-1) protease (Prt) is essential for viral replication and is an effective target for approved antiviral drugs. The HIV Prt cleaves the viral Gag and Gag-Pol polyproteins to produce viral structural proteins (p17, p24, p7 and p6) and the three viral enzymes. Combination therapy with RT inhibitors has proven to be highly effective in suppressing viral replication to unquantifiable levels for a sustained period of time. Also, combination therapy with RT and Prt inhibitors (PI) have shown synergistic effects in suppressing HIV replication. Unfortunately, a high percentage, typically 30 to 50% of patients currently fail combination therapy due to the development of drug resistance, non-compliance with complicated dosing regimens, pharmacokinetic interactions, toxicity, and lack of potency. Therefore, there is a need for new HIV-1 inhibitors that are active against mutant HIV strains, have distinct resistance profiles, fewer side effects, less complicated dosing schedules, and are orally active. In particular, there is a need for a less onerous dosage regimen, such as once per day oral dosing, optimally with as few pills as possible.

The use of combinations of compounds can yield an equivalent antiviral effect with reduced toxicity, or an increase in drug efficacy. Lower overall drug doses can reduce the frequency of occurrence of drug-resistant variants of HIV. Many different methods have been used to examine the effects of combinations of compounds acting together in different assay systems (Furman WO 02/068058). Lower doses predict better patient compliance when pill burden decreases, dosing schedules are simplified and, optionally, if synergy between compounds occurs (Loveday, C. "Nucleoside reverse transcriptase inhibitor resistance" (2001) *JAIDS Journal of Acquired Immune Deficiency Syndromes* 26:S10-S24). AZT (Zidovudine™, 3'-azido, 3'-deoxythymidine)

demonstrates synergistic antiviral activity in vitro in combination with agents that act at HIV-1 replicative steps other than reverse transcription, including recombinant soluble CD4 castanosperinine and recombinant interferon-α. However, it must be noted that combinations of compounds can give rise to increased cytotoxicity. For example, AZT and recombinant interferon-α have an increased cytotoxic effect on normal human bone marrow progenitor cells.

Chemical stability of combinations of antiviral agents is an important aspect of co-formulation success and the present invention provides examples of such combinations.

There is a need for new combinations of orally-active drugs for the treatment of patients infected with certain viruses, e.g. HIV, that provide enhanced therapeutic safety and efficacy, impart lower resistance, and predict higher patient compliance.

SUMMARY OF THE INVENTION

The present invention provides combinations of antiviral compounds, in particular compositions and methods for inhibition of HIV. In an exemplary aspect, the invention includes a composition including tenofovir disoproxil fumarate and emtricitabine which has anti-HIV activity. The composition of tenofovir DF and emtricitabine is both chemically stable and either synergistic and/or reduces the side effects of one or both of tenofovir DF and emtricitabine. Increased patient compliance is likely in view of the lower pill burden and simplified dosing schedule.

The present invention relates to therapeutic combinations of [2-(6-amino-purin-9-yl)-1-methyl-ethoxymethyl]-phosphonic acid diisopropoxycarbonyloxymethyl ester fumarate (tenofovir disoproxil fumarate, tenofovir DF, TDF, Viread®) and (2R,5S,cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine, Emtriva™, (−)-cis FTC) and their use in the treatment of HIV infections including infections with HIV mutants bearing resistance to nucleoside and/or non-nucleoside inhibitors. The present invention is also concerned with pharmaceutical compositions and formulations of said combinations of tenofovir disoproxil fumarate and emtricitabine. Another aspect of the invention is a pharmaceutical formulation comprising a physiologically functional derivative of tenofovir disoproxil fumarate or a physiologically functional derivative of emtricitabine.

Therapeutic combinations and pharmaceutical compositions and formulations of the invention include the combination of PMEA or PMPA (tenofovir) compounds with emtricitabine or (2R,5S,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (3TC, lamivudine, Epivir™), and their use in the treatment of HIV infections.

One aspect of the invention is a method for the treatment or prevention of the symptoms or effects of an HIV infection in an infected animal which comprises administering to, i.e. treating, said animal with a therapeutically effective amount of a combination comprising [2-(6-amino-purin-9-yl)-1-methyl-ethoxymethyl]-phosphonic acid diisopropoxycarbonyloxymethyl ester fumarate (tenofovir DF, TDF) or a physiologically functional derivative thereof, and (2R,5S,cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine) or a physiologically functional derivative thereof.

Another aspect of the invention is a unit dosage form of a therapeutic combination comprising tenofovir disoproxil fumarate and emtricitabine, or physiological functional derivatives thereof. The unit dosage form may be formulated

for administration by oral or other routes and is unexpectedly chemically stable in view of the properties of the structurally diverse components.

Another aspect of the invention is directed to chemically stable combination antiviral compositions comprising tenofovir disoproxil fumarate and emtricitabine. In a further aspect of the invention, the chemically stable combinations of tenofovir disoproxil fumarate and emtricitabine further comprise a third antiviral agent. In this three-component mixture, the unique chemical stability of tenofovir disoproxil fumarate and emtricitabine is taken advantage of in order to enable the combination with the third antiviral agent. Particularly useful third agents include, by way of example and not limitation, those of Table A. Preferably, the third component is an agent approved for antiviral use in humans, more preferably, it is an NNRTI or a protease inhibitor (PI), more preferably yet, it is an NNRTI. In a particularly preferred embodiment, the invention is directed to a combination of the chemically stable mixture of tenofovir disoproxil fumarate and emtricitabine together with efavirenz.

Another aspect of the invention is a patient pack comprising at least one, typically two, and optionally, three active ingredients and other antiviral agents selected from tenofovir disoproxil fumarate and emtricitabine, and an information insert containing directions on the use of tenofovir disoproxil fumarate and emtricitabine together in combination.

Another aspect of the invention is a process for preparing the combinations hereinbefore described, which comprises bringing into association tenofovir DF and emtricitabine of the combination in a medicament to provide an antiviral effect. In a further aspect of the present invention, there is provided the use of a combination of the present invention in the manufacture of a medicament for the treatment of any of the aforementioned viral infections or conditions.

DETAILED DESCRIPTION OF THE INVENTION

While the invention will be described in conjunction with the enumerated claims, it will be understood that they are not intended to limit the invention to those claims. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the scope of the present invention as defined by the claims.

Definitions

Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings:

When tradenames are used herein, applicants intend to independently include the tradename product and the active pharmaceutical ingredient(s) of the tradename product.

The term "chemical stability" means that the two primary antiviral agents in combination are substantially stable to chemical degradation. Preferably, they are sufficiently stable in physical combination to permit commercially useful shelf life of the combination product. Typically, "chemically stable" means that a first component of the mixture does not act to degrade a second component when the two are brought into physical combination to form a pharmaceutical dosage form. More typically, "chemically stable" means that the acidity of a first component does not catalyzes or otherwise accelerate the acid decomposition of a second component. By way of example and not limitation, in one aspect of the invention, "chemically stable" means that tenofovir disoproxil fumarate is not substantially degraded by the acidity of emtricitabine. "Substantially" in this context means at least about less than 10%, preferably less than 1%, more preferably less than 0.1%, more preferably yet, less than 0.01% acid degradation of tenofovir disoproxil fumarate over a 24-hour period when the products are in a pharmaceutical dosage form.

The terms "synergy" and "synergistic" mean that the effect achieved with the compounds used together is greater than the sum of the effects that results from using the compounds separately, i.e. greater than what would be predicted based on the two active ingredients administered separately. A synergistic effect may be attained when the compounds are: (1) co-formulated and administered or delivered simultaneously in a combined formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation therapy, a synergistic effect may be attained when the compounds are administered or delivered sequentially, e.g. in separate tablets, pills or capsules, or by different injections in separate syringes. In general, during alternation therapy, an effective dosage of each active ingredient is administered sequentially, i.e. serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together. A synergistic antiviral effect denotes an antiviral effect which is greater than the predicted purely additive effects of the individual compounds of the combination.

The term "physiologically functional derivative" means a pharmaceutically active compound with equivalent or near equivalent physiological functionality to tenofovir DF or emtricitabine when administered in combination with another pharmaceutically active compound in a combination of the invention. As used herein, the term "physiologically functional derivative" includes any: physiologically acceptable salt, ether, ester, prodrug, solvate, stereoisomer including enantiomer, diastereomer or stereoisomerically enriched or racemic mixture, and any other compound which upon administration to the recipient, is capable of providing (directly or indirectly) such a compound or an antivirally active metabolite or residue thereof.

"Bioavailability" is the degree to which the pharmaceutically active agent becomes available to the target tissue after the agent's introduction into the body. Enhancement of the bioavailability of a pharmaceutically active agent can provide a more efficient and effective treatment for patients because, for a given dose, more of the pharmaceutically active agent will be available at the targeted tissue sites.

The compounds of the combinations of the invention may be referred to as "active ingredients" or "pharmaceutically active agents."

The term "prodrug" as used herein refers to any compound that when administered to a biological system generates the drug substance, i.e. active ingredient, as a result of spontaneous chemical reaction(s), enzyme catalyzed chemical reaction(s), and/or metabolic chemical reaction(s).

"Prodrug moiety" means a labile functional group which separates from the active inhibitory compound during metabolism, systemically, inside a cell, by hydrolysis, enzymatic cleavage, or by some other process (Bundgaard, Hans, "Design and Application of Prodrugs" in *Textbook of Drug Design and Development* (1991), P. Krogsgaard-Larsen and H. Bundgaard, Eds. Harwood Academic Publishers, pp. 113-191). Prodrug moieties can serve to enhance solubility, absorption and lipophilicity to optimize drug delivery, bioavailability and efficacy. A "prodrug" is thus a covalently modified analog of a therapeutically-active compound.

"Alkyl" means a saturated or unsaturated, branched, straight-chain, branched, or cyclic hydrocarbon radical derived by the removal of one hydrogen atom from a single carbon atom of a parent alkane, alkene, or alkyne. Typical alkyl groups consist of 1-18 saturated and/or unsaturated carbons, such as normal, secondary, tertiary or cyclic carbon atoms. Examples include, but are not limited to: methyl, Me (—CH_3), ethyl, Et (—CH_2CH_3), acetylenic (—C≡CH), ethylene, vinyl (—CH═CH_2), 1-propyl, n-Pr, n-propyl (—$CH_2CH_2CH_3$), 2-propyl, i-Pr, i-propyl (—$CH(CH_3)_2$), allyl (—CH_2CH═CH_2), propargyl (—CH_2C≡CH), cyclopropyl (—C_3H_5), 1-butyl, n-Bu, n-butyl (—$CH_2CH_2CH_2CH_3$), 2-methyl-1-propyl, i-Bu, i-butyl (—$CH_2CH(CH_3)_2$), 2-butyl, s-Bu, s-butyl (—$CH(CH_3)CH_2CH_3$), 2-methyl-2-propyl, t-Bu, t-butyl (—$C(CH_3)_3$), 1-pentyl, n-pentyl, (—$CH_2CH_2CH_2CH_2CH_3$), 2-pentyl (—$CH(CH_3)CH_2CH_2CH_3$), 3-pentyl (—$CH(CH_2CH_3)_2$), 2-methyl-2-butyl (—$C(CH_3)_2CH_2CH_3$), cyclopentyl (—C_5H_9), 3-methyl-2-butyl (—$CH(CH_3)CH(CH_3)_2$), 3-methyl-1-butyl (—$CH_2CH_2CH(CH_3)_2$), 2-methyl-1-butyl (—$CH_2CH(CH_3)CH_2CH_3$), 1-hexyl (—$CH_2CH_2CH_2CH_2CH_2CH_3$), 5-hexenyl (—$CH_2CH_2CH_2CH_2CH$═CH_2) 1-hexyl (—$CH(CH_3)CH_2CH_2CH_2CH_3$), 3-hexyl (—$CH(CH_2CH_3)(CH_2CH_2CH_3)$), cyclohexyl (—$C_6H_{11}$), 2-methyl-2-pentyl (—$C(CH_3)_2CH_2CH_2CH_3$), 3-methyl-2-pentyl (—$CH(CH_3)CH(CH_3)CH_2CH_3$), 4-methyl-2-pentyl (—$CH(CH_3)CH_2CH(CH_3)_2$), 3-methyl-3-pentyl (—$C(CH_3)(CH_2CH_3)_2$), 2-methyl-3-pentyl (—$CH(CH_2CH_3)CH(CH_3)_2$), 2,3-dimethyl-2-butyl (—$C(CH_3)_2CH(CH_3)_2$), and 3,3-dimethyl-2-butyl (—$CH(CH_3)C(CH_3)_3$.

"Aryl" means a monovalent aromatic hydrocarbon radical of 6-20 carbon atoms derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system. Typical aryl groups include, but are not limited to, radicals derived from benzene, substituted benzene, naphthalene, anthracene, biphenyl, and the like.

"Arylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with an aryl radical. Typical arylalkyl groups include, but are not limited to, benzyl, 2-phenylethan-1-yl, 2-phenylethen-1-yl, naphthylmethyl, 2-naphthylethan-1-yl, 2-naphthylethen-1-yl, naphthobenzyl, 2-naphthophenylethan-1-yl and the like. The arylalkyl group 6 to 20 carbon atoms e.g., the alkyl moiety, including alkanyl, alkenyl or alkynyl groups, of the arylalkyl group is 1 to 6 carbon atoms and the aryl moiety is 5 to 14 carbon atoms.

"Substituted alkyl", "substituted aryl", and "substituted arylalkyl" mean alkyl, aryl, and arylalkyl respectively, in which one or more hydrogen atoms are each independently replaced with a substituent. Typical substituents include, but are not limited to, —X, —R, —O^-, —OR, —SR, —S^-, —NR_2, —NR_3, ═NR, —CX_3, —CN, —OCN, —SCN, —N═C═O, —NCS, —NO, —NO_2, ═N_2, —N_3, NC(═O)R, —C(═O)R, —C(═O)NRR—$S(═O)_2O^-$, —$S(═O)_2OH$, —$S(═O)_2R$, —$OS(═O)_2OR$, —$S(═O)_2$NR, —S(═O)R, —$OP(═O)O_2RR$—$P(═O)O_2RR$ —$P(═O)(O^-)_2$, —$P(═O)(OH)_2$, —C(═O)R, —C(═O)X, —C(S)R, —C(O)OR, —$C(O)O^-$, —C(S)OR, —C(O)SR, —C(S)SR, —C(O)NRR, —C(S)NRR, —C(NR)NRR, where each X is independently a halogen: F, Cl, Br, or I; and each R is independently —H, alkyl, aryl, heterocycle, or prodrug moiety.

"Heteroaryl" and "Heterocycle" refer to a ring system in which one or more ring atoms is a heteroatom, e.g. nitrogen, oxygen, and sulfur. Heterocycles are described in: Katritzky, Alan R., Rees, C. W., and Scriven, E. *Comprehensive Heterocyclic Chemistry* (1996) Pergamon Press; Paquette, Leo A.; *Principles of Modern Heterocyclic Chemistry* W. A. Benjamin, New York, (1968), particularly Chapters 1, 3, 4, 6, 7, and 9; "The Chemistry of Heterocyclic Compounds, A series of Monographs" (John Wiley & Sons, New York, 1950 to present), in particular Volumes 13, 14, 16, 19, and 28. Exemplary heterocycles include but are not limited to substituents, i.e. radicals, derived from pyrrole, indole, furan, benzofuran, thiophene, benzothiophene, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-quinolyl, 3-quinolyl, 4-quinolyl, 2-imidazole, 4-imidazole, 3-pyrazole, 4-pyrazole, pyridazine, pyrimidine, pyrazine, purine, cinnoline, pthalazine, quinazoline, quinoxaline, 3-(1,2,4-N)-triazolyl, 5-(1,2,4-N)-triazolyl, 5-tetrazolyl, 4-(1-O, 3-N)-oxazole, 5-(1-O,3-N)-oxazole, 4-(1-S, 3-N)-thiazole, 5-(1-S, 3-N)-thiazole, 2-benzoxazole, 2-benzothiazole, 4-(1,2,3-N)-benzotriazole, and benzimidazole.

Stereochemical definitions and conventions used herein generally follow S. P. Parker, Ed., *McGraw-Hill Dictionary of Chemical Terms* (1984) McGraw-Hill Book Company, New York; and Eliel, E. and Wilen, S., *Stereochemistry of Organic Compounds* (1994) John Wiley & Sons, Inc., New York. Many organic compounds exist in optically active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L or R and S are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (−) are employed to designate the sign of rotation of plane-polarized light by the compound, with (−) or l meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, these compounds, called stereoisomers, are identical except that they are mirror images of one another. A specific stereoisomer is also referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture or a racemate. The terms "racemic mixture" and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.

The term "chiral" refers to molecules which have the property of non-superimposability of the mirror image partner, while the term "achiral" refers to molecules which are superimposable on their mirror image partner.

The term "stereoisomers" refers to compounds which have identical chemical constitution, but differ with regard to the arrangement of the atoms or groups in space.

"Diastereomer" refers to a stereoisomer with two or more centers of chirality and whose molecules are not mirror images of one another. Diastereomers have different physical properties, e.g. melting points, boiling points, spectral properties, and reactivities. Mixtures of diastereomers may separate under high resolution analytical procedures such as electrophoresis and chromatography.

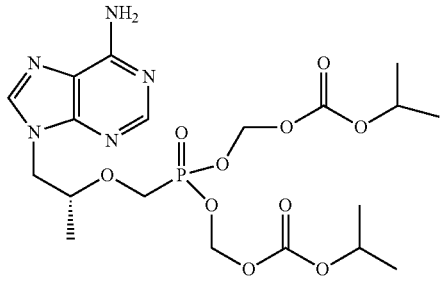
"Enantiomers" refer to two stereoisomers of a compound which are non-superimposable mirror images of one another.

Active Ingredients of the Combinations

The present invention provides novel combinations of two or more active ingredients being employed together. In some embodiments, a synergistic antiviral effect is achieved. In other embodiments, a chemically stable combination is obtained. The combinations include at least one active ingredient selected from (1) tenofovir disoproxil fumarate and physiologically functional derivatives, and at least one active ingredient selected from (2) emtricitabine and physi-

ologically functional derivatives. The term "synergistic antiviral effect" is used herein to denote an antiviral effect which is greater than the predicted purely additive effects of the individual components (a) and (b) of the combination.

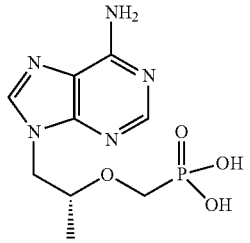
Tenofovir disoproxil fumarate (also known as Viread®, Tenofovir DF, Tenofovir disoproxil, TDF, Bis-POC-PMPA (U.S. Pat. Nos. 5,935,946, 5,922,695, 5,977,089, 6,043,230, 6,069,249) is a prodrug of tenofovir, and has the structure:



and including fumarate salt ($HO_2CCH_2CH_2CO_2^-$).

The chemical names for Tenofovir disoproxil include: [2-(6-amino-purin-9-yl)-1-methyl-ethoxymethyl]-phosphonic acid diisopropoxycarbonyloxymethyl ester; 9-[(R)-2-[[bis[[(isopropoxycarbonyl)oxy]methoxy]phosphinyl] methoxy]propyl]adenine; and 2,4,6,8-tetraoxa-5-phosphanonanedioic acid, 5-[[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy]methyl]-, bis(1-methylethyl)ester, 5-oxide. The CAS Registry numbers include: 201341-05-1; 202138-50-9; 206184-49-8. It should be noted that the ethoxymethyl unit of tenofovir has a chiral center. The R (rectus, right handed configuration) enantiomer is shown. However, the invention also includes the S isomer. The invention includes all enantiomers, diastereomers, racemates, and enriched stereoisomer mixtures of tenofovir (PMPA) and physiologically functional derivatives thereof.

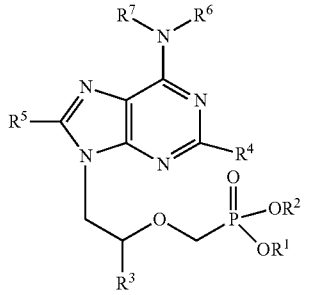
PMPA or tenofovir (U.S. Pat. Nos. 4,808,716, 5,733,788, 6,057,305) has the structure:



The chemical names of PMPA, tenofovir include: (R)-9-(2-phosphonylmethoxypropyl)adenine; and phosphonic acid, [[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy] methyl]. The CAS Registry number is 147127-20-6.

Tenofovir disoproxil fumarate (DF) is a nucleotide reverse transcriptase inhibitor approved in the United States in 2001 for the treatment of HIV-1 infection in combination with other antiretroviral agents. Tenofovir disoproxil fumarate or Viread® (Gilead Science, Inc.) is the fumarate salt of tenofovir disoproxil. Viread® may be named as: 9-[(R)-2-[[bis[[(isopropoxycarbonyl)oxy]methoxy]phosphinyl] methoxy]propyl]adenine fumarate (1:1); or 2,4,6,8-tetraoxa-5-phosphanonanedioic acid, 5-[[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy]methyl]-, bis(1-methylethyl)ester, 5-oxide, (2E)-2-butenedioate (1:1). The CAS Registry number is 202138-50-9.

Physiologically functional derivatives of tenofovir disoproxil fumarate include PMEA (adefovir, 9-((R)-2-(phosphonomethoxy)ethyl)adenine) and PMPA compounds. Exemplary combinations include a PMEA or PMPA compound in combination with emtricitabine or 3TC. PMEA and PMPA compounds have the structures:



where PMEA (R^3 is H) and PMPA (R^3 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, or CH_2OR^8 where R^8 is C_1-C_6 alkyl, C_1-C_6 hydroxyalkyl or C_1-C_6 haloalkyl. R^6 and R^7 are independently H or C_1-C_6 alkyl. R^4 and R^5 are independently H, NH_2, NHR or NR_2 where R is C_1-C_6 alkyl. R^1 and R^2 are independently H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_6-C_{20} arylalkyl, C_6-C_{20} substituted arylalkyl, acyloxymethyl esters $—CH_2OC(=O)R^9$ (e.g. POM) or acyloxymethyl carbonates $—CH_2C(=O)OR^9$ (e.g. POC) where R^9 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl or C_6-C_{20} substituted aryl. For example, R_1 and R_2 may be pivaloyloxymethoxy, POM, $—CH_2C(=O)C(CH_3)_3$; $—CH_2C(=O)OC(CH_3)_3$; or POC, $—CH_2C(=O)OCH(CH_3)_2$. Also for example, tenofovir has the structure where R^3 is CH_3, and R^1, R^2, R^4, R^5, R^6 and R^7 are H. Dialkyl phosphonates may be prepared according to the methods of: Quast et al (1974) *Synthesis* 490; Stowell et al (1990) *Tetrahedron Lett.* 3261; U.S. Pat. No. 5,663,159.

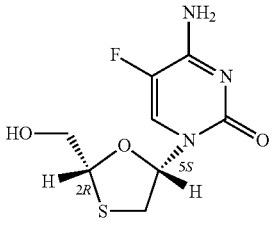
The PMPA compound may be enantiomerically-enriched or purified (single stereoisomer) where the carbon atom bearing R^3 may be the R or S enantiomer. The PMPA compound may be a racemate, i.e. a mixture of R and S stereoisomers.

Adefovir (9-(2-phosphonomethoxyethyl)adenine where R_1-R_7═H) is an exemplary PMEA compound (U.S. Pat. Nos. 4,808,716, 4,724,233). As the bis-pivalate prodrug, Adefovir dipivoxil, also known as bis-POM PMEA, (R_3-R_7═H, R_1 and R_2═$—CH_2OC(=O)C(CH_3)_3$, pivoxil, POM, pivaloyloxymethoxy), is effective against HIV and Hepatitis B infections (U.S. Pat. Nos. 5,663,159, 6,451,340). Adefovir dipivoxil has demonstrated minor to moderate synergistic inhibition of HIV replication in combination with other compounds with anti-HIV activity including PMPA, d4T, ddC, nelfinavir, ritonavir, and saquinavir (Mulato et al (1997) *Antiviral Research* 36:91-97).

The invention includes all enantiomers, diastereomers, racemates, and enriched stereoisomer mixtures of PMEA and PMPA, and physiologically functional derivatives thereof.

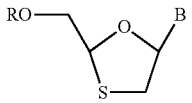
Emtricitabine ((−)-cis-FTC, Emtriva™), a single enantiomer of FTC, is a potent nucleoside reverse transcriptase inhibitor approved for the treatment of HIV (U.S.

Pat. Nos. 5,047,407, 5,179,104, 5,204,466, 5,210,085, 5,486,520, 5,538,975, 5,587,480, 5,618,820, 5,763,606, 5,814,639, 5,914,331, 6,114,343, 6,180,639, 6,215,004; WO 02/070518). The single enantiomer emtricitabine has the structure:



The chemical names for emtricitabine include: (−)-cis-FTC; β-L-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane; (2R,5S)-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine; and 4-amino-5-fluoro-1-(2-hydroxymethyl-[1,3]-(2R,5S)-oxathiolan-5-yl)-1H-pyrimidin-2-one. The CAS Registry numbers include: 143491-57-0; 143491-54-7. It should be noted that FTC contains two chiral centers, at the 2 and 5 positions of the oxathiolane ring, and therefore can exist in the form of two pairs of optical isomers (i.e. enantiomers) and mixtures thereof including racemic mixtures. Thus, FTC may be either a cis or a trans isomer or mixtures thereof. Mixtures of cis and trans isomers are diastereomers with different physical properties. Each cis and trans isomer can exist as one of two enantiomers or mixtures thereof including racemic mixtures. The invention includes all enantiomers, diastereomers, racemates, and enriched stereoisomer mixtures of emtricitabine and physiologically functional derivatives thereof. For example, the invention includes physiological functional derivatives such as the 1:1 racemic mixture of the enantiomers (2R,5S,cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine) and its mirror image (2S,5R,cis)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one, or mixtures of the two enantiomers in any relative amount. The invention also includes mixtures of cis and trans forms of FTC.

Physiologically functional derivatives of emtricitabine include 1,3 oxathiolane nucleosides having the structure:

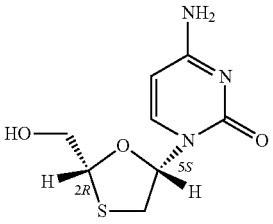


In the 1,3 oxathiolane nucleoside structure above, B is a nucleobase including any nitrogen-containing heterocyclic moiety capable of forming Watson-Crick hydrogen bonds in pairing with a complementary nucleobase or nucleobase analog, e.g. a purine, a 7-deazapurine, or a pyrimidine. Examples of B include the naturally occurring nucleobases: adenine, guanine, cytosine, uracil, thymine, and minor constituents and analogs of the naturally occurring nucleobases, e.g. 7-deazaadenine, 7-deazaguanine, 7-deaza-8-azaguanine, 7-deaza-8-azaadenine, inosine, nebularine, nitropyrrole, nitroindole, 2-aminopurine, 2-amino-6-chloropurine, 2,6-diaminopurine, hypoxanthine, pseudouridine, 5-fluorocytosine, 5-chlorocytosine, 5-bromocytosine, 5-iodocytosine, pseudocytosine, pseudoisocytosine, 5-propynylcytosine, isocytosine, isoguanine, 7-deazaguanine, 2-thiopyrimidine, 6-thioguanine, 4-thiothymine, 4-thiouracil, O^6-methylguanine, N^6-methyladenine, O^4-methylthymine, 5,6-dihydrothymine, 5,6-dihydrouracil, 4-methylindole, pyrazolo[3,4-D]pyrimidines (U.S. Pat. Nos. 6,143,877 and 6,127,121; WO 01/38584), and ethenoadenine (Fasman (1989) in *Practical Handbook of Biochemistry and Molecular Biology*, pp. 385-394, CRC Press, Boca Raton, Fl).

Nucleobases B may be attached in the configurations of naturally-occurring nucleic acids to the 1,3 oxathiolane moiety through a covalent bond between the N-9 of purines, e.g. adenin-9-yl and guanin-9-yl, or N−1 of pyrimidines, e.g. thymin-1-yl and cytosin-1-yl (Blackburn, G. and Gait, M. Eds. "DNA and RNA structure" in *Nucleic Acids in Chemistry and Biology*, 2nd Edition, (1996) Oxford University Press, pp. 15-81).

Also in the 1,3 oxathiolane nucleoside structure above, R is H, C_1-C_{18} alkyl, C_1-C_{18} substituted alkyl, C_2-C_{18} alkenyl, C_2-C_{18} substituted alkenyl, C_2-C_{18} alkynyl, C_2-C_{18} substituted alkynyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_2-C_{20} heterocycle, C_2-C_{20} substituted heterocycle, phosphonate, phosphophosphonate, diphosphophosphonate, phosphate, diphosphate, triphosphate, polyethyleneoxy, or a prodrug moiety.

Physiologically functional derivatives of emtricitabine also include 3TC (lamivudine, Epivir®), a reverse transcriptase inhibitor approved in the United States for the treatment of HIV-1 infection in combination with AZT as Combivir® (GlaxoSmithKline). U.S. Pat. Nos. 5,859,021; 5,905,082; 6,177,435; 5,627,186; 6,417,191. Lamivudine (U.S. Pat. Nos. 5,587,480, 5,696,254, 5,618,820, 5,756,706, 5,744,596, 568,164, 5,466,806, 5,151,426) has the structure:



For example and for some therapeutic uses, 3TC may be a physiologically functional derivative of emtricitabine in combination with tenofovir DF or a physiologically functional derivative of tenofovir DF.

It will be appreciated that tenofovir DF and emtricitabine, and their physiologically functional derivatives may exist in keto or enol tautomeric forms and the use of any tautomeric form thereof is within the scope of this invention. Tenofovir DF and emtricitabine will normally be utilized in the combinations of the invention substantially free of the corresponding enantiomer, that is to say no more than about 5% w/w of the corresponding enantiomer will be present.

Prodrugs

The invention includes all prodrugs of tenofovir and emtricitabine. An exemplary prodrug of tenofovir is tenofovir disoproxil fumarate (TDF, Viread®). A large number of structurally-diverse prodrugs have been described for phosphonic acids (Freeman and Ross in *Progress in Medicinal Chemistry* 34: 112-147 (1997). A commonly used prodrug class is the acyloxyalkyl ester, which was first used as a prodrug strategy for carboxylic acids and then applied to phosphates and phosphonates by Farquhar et al (1983) *J. Pharm. Sci.* 72: 324; also U.S. Pat. Nos. 4,816,570, 4,968,

788, 5,663,159 and 5,792,756. Subsequently, the acyloxyalkyl ester was used to deliver phosphonic acids across cell membranes and to enhance oral bioavailability. A close variant of the acyloxyalkyl ester strategy, the alkoxycarbonyloxyalkyl ester, may also enhance oral bioavailability as a prodrug moiety in the compounds of the combinations of the invention. Aryl esters of phosphorus groups, especially phenyl esters, are reported to enhance oral bioavailability (DeLambert et al (1994) *J. Med. Chem.* 37: 498). Phenyl esters containing a carboxylic ester ortho to the phosphate have also been described (Khamnei and Torrence, (1996) *J. Med. Chem.* 39:4109-4115). Benzyl esters are reported to generate the parent phosphonic acid. In some cases, substituents at the ortho- or para-position may accelerate the hydrolysis. Benzyl analogs with an acylated phenol or an alkylated phenol may generate the phenolic compound through the action of enzymes, e.g. esterases, oxidases, etc., which in turn undergoes cleavage at the benzylic C—O bond to generate the phosphoric acid and the quinone methide intermediate. Examples of this class of prodrugs are described by Mitchell et al (1992) *J. Chem. Soc. Perkin Trans. I* 2345; Brook et al WO 91/19721. Still other benzylic prodrugs have been described containing a carboxylic ester-containing group attached to the benzylic methylene (Glazier et al WO 91/19721). Thio-containing prodrugs are reported to be useful for the intracellular delivery of phosphonate drugs. These proesters contain an ethylthio group in which the thiol group is either esterified with an acyl group or combined with another thiol group to form a disulfide. Deesterification or reduction of the disulfide generates the free thio intermediate which subsequently breaks down to the phosphoric acid and episulfide (Puech et al (1993) *Antiviral Res.,* 22: 155-174; Benzaria et al (1996) *J. Med. Chem.* 39: 4958). Cyclic phosphonate esters have also been described as prodrugs of phosphorus-containing compounds.

Prodrug esters in accordance with the invention are independently selected from the following groups: (1) mono-, di-, and tri-phosphate esters of tenofovir or emtricitabine or any other compound which upon administration to a human subject is capable of providing (directly or indirectly) said mono-, di, or triphosphate ester; (2) carboxylic acid esters (3) sulphonate esters, such as alkyl- or aralkylsulphonyl (for example, methanesulphonyl); (4) amino acid esters (for example, alanine, L-valyl or L-isoleucyl); (5) phosphonate; and (6) phosphonamidate esters.

Ester groups (1)-(6) may be substituted with; straight or branched chain C_1-C_{18} alkyl (for example, methyl, n-propyl, t-butyl, or n-butyl); C_3-C_{12} cycloalkyl; alkoxyalkyl (for example, methoxymethyl); arylalkyl (for example, benzyl); aryloxyalkyl (for example, phenoxymethyl); C_5-C_{20} aryl (for example, phenyl optionally substituted by, for example, halogen, C_1-C_4 alkyl, C_1-C_4 alkoxy, or amino; acyloxymethyl esters —$CH_2C(=O)R^9$ (e.g. POM) or acyloxymethyl carbonates —$CH_2OC(=O)OR^9$ (e.g. POC) where R^9 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl or C_6-C_{20} substituted aryl. For example, ester groups may be: —$CH_2C(=O)C(CH_3)_3$, —$CH_2OC(=O)OC(CH_3)_3$ or —$CH_2C(=O)OCH(CH_3)_2$.

An exemplary aryl moiety present in such esters comprises a phenyl or substituted phenyl group. Many phosphate prodrug moieties are described in U.S. Pat. No. 6,312,662; Jones et al (1995) *Antiviral Research* 27:1-17; Kucera et al (1990) *AIDS Res. Hum. Retro Viruses* 6:491-501; Piantadosi et al (1991) *J. Med. Chem.* 34:1408-14; Hosteller et al (1992) *Antimicrob. Agents Chemother.* 36:2025-29; Hostetler et al (1990) *J. Biol. Chem.* 265:611127; and Siddiqui et al (1999) *J. Med. Chem.* 42:4122-28.

Pharmaceutically acceptable prodrugs refer to a compound that is metabolized in the host, for example hydrolyzed or oxidized, by either enzymatic action or by general acid or base solvolysis, to form an active ingredient. Typical examples of prodrugs of the active ingredients of the combinations of the invention have biologically labile protecting groups on a functional moiety of the active compound. Prodrugs include compounds that can be oxidized, reduced, aminated, deaminated, esterified, deesterified, alkylated, dealkylated, acylated, deacylated, phosphorylated, dephosphorylated, or other functional group change or conversion involving forming or breaking chemical bonds on the prodrug.

Chemical Stability of a Pharmaceutical Formulation

The chemical stability of the active ingredients in a pharmaceutical formulation is of concern to minimize the generation of impurities and ensure adequate shelf-life. The active ingredients, tenofovir disoproxil fumarate and emtricitabine, in the pharmaceutical formulations of the invention have relatively low pKa values, indicative of the potential to cause acidic hydrolysis of the active ingredients. Emtricitabine, with a pKa of 2.65 (Emtriva™ Product Insert, Gilead Sciences, Inc. 2003, available at gilead.com) is subject to hydrolytic deamination of the 5-fluoro cytosine nucleobase to form the 5-fluoro uridine nucleobase. Tenofovir disoproxil fumarate, with a pKa of 3.75 (Yuan L. et al "Degradation Kinetics of Oxycarbonyloxymethyl Prodrugs of Phosphonates in Solution", *Pharmaceutical Research* (2001) Vol. 18, No. 2, 234-237), is subject also to hydrolytic deamination of the exocyclic amine of the adenine nucleobase, and to hydrolysis of one or both of the POC ester groups (U.S. Pat. No. 5,922,695). It is desirable to formulate a therapeutic combination of tenofovir disoproxil fumarate and emtricitabine, and the physiological functional derivatives thereof, with a minimum of impurities and adequate stability.

The combinations of the present invention provide combination pharmaceutical dosage forms which are chemically stable to acid degradation of: (1) a first component (such as tenofovir disoproxil fumarate, and physiological functional derivatives; (2) a second component (such as emtricitabine, and physiological functional derivatives; and (3) optionally a third component having antiviral activity. The third component includes anti-HIV agents and include: protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), and integrase inhibitors. Exemplary third active ingredients to be administered in combination with first and second components are shown in Table A. First and second components are as defined in the above section entitled: ACTIVE INGREDIENTS OF THE COMBINATIONS.

Salts

Any reference to any of the compounds in the compositions of the invention also includes any physiologically acceptable salt thereof. Examples of physiologically acceptable salts of tenofovir DF, emtricitabine and their physiologically functional derivatives include salts derived from an appropriate base, such as an alkali metal (for example, sodium), an alkaline earth (for example, magnesium), ammonium and NX_4^+ (wherein X is C_1-C_4 alkyl), or an organic acid such as fumaric acid, acetic acid, succinic acid. Physiologically acceptable salts of an hydrogen atom or an amino group include salts of organic carboxylic acids such as acetic, benzoic, lactic, fumaric, tartaric, maleic, malonic, malic, isethionic, lactobionic and succinic acids; organic

sulfonic acids, such as methanesulfonic, ethanesulfonic, benzenesulfonic and p-toluenesulfonic acids; and inorganic acids, such as hydrochloric, sulfuric, phosphoric and sulfamic acids. Physiologically acceptable salts of a compound of an hydroxy group include the anion of said compound in combination with a suitable cation such as Na^+ and NX_4^+ (wherein X is independently selected from H or a C_1-C_4 alkyl group).

For therapeutic use, salts of active ingredients of the combinations of the invention will be physiologically acceptable, i.e. they will be salts derived from a physiologically acceptable acid or base. However, salts of acids or bases which are not physiologically acceptable may also find use, for example, in the preparation or purification of a physiologically acceptable compound. All salts, whether or not derived from a physiologically acceptable acid or base, are within the scope of the present invention.

Administration of the Formulations

While it is possible for the active ingredients of the combination to be administered alone and separately as monotherapies, it is preferable to administer them as a pharmaceutical co-formulation. A two-part or three-part combination may be administered simultaneously or sequentially. When administered sequentially, the combination may be administered in one, two, or three administrations.

Preferably, two-part or three-part combinations are administered in a single pharmaceutical dosage form. More preferably, a two-part combination is administered as a single oral dosage form and a three-part combination is administered as two identical oral dosage forms. Examples include a single tablet of tenofovir disoproxil fumarate and emtricitabine, or two tablets of tenofovir disoproxil fumarate, emtricitabine, and efavirenz.

It will be appreciated that the compounds of the combination may be administered: (1) simultaneously by combination of the compounds in a co-formulation or (2) by alternation, i.e. delivering the compounds serially, sequentially, in parallel or simultaneously in separate pharmaceutical formulations. In alternation therapy, the delay in administering the second, and optionally a third active ingredient, should not be such as to lose the benefit of a synergistic therapeutic effect of the combination of the active ingredients. By either method of administration (1) or (2), ideally the combination should be administered to achieve peak plasma concentrations of each of the active ingredients. A one pill once-per-day regimen by administration of a combination co-formulation may be feasible for some HIV-positive patients. Effective peak plasma concentrations of the active ingredients of the combination will be in the range of approximately 0.001 to 100 μM. Optimal peak plasma concentrations may be achieved by a formulation and dosing regimen prescribed for a particular patient. It will also be understood that tenofovir DF and emtricitabine, or the physiologically functional derivatives of either thereof, whether presented simultaneously or sequentially, may be administered individually, in multiples, or in any combination thereof. In general, during alternation therapy (2), an effective dosage of each compound is administered serially, where in co-formulation therapy (1), effective dosages of two or more compounds are administered together.

Formulation of the Combinations

When the individual components of the combination are administered separately they are generally each presented as a pharmaceutical formulation. The references hereinafter to formulations refer unless otherwise stated to formulations containing either the combination or a component compound thereof. It will be understood that the administration of the combination of the invention by means of a single patient pack, or patient packs of each formulation, within a package insert diverting the patient to the correct use of the invention is a desirable additional feature of this invention. The invention also includes a double pack comprising in association for separate administration, formulations of tenofovir disoproxil fumarate and emtricitabine, or a physiologically functional derivative of either or both thereof.

The combination therapies of the invention include: (1) a combination of tenofovir DF and emtricitabine or (2) a combination containing a physiologically functional derivative of either or both thereof.

The combination may be formulated in a unit dosage formulation comprising a fixed amount of each active pharmaceutical ingredient for a periodic, e.g. daily, dose or subdose of the active ingredients.

Pharmaceutical formulations according to the present invention comprise a combination according to the invention together with one or more pharmaceutically acceptable carriers or excipients and optionally other therapeutic agents. Pharmaceutical formulations containing the active ingredient may be in any form suitable for the intended method of administration. When used for oral use for example, tablets, troches, lozenges, aqueous or oil suspensions, dispersible powders or granules, emulsions, hard or soft capsules, syrups or elixirs may be prepared (*Remington's Pharmaceutical Sciences* (Mack Publishing Co., Easton, Pa.). Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents including antioxidants, sweetening agents, flavoring agents, coloring agents and preserving agents, in order to provide a palatable preparation. Tablets containing the active ingredient in admixture with non-toxic pharmaceutically acceptable excipient which are suitable for manufacture of tablets are acceptable. These excipients may be, for example, inert diluents, such as calcium or sodium carbonate, lactose, lactose monohydrate, croscarmellose sodium, povidone, calcium or sodium phosphate; granulating and disintegrating agents, such as maize starch, or alginic acid; binding agents, such as cellulose, microcrystalline cellulose, starch, gelatin or acacia; and lubricating agents, such as magnesium stearate, stearic acid or talc. Tablets may be uncoated or may be coated by known techniques including microencapsulation to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate alone or with a wax may be employed.

Formulations for oral use may be also presented as hard gelatin capsules where the active ingredient is mixed with an inert solid diluent, for example pregelatinized starch, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, such as peanut oil, liquid paraffin or olive oil.

Aqueous suspensions of the invention contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients include a suspending agent, such as sodium carboxymethylcellulose, methylcellulose, hydroxypropyl methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as a naturally occurring phosphatide (e.g., lecithin), a condensation product of an alkylene oxide with a fatty acid (e.g., polyoxyethylene stearate), a condensation product of ethyl-

ene oxide with a long chain aliphatic alcohol (e.g., heptadecaethyleneoxycetanol), a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol anhydride (e.g., polyoxyethylene sorbitan monooleate). The aqueous suspension may also contain one or more preservatives such as ethyl or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents, such as sucrose, sucralose or saccharin.

Oil suspensions may be formulated by suspending the active ingredient in a vegetable oil, such as arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oral suspensions may contain a thickening agent, such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents, such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid, BHT, etc.

Dispersible powders and granules of the invention suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, a suspending agent, and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those disclosed above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions or liposome formulations. The oily phase may be a vegetable oil, such as olive oil or arachis oil, a mineral oil, such as liquid paraffin, or a mixture of these. Suitable emulsifying agents include naturally-occurring gums, such as gum acacia and gum tragacanth, naturally occurring phosphatides, such as soybean lecithin, esters or partial esters derived from fatty acids and hexitol anhydrides, such as sorbitan monooleate, and condensation products of these partial esters with ethylene oxide, such as polyoxyethylene sorbitan monooleate. The emulsion may also contain sweetening and flavoring agents. Syrups and elixirs may be formulated with sweetening agents, such as glycerol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, a flavoring or a coloring agent.

The pharmaceutical compositions of the invention may be in the form of a sterile injectable preparation, such as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, such as a solution in 1,3-butane-diol or prepared as a lyophilized powder. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile fixed oils may conventionally be employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid may likewise be used in the preparation of injectables.

The pharmaceutical compositions of the invention may be injected parenterally, for example, intravenously, intraperitoneally, intrathecally, intraventricularly, intrastemally, intracranially, intramuscularly or subcutaneously, or they may be administered by infusion techniques. They are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art.

The pharmaceutical compositions of the invention may also be administered intranasally or by inhalation and are conveniently delivered in the form of a dry powder inhaler or an aerosol spray presentation from a pressurized container or a nebuliser with the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFC 134a), carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurized container or nebuliser may contain a solution or suspension of the composition, e.g. using a mixture of ethanol and the propellant as the solvent, which may additional contain a lubricant, e.g. sorbitan trioleate. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated to contain a powder mix of a compound of the formula (I) and a suitable powder base such as lactose or starch. Aerosol or dry powder formulations are preferably arranged so that each metered dose or "puff" contains from 20 μg to 20 mg of a composition for delivery to the patient. The overall daily dose with an aerosol will be in the range of from 20 μg to 20 mg which may be administered in a single dose or, more usually, in divided doses throughout the day.

The amount of active ingredient that may be combined with the carrier material to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a time-release formulation intended for oral administration to humans may contain approximately 1 to 1000 mg of active material compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95% of the total compositions (weight:weight). The pharmaceutical composition can be prepared to provide easily measurable amounts for administration. For example, an aqueous solution intended for intravenous infusion may contain from about 3 to 500 μg of the active ingredient per milliliter of solution in order that infusion of a suitable volume at a rate of about 30 mL/hr can occur. As noted above, formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be administered as a bolus, electuary or paste.

The combinations of the invention may conveniently be presented as a pharmaceutical formulation in a unitary dosage form. A convenient unitary dosage formulation contains the active ingredients in any amount from 1 mg to 1 g each, for example but not limited to, 10 mg to 300 mg. The synergistic effects of tenofovir DF in combination with emtricitabine may be realized over a wide ratio, for example 1:50 to 50:1 (tenofovir DF:emtricitabine). In one embodiment, the ratio may range from about 1:10 to 10:1. In another embodiment, the weight/weight ratio of tenofovir to emtricitabine in a co-formulated combination dosage form, such as a pill, tablet, caplet or capsule will be about 1, i.e. an approximately equal amount of tenofovir DF and emtricitabine. In other exemplary co-formulations, there may be more or less tenofovir than FTC. For example, 300 mg

tenofovir DF and 200 mg emtricitabine can be co-formulated in a ratio of 1.5:1 (tenofovir DF: emtricitabine). In one embodiment, each compound will be employed in the combination in an amount at which it exhibits antiviral activity when used alone. Exemplary Formulations A, B, C, D, E, and F (Examples) have ratios of 12:1 to 1:1 (tenofovir DF:emtricitabine). Exemplary Formulations A, B, C, D, E, and F use amounts of tenofovir DF and emtricitabine ranging from 25 mg to 300 mg. Other ratios and amounts of the compounds of said combinations are contemplated within the scope of the invention.

A unitary dosage form may further comprise tenofovir DF and emtricitabine, or physiologically functional derivatives of either thereof, and a pharmaceutically acceptable carrier.

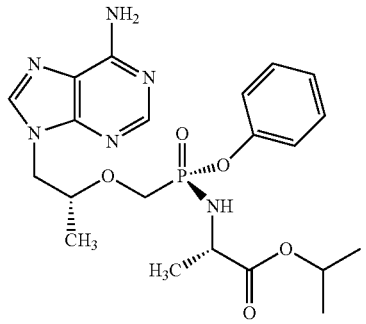
It will be appreciated by those skilled in the art that the amount of active ingredients in the combinations of the invention required for use in treatment will vary according to a variety of factors, including the nature of the condition being treated and the age and condition of the patient, and will ultimately be at the discretion of the attending physician or health care practitioner. The factors to be considered include the route of administration and nature of the formulation, the animal's body weight, age and general condition and the nature and severity of the disease to be treated. For example, in a Phase I/II monotherapy study of emtricitabine, patients received doses ranging from 25 mg to 200 mg twice-a-day for two weeks. At each dose regimen greater or equal to 200 mg, a 98-percent (1.75 log 10) or greater viral suppression was observed. A once-a-day dose of 200 mg of emtricitabine reduced the viral load by an average of 99 percent (1.92 log 10). Viread® (tenofovir DF) has been approved by the FDA for the treatment and prophylaxis of HIV infection as a 300 mg oral tablet. Emtriva™ (emtricitabine) has been approved by the FDA for the treatment of HIV as a 200 mg oral tablet.

It is also possible to combine any two of the active ingredients in a unitary dosage form for simultaneous or sequential administration with a third active ingredient. The three-part combination may be administered simultaneously or sequentially. When administered sequentially, the combination may be administered in two or three administrations. Third active ingredients have anti-HIV activity and include protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), and integrase inhibitors. Exemplary third active ingredients to be administered in combination with tenofovir DF, emtricitabine, and their physiological functional derivatives, are shown in Table A.

TABLE A

5,6 dihydro-5-azacytidine
5-aza 2'deoxycytidine
5-azacytidine
5-yl-carbocyclic 2'-deoxyguanosine (BMS200,475)
9 (arabinofuranosyl)guanine; 9-(2' deoxyribofuranosyl)guanine
9-(2'-deoxy 2'fluororibofuranosyl)-2,6-diaminopurine
9-(2'-deoxy 2'fluororibofuranosyl)guanine
9-(2'-deoxyribofuranosyl)-2,6 diaminopurine
9-(arabinofuranosyl)-2,6 diaminopurine
Abacavir, Ziagen ®
Acyclovir, ACV; 9-(2-hydroxyethoxylmethyl)guanine
Adefovir dipivoxil, Hepsera ®
amdoxivir, DAPD
Amprenavir, Agenerase ®
araA; 9-β-D-arabinofuranosyladenine (Vidarabine)
atazanivir sulfate (Reyataz ®)
AZT; 3'-azido-2',3'-dideoxythymdine, Zidovudine, (Retrovir ®)
BHCG; (.+−.)-(1a,2b,3a)-9-[2,3-bis(hydroxymethyl)cyclobutyl]guanine
BMS200,475; 5-yl-carbocyclic 2'-deoxyguanosine
Buciclovir; (R) 9-(3,4-dihydroxybutyl)guanine
BvaraU; 1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil (Sorivudine)
Calanolide A
Capravirine
CDG; carbocyclic 2'-deoxyguanosine
Cidofovir, HPMPC; (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine
Clevudine, L-FMAU; 2'-Fluoro-5-methyl-β-L-arabino-furanosyluracil
Combivir ® (lamivudine/zidovudine)
Cytallene; [1-(4'-hydroxy-1',2'-butadienyl)cytosine]
d4C; 3'-deoxy-2',3'-didehydrocytidine
DAPD; (−)-β-D-2,6-diaminopurine dioxolane
ddA; 2',3'-dideoxyadenosine
ddAPR; 2,6-diaminopurine-2',3'-dideoxyriboside
ddC; 2',3'-dideoxycytidine (Zalcitabine)
ddI; 2',3'-dideoxyinosine, didanosine, (Videx ®, Videx ® EC)
Delavirdine, Rescriptor ®
Didanosine, ddI, Videx ®; 2',3'-dideoxyinosine
DXG; dioxolane guanosine
E-5-(2-bromovinyl)-2'-deoxyuridine
Efavirenz, Sustiva ®
Enfuvirtide, Fuzeon ®
F-ara-A; fluoroarabinosyladenosine (Fludarabine)
FDOC; (−)-β-D-5-fluoro-1-[2-(hydroxymethyl)-1,3-dioxolane]cytosine
FEAU; 2'-deoxy-2'-fluoro-1-β-D-arabinofuranosyl-5-ethyluracil
FIAC; 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine
FIAU; 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouridine
FLG; 2',3'-dideoxy-3'-fluoroguanosine
FLT; 3'-deoxy-3'-fluorothymidine
Fludarabine; F-ara-A; fluoroarabinosyladenosine
FMAU; 2'-Fluoro-5-methyl-β-L-arabino-furanosyluracil
FMdC
Foscarnet; phosphonoformic acid, PFA
FPMPA; 9-(3-fluoro-2-phosphonylmethoxypropyl)adenine
Gancyclovir, GCV; 9-(1,3-dihydroxy-2-propoxymethyl)guanine
GS-7340; 9-[R-2-[[(S)-[[(S)-1-(isopropoxycarbonyl)ethyl]amino]-phenoxyphosphinyl]methoxy]propyl]adenine
HPMPA; (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine
HPMPC; (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (Cidofovir)
Hydroxyurea, Droxia ®
Indinavir, Crixivan ®
Kaletra ® (lopinavir/ritonavir)
Lamivudine, 3TC, Epivir ™; (2R, 5S, cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one
L-d4C; L-3'-deoxy-2',3'-didehydrocytidine
L-ddC; L-2',3'-dideoxycytidine
L-Fd4C; L-3'-deoxy-2',3'-didehydro-5-fluorocytidine
L-FddC; L-2',3'-dideoxy-5-fluorocytidine
Lopinavir
Nelfinavir, Viracept ®
Nevirapine, Viramune ®
Oxetanocin A; 9-(2-deoxy-2-hydroxymethyl-β-D-erythro-oxetanosyl)adenine
Oxetanocin G; 9-(2-deoxy-2-hydroxymethyl-β-D-erythro-oxetanosyl)guanine
Penciclovir
PMEDAP; 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine
PMPA, tenofovir; (R)-9-(2-phosphonylmethoxypropyl)adenine
PPA; phosphonoacetic acid
Ribavirin; 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide
Ritonavir, Norvir ®
Saquinavir, Invirase ®, Fortovase ®
Sorivudine, BvaraU; 1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil
Stavudine, d4T, Zerit ®; 2',3'-didehydro-3'-deoxythymidine
Trifluorothymidine, TFT; Trifluorothymidine
Trizivir ® (abacavir sulfate/lamivudine/zidovudine)
Vidarabine, araA; 9-β-D-arabinofuranosyladenine
Zalcitabine, Hivid ®, ddC; 2',3'-dideoxycytidine
Zidovudine, AZT, Retrovir ®; 3'-azido-2',3'-dideoxythymdine
Zonavir; 5-propynyl-1-arabinosyluracil

Another aspect of the present invention is a three-part combination comprising tenofovir DF, FTC, and 9-[(R)-2-[[(S)-[[(S)-1-(isopropoxycarbonyl)ethyl]amino]phenoxy-phosphinyl]methoxy]propyl]adenine, also designated herein as GS-7340, which has the structure:



GS-7340 is a prodrug of tenofovir and the subject of commonly owned, pending application, U.S. Ser. No. 09/909,560, filed Jul. 20, 2001 and Becker et al WO 02/08241.

For example, a ternary unitary dosage may contain 1 mg to 1000 mg of tenofovir disoproxil fumarate, 1 mg to 1000 mg of emtricitabine, and 1 mg to 1000 mg of the third active ingredient. As a further feature of the present invention, a unitary dosage form may further comprise tenofovir DF, emtricitabine, the third active ingredient, or physiologically functional derivatives of the three active ingredients thereof, and a pharmaceutically acceptable carrier.

Combinations of the present invention enable patients greater freedom from multiple dosage medication regimens and ease the needed diligence required in remembering and complying with complex daily dosing times and schedules. By combining tenofovir disoproxil fumarate and emtricitabine into a single dosage form, the desired daily regimen may be presented in a single dose or as two or more sub-doses per day. The combination of co-formulated tenofovir DF and emtricitabine may be administered as a single pill, once per day.

A further aspect of the invention is a patient pack comprising at least one active ingredient: tenofovir disoproxil fumarate, emtricitabine, or a physiologically functional derivative of either of the combination and an information package or product insert containing directions on the use of the combination of the invention.

Segregation of active ingredients in pharmaceutical powders and granulations is a widely recognized problem that can result in inconsistent dispersions of the active ingredients in final dosage forms. Some of the main factors contributing to segregation are particle size, shape and density. Segregation is particularly troublesome when attempting to formulate a single homogenous tablet containing multiple active ingredients having different densities and different particle sizes. Glidants are substances that have traditionally been used to improve the flow characteristics of granulations and powders by reducing interparticulate friction. See Lieberman, Lachman, & Schwartz, *Pharmaceutical Dosage Forms: Tablets, Volume* 1, p. 177-178 (1989), incorporated herein by reference. Glidants are typically added to pharmaceutical compositions immediately prior to tablet compression to facilitate the flow of granular material into the die cavities of tablet presses. Glidants include: colloidal silicon dioxide, asbestos free talc, sodium aluminosilicate, calcium silicate, powdered cellulose, microcrystalline cellulose, corn starch, sodium benzoate, calcium carbonate, magnesium carbonate, metallic stearates, calcium stearate, magnesium stearate, zinc stearate, stearowet C, starch, starch 1500, magnesium lauryl sulfate, and magnesium oxide. Exemplary Tablet Formulation A has colloidal silicon dioxide (Examples). Glidants can be used to increase and aid blend composition homogeneity in formulations of anti-HIV drugs (U.S. Pat. No. 6,113,920). The novel compositions of the present invention may contain glidants to effect and maintain homogeneity of active ingredients during handling prior to tablet compression.

The present invention provides pharmaceutical formulations combining the active ingredients tenofovir DF and emtricitabine, or physiologically functional derivatives thereof, in a sufficiently homogenized form, and a method for using this pharmaceutical formulation. An object of the present invention is to utilize glidants to reduce the segregation of active ingredients in pharmaceutical compositions during pre-compression material handling. Another object of the present invention is to provide a pharmaceutical formulation combining the active ingredients tenofovir DF and emtricitabine, or physiologically functional derivatives thereof, with a pharmaceutically acceptable glidant, resulting in a mixture characterized by a pharmaceutically acceptable measure of homogeneity.

Formulations include those suitable for oral, rectal, nasal, topical (including transdermal, buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods represent a further feature of the present invention and include the step of bringing into association the active ingredients with the carrier, which constitutes one or more accessory ingredients, and maintaining chemical stability. In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, caplets, cachets or tablets each containing a predetermined amount of the active ingredients; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. povidone, gelatin, hydroxypropyl methylcellulose), lubricant, inert diluent, preservative, disintegrant (e.g. sodium starch glycollate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose) surface-active or dispersing agent. Molded tablets may be made by molding a mixture of the powdered compound moistened with an inert liquid diluent in a suitable machine. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredients therein using, for example, cellulose ether derivatives (e.g., hydroxypropyl methylcellulose) or methacrylate derivatives in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredients in a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and

mouthwashes comprising the active ingredient in a suitable liquid carrier. Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylates. Topical administration may also be by means of a transdermal iontophoretic device.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for penile administration for prophylactic or therapeutic use may be presented in condoms, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Pharmaceutical formulations suitable for rectal administration wherein the carrier is a solid are most preferably presented as unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art. The suppositories may be conveniently formed by admixture of the active combination with the softened or melted carrier(s) followed by chilling and shaping in moulds.

Formulations suitable for parenteral administration include aqueous and nonaqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents; and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Exemplary unit dosage formulations are those containing a daily dose or daily subdose of the active ingredients, as hereinbefore recited, or an appropriate fraction thereof. It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include such further agents as sweeteners, thickeners and flavoring agents.

The compounds of the combination of the present invention may be obtained in a conventional manner, known to those skilled in the art. Tenofovir disoproxil fumarate can be prepared, for example, as described in U.S. Pat. No. 5,977,089. Methods for the preparation of FTC are described in WO 92/14743, incorporated herein by reference.

Composition Use

Compositions of the present invention are administered to a human or other mammal in a safe and effective amount as described herein. These safe and effective amounts will vary according to the type and size of mammal being treated and the desired results of the treatment. Any of the various methods known by persons skilled in the art for packaging tablets, caplets, or other solid dosage forms suitable for oral administration, that will not degrade the components of the present invention, are suitable for use in packaging. The combinations may be packaged in glass and plastic bottles. Tablets, caplets, or other solid dosage forms suitable for oral administration may be packaged and contained in various packaging materials optionally including a dessicant, e.g. silica gel. Packaging may be in the form of unit dose blister packaging. For example, a package may contain one blister tray of tenofovir DF and another blister tray of emtricitabine pills, tablets, caplets, or capsule. A patient would take one dose, e.g. a pill, from one tray and one from the other. Alternatively, the package may contain a blister tray of the co-formulated combination of tenofovir DF and emtricitabine in a single pill, tablet, caplet or capsule. As in other combinations and packaging thereof, the combinations of the invention include physiological functional derivatives of tenofovir DF and FTC.

The packaging material may also have labeling and information related to the pharmaceutical composition printed thereon. Additionally, an article of manufacture may contain a brochure, report, notice, pamphlet, or leaflet containing product information. This form of pharmaceutical information is referred to in the pharmaceutical industry as a "package insert." A package insert may be attached to or included with a pharmaceutical article of manufacture. The package insert and any article of manufacture labeling provides information relating to the pharmaceutical composition. The information and labeling provides various forms of information utilized by health-care professionals and patients, describing the composition, its dosage and various other parameters required by regulatory agencies such as the United States Food and Drug Agency.

Assays of the Combinations

The combinations of the inventions may be tested for in vitro activity against HIV and sensitivity, and for cytotoxicity in laboratory adapted cell lines, e.g. MT2 and in peripheral blood mononuclear cells (PBMC) according to standard assays developed for testing anti-HIV compounds, such as WO 02/068058 and U.S. Pat. No. 6,475,491. Combination assays may be performed at varying concentrations of the compounds of the combinations to determine EC_{50} by serial dilutions.

Exemplary Formulations

The following examples further describe and demonstrate particular embodiments within the scope of the present invention. Techniques and formulations generally are found in *Remington's Pharmaceutical Sciences* (Mack Publishing Co., Easton, Pa.). The examples are given solely for illustration and are not to be construed as limitations as many variations are possible without departing from spirit and scope of the Invention. The following examples are intended for illustration only and are not intended to limit the scope of the invention in any way. "Active ingredient" denotes tenofovir disoproxil fumarate, emtricitabine, or a physiologically functional derivative of either thereof.

Tablet Formulation

The following exemplary formulations A, B, C, D, E, and F are prepared by wet granulation of the ingredients with an aqueous solution, addition of extragranular components and then followed by addition of magnesium stearate and compression.

Formulation A:

	mg/tablet
Tenofovir Disoproxil Fumarate	300
emtricitabine	200
Microcrystalline Cellulose	200

-continued

	mg/tablet
Lactose Monohydrate	175
Croscarmellose Sodium	60
Pregelatinized Starch	50
Colloidal silicon dioxide	5
Magnesium Stearate	10
total:	1000

Formulation B:

	mg/tablet
Tenofovir Disoproxil fumarate	300
emtricitabine	100
Microcrystalline Cellulose	200
Lactose Monohydrate	180
Sodium Starch Glycollate	60
Pregelatinized Starch	50
Magnesium Stearate	10
total:	900

Formulation C:

	mg/tablet
Tenofovir Disoproxil fumarate	200
emtricitabine	200
Microcrystalline Cellulose	200
Lactose Monohydrate	180
Sodium Starch Glycollate	60
Pregelatinized Starch	50
Magnesium Stearate	10
total:	900

Formulation D:

	mg/tablet
Tenofovir Disoproxil fumarate	300
emtricitabine	25
Microcrystalline Cellulose	200
Lactose Monohydrate	180
Sodium Starch Glycollate	60
Pregelatinized Starch	50
Magnesium Stearate	10
total:	825

Formulation E:

	mg/tablet
Tenofovir Disoproxil fumarate	200
emtricitabine	25
Microcrystalline Cellulose	200
Lactose Monohydrate	180
Sodium Starch Glycollate	60
Pregelatinized Starch	50
Magnesium Stearate	10
total:	725

Formulation F:

	mg/tablet
Tenofovir Disoproxil fumarate	100
emtricitabine	100
Microcrystalline Cellulose	200
Lactose Monohydrate	180
Sodium Starch Glycollate	60
Pregelatinized Starch	50
Magnesium Stearate	10
total:	700

Formulation G (Controlled Release Formulation):

This formulation is prepared by wet granulation of the ingredients with an aqueous solution, followed by the addition of magnesium stearate and compression.

	mg/tablet
Tenofovir Disoproxil fumarate	300
emtricitabine	200
Hydroxypropyl Methylcellulose	112
Lactose B.P.	53
Pregelatinized Starch B.P.	28
Magnesium Stearate	7
total:	700

Drug release takes place over a period of about 6-8 hours and is complete after 12 hours.

Capsule Formulations

Formulation H:

A capsule formulation is prepared by admixing the ingredients and filling into a two-part hard gelatin or hydroxypropyl methylcellulose capsule.

	mg/capsule
Active Ingredient	500
Microcrystalline Cellulose	143
Sodium Starch Glycollate	25
Magnesium Stearate	2
total:	670

Formulation I (Controlled Release Capsule):

The following controlled release capsule formulation is prepared by extruding ingredients a, b, and c using an extruder, followed by spheronization of the extrudate and drying. The dried pellets are then coated with release-controlling membrane (d) and filled into a two-piece, hard gelatin or hydroxypropyl methylcellulose capsule.

	mg/capsule
(a) Active Ingredient	500
(b) Microcrystalline Cellulose	125
(c) Lactose B.P.	125
(d) Ethyl Cellulose	13
total:	763

Formulation J (Oral Suspension):

The active ingredients are admixed with the ingredients and filling them as dry powder. Purified water is added and mixed well before use.

Active Ingredient	500 mg
Confectioner's Sugar	2000 mg
Simethicone	300 mg
Methylparaben	30 mg
Propylparaben	10 mg
Flavor, Peach	500 mg
Purified Water q.s. to	5.00 ml

Formulation K (Suppository):

One-fifth of the Witepsol H15 is melted in a steam jacketed pan at 45° C. maximum. The active ingredients are sifted through a 200 micron sieve and added to the molten base with mixing, using a Silverson fitted with a cutting head, until a smooth dispersion is achieved. Maintaining the mixture at 45° C., the remaining Witepsol H15 is added to the suspension and stirred to ensure a homogenous mix. The entire suspension is passed through a 250 micron stainless steel screen and, with continuous stirring, is allowed to cool to 40° C. At a temperature of 38° C. to 40° C., 2.02 g of the mixture is filled into suitable, 2 ml plastic molds. The suppositories are allowed to cool to room temperature.

	mg/Suppository
Active Ingredient	500
Hard Fat, B.P. (Witepsol H15-Dynamit Nobel)	1770
total	2270

Fixed Dose Combination Tablet

A fixed dose combination tablet of tenofovir disoproxil fumarate (TDF) 300 mg/emtricitabine 200 mg was formulated using a wet granulation/fluid-bed drying process using conventional methods. See: U.S. Pat. No. 5,935,946; L. Young (editor). Tableting Specification Manual 5th ed., American Pharmaceutical Association, Washington, D.C., (2001); L. Lachman, H. Lieberman (editors). Pharmaceutical Dosage Forms: Tablets (Vol 2), Marcel Dekker Inc., New York, 185-202 (1981); J. T. Fell and J. M. Newton, J. Pharm. Pharmacol. 20, 657-659 (1968); US Pharmacopeia 24-National Formulary 19, "Tablet Friability", Chapter <1216>, Page 2148 (2000).

The effects of granulation water level (ranging from 40% to 50% w/w) and wet massing time were studied on the physicochemical properties of the final powder blend and its performance with respect to blend uniformity and compressibility (tablet compatibility). In addition, content uniformity, assay, stability and dissolution performance was evaluated for the TDF/emtricitabine fixed dose combination tablets.

Formulation Equipment

Equipment included a high shear mixer equipped with a pressure tank and spray nozzle tip to add the granulating water, a fluid-bed dryer, a mill, a tumble blender, a rotary tablet press, and a tablet deduster.

Formulation Process

The dried, milled powder was blended with the extragranular microcrystalline cellulose and croscarmellose sodium and then blended with magnesium stearate. Powder samples were removed after mixing with the magnesium stearate. The blend samples were evaluated for, bulk density, mesh analysis and compressibility. The powder blend mixed with the magnesium stearate was compressed into tablets on a press setup.

Materials

The following Table 1 lists the quantitative composition of the TDF/emtricitabine tablet formulation.

TABLE 1

Ingredient	% w/w	Unit Formula for tablet cores (mg/tablet)	Quantity per 12 kg Batch (kg)
Tenofovir Disoproxil Fumarate[a]	30.0	300.0	3.60
Emtricitabine[a]	20.0	200.0	2.40
Pregelatinized Starch, NF/EP	5.0	50.0	0.60
Croscarmellose Sodium, NF/EP	6.0	60.0	0.72
Lactose Monohydrate, NF/EP[a]	8.0	80.0	0.96
Microcrystalline Cellulose, NF/EP[c]	30.0	300.0	3.60
Magnesium Stearate, NF/EP	1.0	10.0	0.12
Purified Water, USP/EP	[b]	[b]	[b]
Totals	100.0	1000.0	12.00

[a]Actual weight is adjusted based on the Drug Content Factor (DCF) of tenofovir disoproxil fumarate and emtricitabine.
[b]Water removed during drying.

Characterization Equipment

Moisture content was measured by loss on drying using a heat lamp/balance system. The powder blend was sampled with a sampling thief fitted with chambers to determine powder blend uniformity. Duplicate samples were removed from each of several locations in the blender. Blend uniformity analysis was performed on one sample from each location.

Particle size analysis of the final powder blend was determined by sifting a multi-gram sample through a screen using a sonic sifter. The quantity of final powder blend retained on each sieve and the fines collector was determined by calculating the difference in weight between the sieves and fines collector before and after the test. The geometric mean diameter particle size was calculated by logarithmic weighting of the sieved distribution.

Bulk density was determined by filling a graduated cylinder with the final powder blend and measuring the weight differential between the empty and filled graduate cylinder per unit volume.

Tablets were characterized for friability using a friabilator, a hardness tester, a thickness micrometer equipped with a printer, and a weighing balance.

Compression characteristics were determined using a rotary tablet press equipped with a flat-faced, beveled edged punch to a target weight of 400 mg. The powder blends were compressed using target upper punch pressures ranging from approximately 100 to 250 MPa. The apparent normalized ejection force was determined and normalized for tablet thickness and diameter.

Tablet hardness was determined using a hardness tester. Tablet thickness was determined using a micrometer, and tablet weights were determined using a top loading balance.

Wet Granulation

The powders were blended in a granulator and then granulated using water. The impeller and chopper speeds were kept constant in the blender at a low setting during the granulation and wet massing operations. After water addition, the impeller and chopper were stopped and the granulator bowl was opened to observe the granulation consistency and texture. The lid was closed and the wet massing phase was performed. Acceptable granules had 40% w/w and 60% w/w water, respectively.

Wet Milling

To facilitate a uniform drying process, each wet granulation was deagglomerated with a mill fitted with a screen and an impeller. The milled wet granules were charged into a fluid-bed dryer immediately following wet milling.

Fluid-Bed Drying

Milled wet granules were dried using an inlet air setpoint temperature of about 70° C. and airflow of approximately 100 cfm. The target LOD was about 1.0% with a range of not more than (NMT) 1.5%. The total fluid-bed drying time ranged from 53 to 75 minutes. Final LOD ranged from 0.4% to 0.7% for all of the batches dried. The final exhaust temperatures for all the batches ranged from 47° C. to 50° C.

Dry Milling

All dried granules were milled through a perforated screen. The mill was equipped with a square impeller and operated. The lots were milled and manually transferred to the V-blender.

Blending

Each lot was blended using the V-blender. In one set of three formulations, starting with 12 kg materials, final powder blend yield available for compression after blending ranged from 10.5 kg (87.5%) to 11.1 kg (92.5%). The final powder blend bulk density ranged from 0.48 to 0.58 g/cc and the geometric mean diameter particle size ranged from 112 to 221 μm. Percent water and wet massing time affect final powder blend particle size and bulk density.

The powder blending for both tenofovir DF and emtricitabine gave a mean (n=10) strength value for tenofovir DF ranged from 100.6% to 102.8% of target strength for the lots and the relative standard deviation (RSD) was from 0.5% to 1.7%. The mean (n=10) strength value for emtricitabine ranged from 101.3% to 104.1% of target strength for the lots with the relative standard deviation (RSD) ranged from 0.6% to 1.7%. The final powder blend moisture level ranged from 0.8% to 1.1% LOD.

Tablet Compression

The final blends were compressed using a rotary tablet press and the tablets were film-coated.

Three 300 gm formulations (Table 2) were granulated in a granulator equipped with a 1-L bowl. The quantities of intragranular components were based on a 300 g total batch size. The formulations in lots 1 and 2 differed in the amount of microcrystalline cellulose 30% vs. 20% w/w, respectively. Lots 2 and 3 were identical except for the type of binder. Lot 2 contained 5% w/w of pregelatinized starch and lot 3 contained 5% w/w povidone as binder.

TABLE 2

Ingredient	Lot 1 % w/w	Lot 2 % w/w	Lot 3 % w/w
Tenofovir Disoproxil Fumarate	30.0	30.0	30.0
Emtricitabine	20.0	20.0	20.0
Pregelatinized Starch, NF/EP	5.0	5.0	N/A
Povidone, USP/NF (C-30)	N/A	N/A	5.0
Croscarmellose Sodium, NF/EP	6.0	6.0	6.0
Lactose Monohydrate, NF/EP	8.0	18.0	18.0
Microcrystalline Cellulose, NF/EP[a]	30.0	20.0	20.0
Magnesium Stearate, NF/EP	1.0	1.0	1.0
Purified Water, USP/EP	[a]	[a]	[a]
Total	100.0	100.0	100.0

[a]Water removed during drying.

After water addition, the impeller and chopper were stopped and the granulator bowl was opened to observe the granulation consistency and texture. To achieve similar granulation consistency, lots 1, 2, and 3 were granulated with 45%, 40%, and 30% w/w water, respectively. The lid was closed and the wet massing phase was performed. All lots had a 30 sec wet massing resulting in acceptable granulations. The wet granulations from all batches were hand screened through a sieve to deagglomerate. The resulting granulations were tray dried in a convection oven set at 60° C. for approximately 20 hours to an LOD <1.0%. The dried granulations from all batches were hand screened through a sieve. In order to fit the granulation into the small scale (300 mL) V-blender, the final blend batch size was adjusted to 100 g. A portion, 81 g of the resulting blend from Lot 1 was blended with 15 g microcrystalline cellulose, 3 g croscarmellose sodium and 1 g magnesium stearate. 86 g of the resulting granulation from Lot 2 and Lot 3 were each blended with 10 g microcrystalline cellulose, 3 g croscarmellose sodium and 1 g magnesium stearate.

Purity analysis was conducted by reverse-phase HPLC (high performance liquid chromatography). Impurities related to tenofovir disoproxil fumarate and emtricitabine were characterized and measured in the bulk API (active pharmaceutical ingredient) before formulation in the three lots of Table 2, and again after formulation in the resulting tablets. The impurities include by-products from hydrolysis of the exocyclic amino groups of tenofovir disoproxil fumarate and emtricitabine, and the hydrolysis of the disoproxil (POC) esters of tenofovir disoproxil fumarate. In each lot, the sum total of impurities related to tenofovir disoproxil fumarate and emtricitabine was less than 1% after formulation and tablet manufacture.

The physicochemical properties of tenofovir disoproxil fumarate and emtricitabine tablets were evaluated by visual appearance, water content, label strength, impurity and degradation product contents, and tablet dissolution. Stability studies were conducted on drug product packaged in container-closure systems that are identical to the intended clinical and commercial container-closure system. There was no sign of discoloration or tablet cracking during the course of the stability study. Film-coated tenofovir disoproxil fumarate and emtricitabine tablets exhibited satisfactory stability at 40° C./75% RH (relative humidity) for up to six months when packaged and stored with silica gel desiccant. No significant loss (defined as ≥5% degradation) in % label strength of tenofovir DF or emtricitabine was observed after six months at 40° C./75% RH. when packaged and stored with desiccant. The increase in the total degradation products was 1.5% for tenofovir DF and 0.6-0.7% for emtricitabine after six months at 40° C./75% RH when packaged and stored with 3 grams of desiccant.

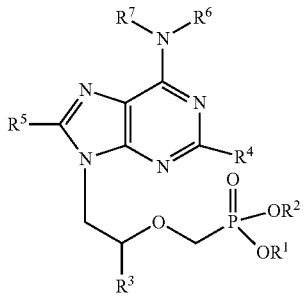
All publications and patent applications cited herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although certain embodiments are described in detail above, those having ordinary skill in the art will clearly understand that many modifications are possible in the

claims without departing from the teachings thereof. All such modifications are intended to be encompassed within the claims of the invention.

Embodiments of the Invention:

A1. A pharmaceutical composition comprising an effective amount of a compound of the formula:



(1)

wherein R^1 and R^2 are independently selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_6-C_{20} arylalkyl, C_6-C_{20} substituted arylalkyl, acyloxymethyl esters $—CH_2C(=O)R^9$ and acyloxymethyl carbonates $—CH_2C(=O)OR^9$ where R^9 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl and C_6-C_{20} substituted aryl;

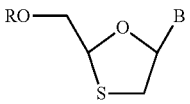
R^3 is selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, or CH_2OR^8 where R^8 is C_1-C_6 alkyl, C_1-C_6 hydroxyalkyl and C_1-C_6 haloalkyl;

R^4 and R^5 are independently selected from H, NH_2, NHR and NR_2 where R is C_1-C_6 alkyl; and

R^6 and R^7 are independently selected from H and C_1-C_6 alkyl;

or a physiologically functional derivative thereof;

in combination with an effective amount of a compound of the formula



(2)

wherein B is selected from adenine, guanine, cytosine, uracil, thymine, 7-deazaadenine, 7-deazaguanine, 7-deaza-8-azaguanine, 7-deaza-8-azaadenine, inosine, nebularine, nitropyrrole, nitroindole, 2-aminopurine, 2-amino-6-chloropurine, 2,6-diaminopurine, hypoxanthine, pseudouridine, 5-fluorocytosine, 5-chlorocytosine, 5-bromocytosine, 5-iodocytosine, pseudocytosine, pseudoisocytosine, 5-propynylcytosine, isocytosine, isoguanine, 7-deazaguanine, 2-thiopyrimidine, 6-thioguanine, 4-thiothymine, 4-thiouracil, O^6-methylguanine, N^6-methyladenine, O^4-methylthymine, 5,6-dihydrothymine, 5,6-dihydrouracil, 4-methylindole, and a pyrazolo[3,4-D]pyrimidine; and

R is selected from H, C_1-C_{18} alkyl, C_1-C_{18} substituted alkyl, C_2-C_{18} alkenyl, C_2-C_{18} substituted alkenyl, C_2-C_{18} alkynyl, C_2-C_{18} substituted alkynyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_2-C_{20} heterocycle, C_2-C_{20} substituted heterocycle, phosphonate, phosphophosphonate, diphosphophosphonate, phosphate, diphosphate, triphosphate, polyethyleneoxy or a physiologically functional derivative thereof; and

a pharmaceutically acceptable carrier.

B2. A composition of embodiment A1 wherein, in formula 1, R^1 and R^2 are independently selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_6-C_{20} arylalkyl, C_6-C_{20} substituted arylalkyl, acyloxymethyl esters $—CH_2C(=O)R^9$ and acyloxymethyl carbonates $—CH_2C(=O)OR^9$ where R^9 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl and C_6-C_{20} substituted aryl; and R^3, R^4, R^5, R^6 and R^7 are independently H or C_1-C_6 alkyl.

C3. A composition of embodiment A1 wherein, in formula 2, B is cytosine or a 5-halocytosine.

D4. A composition of embodiment A1 wherein, in formula 1, R^1 and R^2 are independently selected from H, C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl, C_6-C_{20} substituted aryl, C_6-C_{20} arylalkyl, C_6-C_{20} substituted arylalkyl, acyloxymethyl esters $—CH_2C(=O)R^9$ and acyloxymethyl carbonates $—CH_2C(=O)OR^9$ where R^9 is C_1-C_6 alkyl, C_1-C_6 substituted alkyl, C_6-C_{20} aryl and C_6-C_{20} substituted aryl; and R^3, R^4, R^5, R^6 and R^7 are independently H or C_1-C_6 alkyl; and, in formula 2, B is cytosine or a 5-halocytosine.

E5. A composition of embodiment D 4 wherein, in formula 1, R^1 and R^2 are independently selected from H, acyloxymethyl esters $—CH_2C(=O)R^9$ and acyloxymethyl carbonates $—CH_2C(=O)OR^9$ where R^9 is C_1-C_6 alkyl; and R^3, R^4, R^5, R^6 and R^7 are independently H or C_1-C_6 alkyl; and, in formula 2, B is cytosine or a 5-halocytosine and R is H.

F6. A composition of embodiment E5 wherein, in formula 1, R^1 and R^2 are independently selected from H and $—CH_2C(=O)OCH(CH_3)_2$; R^3 is $—CH_3$; and R^4, R^5, R^6 and R^7 are H; and, in formula 2, B is 5-fluorocytosine and R is H.

G7. A pharmaceutical composition comprising a pharmaceutically effective amount of [2-(6-amino-purin-9-yl)-1-methyl-ethoxymethyl]-phosphonic acid diisopropoxycarbonyloxymethyl ester fumarate (tenofovir disoproxil fumarate) or a physiologically functional derivative thereof and a pharmaceutically effective amount of (2R, 5S)-4-amino-5-fluoro-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (emtricitabine) or a physiologically functional derivative thereof; and a pharmaceutically acceptable carrier.

H8. A pharmaceutical formulation of embodiment A1 to G7 further comprising a third active ingredient selected from the group consisting of a protease inhibitor, a nucleoside or nucleotide reverse transcriptase inhibitor, a non-nucleoside reverse transcriptase inhibitor, and an integrase inhibitor.

I9. A pharmaceutical formulation of embodiments A1 to H8 in unit dosage form.

J10. A method for the treatment or prevention of the symptoms or effects of an HIV infection in an infected animal which comprises administering to said animal a pharmaceutical composition of embodiments claims A1 to I9.

The invention claimed is:

1. A fixed-dose combination comprising 300 mg of tenofovir disoproxil fumarate and 200 mg of emtricitabine wherein the combination exhibits equal to or less than 5% degradation of the tenofovir disoproxil fumarate and emtricitabine after six months at 40° C./75% relative humidity when packaged and stored with silica gel desiccant, and wherein the fixed-dose combination is a tablet.

2. The fixed-dose combination of claim **1** where there is less than 10% degradation of tenofovir disoproxil fumarate over a 24-hour period.

3. The fixed-dose combination of claim 1 where there is less than 1% degradation of tenofovir disoproxil fumarate over a 24-hour period.

4. The fixed-dose combination of claim 1 where there is less than 0.1% degradation of the tenofovir disoproxil fumarate over a 24-hour period.

5. The fixed-dose combination of claim 1 where there is less than 0.01% degradation of the tenofovir disoproxil fumarate over a 24-hour period.

6. The fixed-dose combination of claim 1 comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, pregelatinized starch, croscarmellose sodium, lactose monohydrate, microcrystalline cellulose, magnesium stearate, and colloidal silicon dioxide.

7. The fixed-dose combination of claim 1 comprising less than 1% of impurities related to tenofovir disoproxil fumarate and emtricitabine.

8. The fixed-dose combination of claim 1, further comprising a third anti-viral agent.

9. The fixed-dose combination of claim 8, wherein the third antiviral agent is selected from the group consisting of protease inhibitors, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and integrase inhibitors.

10. The fixed-dose combination of claim 9, wherein the third antiviral agent is efavirenz.

11. A method for the treatment of the symptoms or effects of an HIV infection in an infected animal which comprises administering to said animal the fixed-dose combination of claim 1.

12. A method for the treatment of the symptoms or effects of an HIV infection in an infected animal which comprises administering to said animal the fixed-dose combination of claim 10.

13. A fixed-dose combination comprising 300 mg of tenofovir disoproxil fumarate and 200 mg of emtricitabine wherein the combination exhibits less than 10% degradation of tenofovir disoproxil fumarate over a 24-hour period.

14. The fixed-dose combination of claim 13, wherein there is less than 1% degradation of tenofovir disoproxil fumarate.

15. The fixed-dose combination of claim 13, wherein there is less than 0.1% degradation of tenofovir disoproxil fumarate.

16. The fixed-dose combination of claim 13, wherein there is less than 0.01% degradation of tenofovir disoproxil fumarate.

17. The fixed-dose combination of claim 13 wherein the combination exhibits equal to or less than 5% degradation of the tenofovir disoproxil fumarate and emtricitabine after six months at 40° C./75% relative humidity when packaged and stored with silica gel desiccant.

18. The fixed-dose combination of claim 13 comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, pregelatinized starch, croscarmellose sodium, lactose monohydrate, microcrystalline cellulose, and magnesium stearate.

19. The fixed-dose combination of claim 18 comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, 50 mg pregelatinized starch, 60 mg croscarmellose sodium, 80 mg lactose monohydrate, 300 mg microcrystalline cellulose, and 10 mg magnesium stearate.

20. The fixed-dose combination of claim 18 comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, 50 mg pregelatinized starch, 60 mg croscarmellose sodium, 180 mg lactose monohydrate, 200 mg microcrystalline cellulose, and 10 mg magnesium stearate.

21. The fixed-dose combination of claim 13 comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, pregelatinized starch, croscarmellose sodium, lactose monohydrate, microcrystalline cellulose, and magnesium stearate.

22. The fixed-dose combination of claim 21 comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, 50 mg pregelatinized starch, 60 mg croscarmellose sodium, 80 mg lactose monohydrate, 300 mg microcrystalline cellulose, and 10 mg magnesium stearate.

23. The fixed-dose combination of claim 21 comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, 50 mg pregelatinized starch, 60 mg croscarmellose sodium, 180 mg lactose monohydrate, 200 mg microcrystalline cellulose, and 10 mg magnesium stearate.

24. The fixed-dose combination of claim 13 comprising 300 mg tenofovir disoproxil fumarate, 200 mg emtricitabine, pregelatinized starch, croscarmellose sodium, lactose monohydrate, microcrystalline cellulose, magnesium stearate, and colloidal silicon dioxide.

25. The fixed-dose combination of claim 13 comprising less than 1% of impurities related to tenofovir disoproxil fumarate and emtricitabine.

26. The fixed-dose combination of claim 13, further comprising a third anti-viral agent.

27. The fixed-dose combination of claim 26, wherein the third antiviral agent is selected from the group consisting of protease inhibitors, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and integrase inhibitors.

28. The fixed-dose combination of claim 27, wherein the third antiviral agent is efavirenz.

29. A method for the treatment of the symptoms or effects of an HIV infection in an infected animal which comprises administering to said animal the fixed-dose combination of claim 13.

30. A method for the treatment of the symptoms or effects of an HIV infection in an infected animal which comprises administering to said animal the fixed-dose combination of claim 28.

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