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**UNITED STATES DISTRICT COURT
DISTRICT OF NEW JERSEY**

CELGENE CORPORATION,

Plaintiff,

v.

BARR LABORATORIES, INC.,

Defendant.

Civil Action No. 07-286 (SDW)(MCA)

**Hon. Susan D. Wigenton, U.S.D.J.
Hon. Madeline C. Arleo, U.S.M.J.**

**CELGENE CORPORATION and
CHILDREN'S MEDICAL CENTER
CORPORATION,**

Plaintiffs,

v.

**BARR LABORATORIES, INC. and BARR
PHARMACEUTICALS, INC.,**

Defendants.

Civil Action No. 08-3357 (SDW)(MCA)

**Hon. Susan D. Wigenton, U.S.D.J.
Hon. Madeline C. Arleo, U.S.M.J.**

FIRST AMENDED COMPLAINT

(Filed Electronically)

Plaintiffs Celgene Corporation ("Celgene") and Children's Medical Center Corporation ("CMCC") (collectively, "Plaintiffs"), by their undersigned attorneys, amend their allegations in

this action against defendants, Barr Laboratories, Inc. and Barr Pharmaceuticals, Inc. (collectively, “Barr” or “Defendants”), for patent infringement and allege as follows:

Nature of the Action

1. This is an action for patent infringement under the patent laws of the United States, 35 United States Code, arising from Barr Laboratories, Inc.’s filing of an Abbreviated New Drug Application (“ANDA”) with the United States Food and Drug Administration (“FDA”) seeking approval to commercially market a generic version of Celgene’s Thalomid[®] prior to the expiration of United States Patent Nos. 5,629,327 (the “327 patent”), 6,235,756 (the “756 patent”), and 7,435,745 (the “745 patent”) owned by CMCC and exclusively licensed to Celgene (collectively, “the patents-in-suit”).

The Parties

2. Plaintiff Celgene is a corporation organized and existing under the laws of the State of Delaware, having a principal place of business at 86 Morris Avenue, Summit, New Jersey 07901.

3. Plaintiff CMCC is a Massachusetts not-for-profit corporation, having a principal place of business at 55 Shattuck Street, Boston, Massachusetts 02115. CMCC is the sole member of Children’s Hospital Boston, also a Massachusetts not-for-profit corporation and the primary pediatric teaching hospital of Harvard Medical School.

4. On information and belief, defendant Barr Laboratories, Inc. is a corporation, having its principal place of business at 223 Quaker Road, Pomona, New York 10970.

5. On information and belief, defendant Barr Pharmaceuticals, Inc. is a corporation organized and existing under the laws of the State of Delaware, having a place of business at 400 Chestnut Ridge Road, Woodcliff Lake, New Jersey 07677.

6. On information and belief, defendant Barr Laboratories, Inc. is a subsidiary of defendant Barr Pharmaceuticals, Inc.

7. On information and belief, Barr Laboratories, Inc. and Barr Pharmaceuticals, Inc. are registered to do business in New Jersey. Further, on information and belief, Barr Laboratories, Inc. and Barr Pharmaceuticals, Inc. maintain executive offices and a manufacturing facility and otherwise transact business within this District.

8. On information and belief, the acts of Barr Laboratories, Inc. complained of herein were done at the direction of, with the authorization of, or with the cooperation, participation, or assistance of, or at least in part for the benefit of, Barr Pharmaceuticals, Inc.

9. Barr Laboratories, Inc. and Barr Pharmaceuticals, Inc. are referred to hereinafter, collectively, as “Barr.”

Jurisdiction and Venue

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

11. This Court has personal jurisdiction over Barr by virtue of the fact that Barr has availed itself of the laws of New Jersey and conducts business in New Jersey.

12. Venue is proper in this District pursuant to 28 U.S.C. §§ 1391 and 1400(b).

The Patents in Suit

13. On May 13, 1997, the USPTO duly and lawfully issued the '327 patent, entitled “Methods and Compositions for Inhibition of Angiogenesis” to CMCC as assignee of the inventor Robert D’Amato. A copy of the '327 patent is attached hereto as Exhibit A.

14. On May 22, 2001, the USPTO duly and lawfully issued the '756 patent, entitled “Methods and Compositions for Inhibition of Angiogenesis by Thalidomide” to CMCC as

assignee of the inventor Robert D'Amato. A copy of the '756 patent is attached hereto as Exhibit B.

15. On October 14, 2008, the USPTO duly and lawfully issued the '745 patent, entitled "Methods and Compositions for Inhibition of Angiogenesis," to CMCC as assignee of the inventor Robert D'Amato. A copy of the '745 patent is attached hereto as Exhibit C.

16. Celgene is an exclusive licensee under the '327, '756, and '745 patents.

The THALOMID[®] Drug Product

17. Celgene holds an approved New Drug Application ("NDA") under Section 505(a) of the Federal Food Drug and Cosmetic Act ("FFDCA"), 21 U.S.C. § 355(a) for thalidomide capsules (NDA No. 20-785), which it sells under the trade name THALOMID[®]. The claims of the '327, '756 and '745 patents cover methods of treatment by administering compositions containing thalidomide, either alone or in combination with other compounds.

18. Pursuant to 21 U.S.C. § 355(b)(1) and attendant FDA regulations, the '327, '756, and '745 patents are listed in the FDA publication, "Approved Drug Products with Therapeutic Equivalence Evaluations" (the "Orange Book"), with respect to THALOMID[®].

Acts Giving Rise to this Suit

19. Pursuant to Section 505 of the FFDCA, Barr filed ANDA No. 78-505 and amendments thereto for thalidomide capsules, seeking approval to engage in the commercial use, manufacture, sale, offer for sale or importation of thalidomide capsules 50 mg, 100 mg, and 200 mg ("Barr's Proposed Products"), before the patents-in-suit expire. Barr initially also sought approval to market thalidomide capsules 150 mg, but subsequently withdrew its request to market that dosage strength.

20. In connection with the filing of its ANDA as described in the preceding paragraph, Barr has provided written certifications to the FDA, as called for by Section 505 of

the FDCA, alleging that the claims of the '327 and '756 patents are invalid, unenforceable, and/or will not be infringed by the activities described in Barr's ANDA.

21. On May 21, 2008, Barr amended its ANDA to seek approval to engage in the commercial use, manufacture, sale, offer for sale or importation into the United States of Barr's Proposed Products before the '327 and '756 patents expire. The '327 and '756 patents were listed in the Orange Book prior to Barr's filing of its ANDA. After amending its ANDA, Barr was required to send Celgene and CMCC notification pursuant to 21 U.S.C. § 355(j)(2)(B)(ii).

22. No earlier than May 22, 2008, Barr sent written notice of its ANDA amendment to Celgene and CMCC ("Barr's Supplemental Notice Letter"). Barr's Supplemental Notice Letter alleged that the claims of the '327 and '756 patents are invalid, unenforceable, and/or will not be infringed by the activities described in Barr's ANDA. Barr's Supplemental Notice Letter also informed Celgene and CMCC that Barr seeks approval to market Barr's Proposed Products before the '327 and '756 patents expire.

23. In response, on July 3, 2008, Celgene and CMCC filed a Complaint pursuant to 21 U.S.C. § 355(i)(5)(B)(ii).

24. Shortly after issuance, the '745 patent was listed in the Orange Book with respect to THALOMID[®].

25. Barr subsequently amended its ANDA to seek approval to engage in the commercial use, manufacture, sale, offer for sale or importation into the United States of Barr's Proposed Products before the '745 patent expires. After amending its ANDA, Barr was required to send Celgene notification pursuant to 21 U.S.C. § 355(j)(2)(B)(ii).

26. No earlier than November 7, 2008, Barr sent written notice of its ANDA Amendment to Celgene and CMCC ("Barr's Second Supplemental Notice Letter"). Barr's

Second Supplemental Notice Letter alleged that the claims of the '745 patents are invalid, unenforceable, and/or will not be infringed by the activities described in Barr's ANDA. Barr's Second Supplemental Notice Letter also informed Celgene and CMCC that Barr seeks approval to market Barr's Proposed Products before the '745 patent expires.

27. In response, Plaintiffs hereby amend their original Complaint to assert the '745 patent against Barr, pursuant to 21 U.S.C. § 355(i)(5)(B)(ii).

Count I: Barr's Filing of the ANDA Infringes the '327 Patent

28. Plaintiffs repeat and reallege the allegations of paragraphs 1-27 as though fully set forth herein.

29. Barr's submission and amendment of its ANDA to obtain approval to engage in the commercial use, manufacture, sale, offer for sale, or importation of thalidomide capsules, prior to the expiration of the '327 patent, constitutes infringement of one or more of the claims of that patent under 35 U.S.C. § 271(e)(2)(A).

30. There is a justiciable controversy between the parties hereto as to the infringement of the '327 patent.

31. Unless enjoined by this Court, Barr, upon FDA approval of Barr's ANDA, will infringe the '327 patent by making, using, offering to sell, importing, and selling Barr's Proposed Products in the United States.

32. Celgene and CMCC will be substantially and irreparably damaged and harmed if Barr's infringement of the '327 patent is not enjoined.

33. Celgene and CMCC do not have an adequate remedy at law.

34. This case is an exceptional one, and Celgene and CMCC are entitled to an award of their reasonable attorneys' fees under 35 U.S.C. § 285.

Count II: Barr's Filing of the ANDA Infringes the '756 Patent

35. Plaintiffs repeat and reallege the allegations of paragraphs 1-34 as though fully set forth herein.

36. Barr's submission and amendment of its ANDA to obtain approval to engage in the commercial use, manufacture, sale, offer for sale, or importation of thalidomide capsules, prior to the expiration of the '756 patent, constitutes infringement of one or more of the claims of that patent under 35 U.S.C. § 271(e)(2)(A).

37. There is a justiciable controversy between the parties hereto as to the infringement of the '756 patent.

38. Unless enjoined by this Court, Barr, upon FDA approval of Barr's ANDA, will infringe the '756 patent by making, using, offering to sell, importing, and selling Barr's Proposed Products in the United States.

39. Celgene and CMCC will be substantially and irreparably damaged and harmed if Barr's infringement of the '756 patent is not enjoined.

40. Celgene and CMCC do not have an adequate remedy at law.

41. This case is an exceptional one, and Celgene and CMCC are entitled to an award of their reasonable attorneys' fees under 35 U.S.C. § 285.

Count III: Barr's Filing of the ANDA Infringes the '745 Patent

42. Plaintiffs repeat and reallege the allegations of paragraphs 1-41 as though fully set forth herein.

43. Barr's submission and amendment of its ANDA to obtain approval to engage in the commercial use, manufacture, sale, offer for sale, or importation of thalidomide capsules, prior to the expiration of the '745 patent, constitutes infringement of one or more of the claims of that patent under 35 U.S.C. § 271(e)(2)(A).

44. There is a justiciable controversy between the parties hereto as to the infringement of the '745 patent.

45. Unless enjoined by this Court, Barr, upon FDA approval of Barr's ANDA, will infringe the '745 patent by making, using, offering to sell, importing, and selling Barr's Proposed Products in the United States.

46. Celgene and CMCC will be substantially and irreparably damaged and harmed if Barr's infringement of the '745 patent is not enjoined.

47. Celgene and CMCC do not have an adequate remedy at law.

48. This case is an exceptional one, and Celgene and CMCC are entitled to an award of their reasonable attorneys' fees under 35 U.S.C. § 285.

Count IV: Inducing Infringement

49. Plaintiffs repeat and reallege the allegations of paragraphs 1-48 as though fully set forth herein.

50. Upon information and belief, Barr Pharmaceuticals, Inc. has infringed the '327, '756, and '745 patents under 35 U.S.C. § 271(b) by actively inducing Barr Laboratories, Inc. to infringe the '327, '756 and '745 patents.

PRAYER FOR RELIEF

WHEREFORE, Plaintiffs Celgene and CMCC respectfully request the following relief:

(A) A Judgment be entered that Defendants have infringed the '327, '756, and '745 patents by submitting the aforementioned ANDA and amendments thereto;

(B) A Judgment be entered that Defendants have infringed, and that Defendants' making, using, selling, offering to sell, or importing Barr's Proposed Products will infringe one or more claims of the '327, '756, and '745 patents;

(C) An Order that the effective date of FDA approval of ANDA No. 78-505 be a date which is not earlier than the later of the expiration of the '327, '756, and '745 patents or any later expiration of exclusivity to which Plaintiffs are or become entitled;

(D) Preliminary and permanent injunctions enjoining Defendants and their officers, agents, attorneys and employees, and those acting in privity or concert with them, from making, using, selling, offering to sell, or importing Barr's Proposed Products until after the expiration of the '327, '756, and '745 patents, or any later expiration of exclusivity to which Plaintiffs are or become entitled;

(E) A permanent injunction be issued, pursuant to 35 U.S.C. § 271(e)(4)(B), restraining and enjoining Defendants, their officers, agents, attorneys and employees, and those acting in privity or concert with them, from practicing any methods or compositions as claimed in the '327, '756, and '745 patents, or from actively inducing or contributing to the infringement of the '327, '756, and '745 patents, or any later expiration of exclusivity to which Plaintiffs are or become entitled;

(F) A Declaration that the commercial manufacture, use, importation into the United States, sale, or offer for sale of Barr's Proposed Products will directly infringe or induce and/or contribute to infringement of the '327, '756, and '745 patents or any later expiration of exclusivity to which Plaintiffs are or become entitled;

(G) To the extent that Defendants have committed any acts with respect to the methods or compositions claimed in the '327, '756, and '745 patents, other than those acts expressly exempted by 35 U.S.C. § 271(e)(1), Plaintiffs be awarded damages for such acts;

(H) If Defendants engage in the commercial manufacture, use, importation into the United States, sale, or offer for sale of Barr's Proposed Products prior to the expiration of the

'327, '756, and '745 patents, a Judgment awarding damages to Plaintiffs resulting from such infringement together with interest;

- (I) Attorneys' fees in this action as an exceptional case pursuant to 35 U.S.C. § 285;
- (J) Costs and expenses in this action; and
- (K) Such further and other relief as this Court may deem just and proper.

Dated: December 11, 2008

By: s/ Charles M. Lizza

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EXHIBIT A

United States Patent

[11] Patent Number: **5,629,327**

D'Amato

[45] Date of Patent: **May 13, 1997**

- [54] **METHODS AND COMPOSITIONS FOR INHIBITION OF ANGIOGENESIS**
- [75] Inventor: **Robert D'Amato**, Lancaster, Pa.
- [73] Assignee: **Childrens Hospital Medical Center Corp.**, Boston, Mass.
- [21] Appl. No.: **168,817**
- [22] Filed: **Dec. 15, 1993**

Related U.S. Application Data

- [63] Continuation-in-part of Ser. No. 25,046, Mar. 1, 1993, abandoned.
- [51] Int. Cl.⁶ **A61K 31/445**; A61K 31/16; A61K 31/19
- [52] U.S. Cl. **514/323**; 514/557; 514/625
- [58] Field of Search 514/323, 417, 514/414, 557, 625

References Cited

U.S. PATENT DOCUMENTS

4,552,888	11/1985	Koppel et al.	514/474
4,994,443	2/1991	Folkman et al.	514/56
5,001,116	3/1991	Folkman et al.	514/56
5,021,404	6/1991	Folkman et al.	514/26

OTHER PUBLICATIONS

- D'Amato et al., "Thalidomide is an inhibitor of angiogenesis," *Proc. Natl. Acad. Sci., USA*, vol. 91, pp. 4082-4085, 1994.
- Belaube et al., "Faut-il reabilitier la thalidomide,?" [Should Thalidomide be Rehabilitated?], *Sem. Hôp. Paris*, 59(45):3101-3104 (Dec. 8, 1983); Medline, accession No. 84121350. Translation attached.
- Waters, et al., "Treatment of Ulcerative Colitis with Thalidomide," *British Medical Journal*, p. 792 (Mar. 24, 1979).
- Brem et al., *J. Neurosurg.*, 74: pp. 441-446 (1991).
- Crum et al., *Science*, 230:1375 et seq. (1985).

- Taylor et al., *Nature*, 297:p. 307 (1982).
- Folkman et al., *Science*, 221: p. 719 (1983).
- Sidky et al., *Cancer Res.*, 47: pp. 5155-5161 (1987).
- White et al., *New England J. Med.*, 320: pp. 1197-1200 (1989).
- Eger, K. et al., Synthesis, *Central Nervous System Activity and Teratogenicity of a Homothalidomide*, *Arzneim.-Forsch/Drug Res.* 40 (II). Nr. 10 (1990), pp. 1073-1075.
- Fabro, S. et al., *Teratogenic Activity of Thalidomide and Related Compounds*, *Life Sciences* vol. 3, pp. 987-992, 1964.
- Eriksson, Sven O., *Synthesis and Alkaline Hydrolysis of some N-substituted Phthalimides*, *Acta Pharm. Succica* 10, pp. 63-74 (1973).
- Fickentscher, K., *Stereochemical Properties and Teratogenic Activity of Some Tetrahydrophthalimides*, *Mol. Pharmacology* 13, pp. 133-141. 1977.
- Jonsson, N. Ake, et al. *Chemical Structure and Teratogenic Properties*, *Acta Pharm. Succica* 9, pp. 521-542 (1972).
- Matsuyama, Toshifumi, Cytokines and HIV infection: is AIDS a Tumor Necrosis Factor Disease?, *AIDS* 1991, vol. 5 No. 12, pp. 1405-1417.
- Jonsson, N. Ake, et al. *Chemical Structure and Teratogenic Properties*, *Acta Pharm. Succica* 9, pp. 431-446 (1972).
- Hendler, Sheldon S. et al., *Thalidomide for Autoimmune Disease*, *Medical Hypotheses* 10:pp. 437-443 (1983).
- Jonsson, N. Ake, et al. *Chemical Structure and Teratogenic Properties*, *Acta Pharm. Succica* 9, pp. 543-562 (1972).

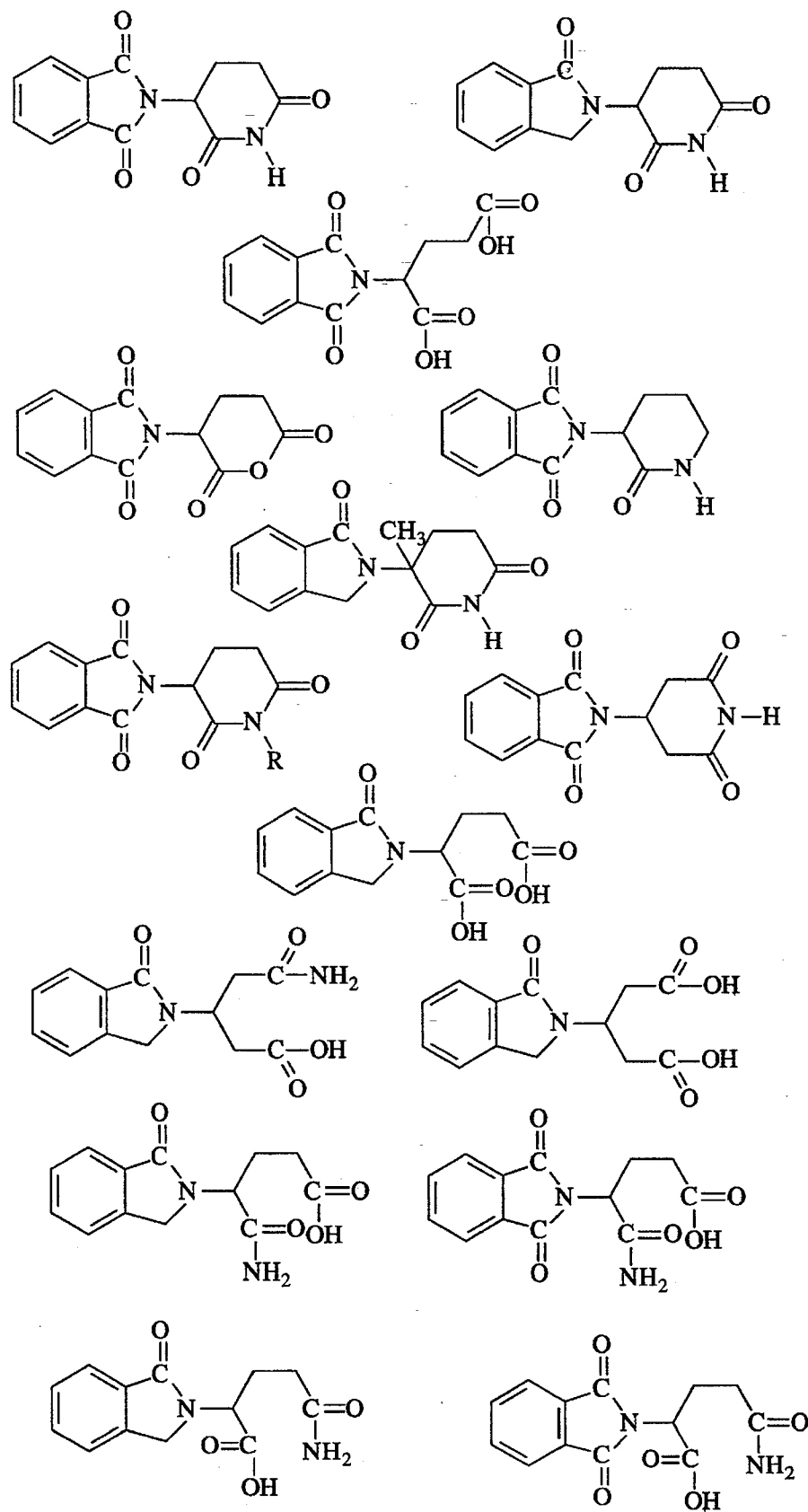
Primary Examiner—José G. Dees
Assistant Examiner—Mary C. Cebulak
Attorney, Agent, or Firm—Jones & Askew

[57] ABSTRACT

The present invention comprises a group of compounds that effectively inhibit angiogenesis. More specifically, thalidomide and various related compounds such as thalidomide precursors, analogs, metabolites and hydrolysis products have been shown to inhibit angiogenesis. Importantly, these compounds can be administered orally.

3 Claims, 5 Drawing Sheets

FIGURE 1



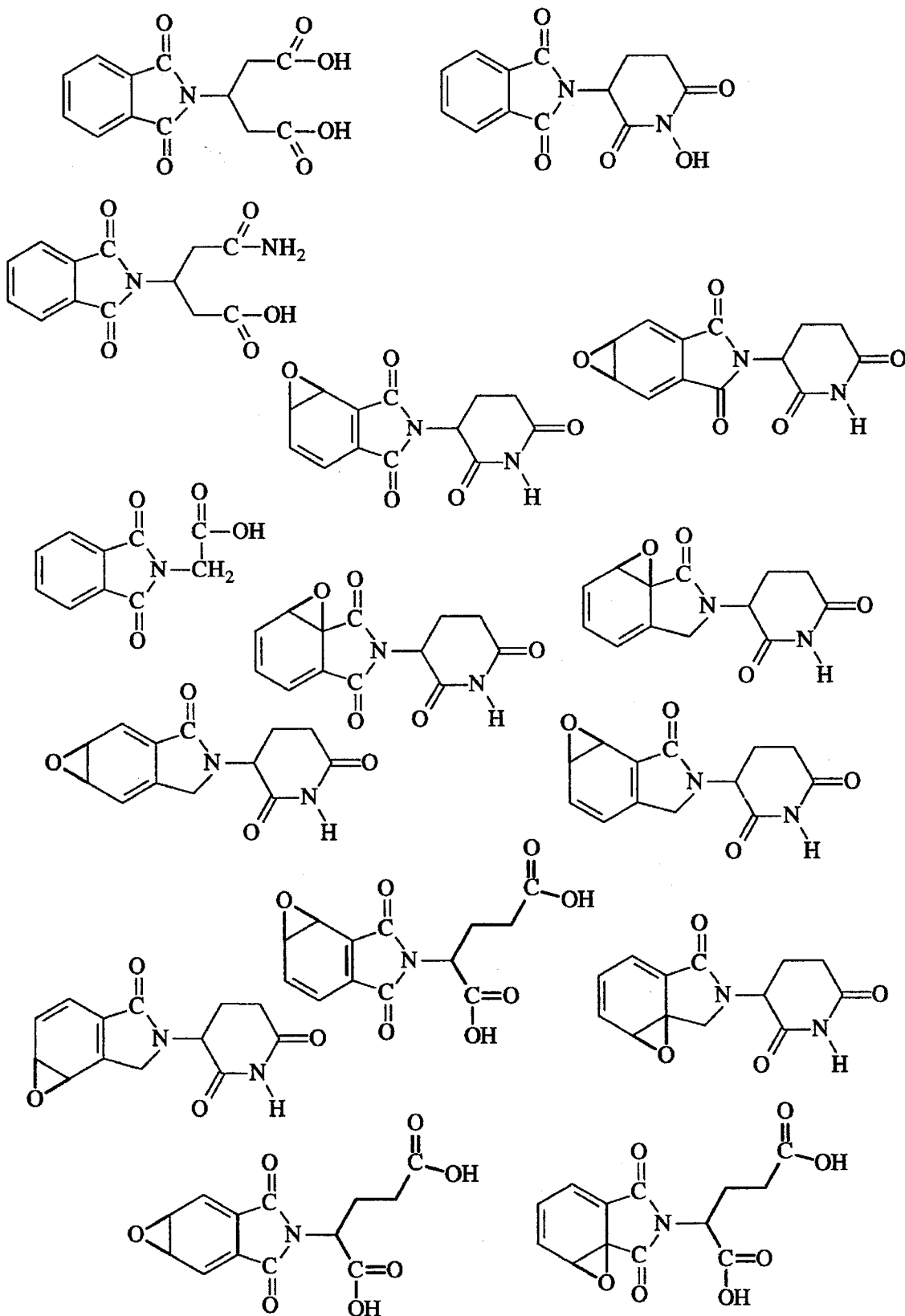


Figure 2

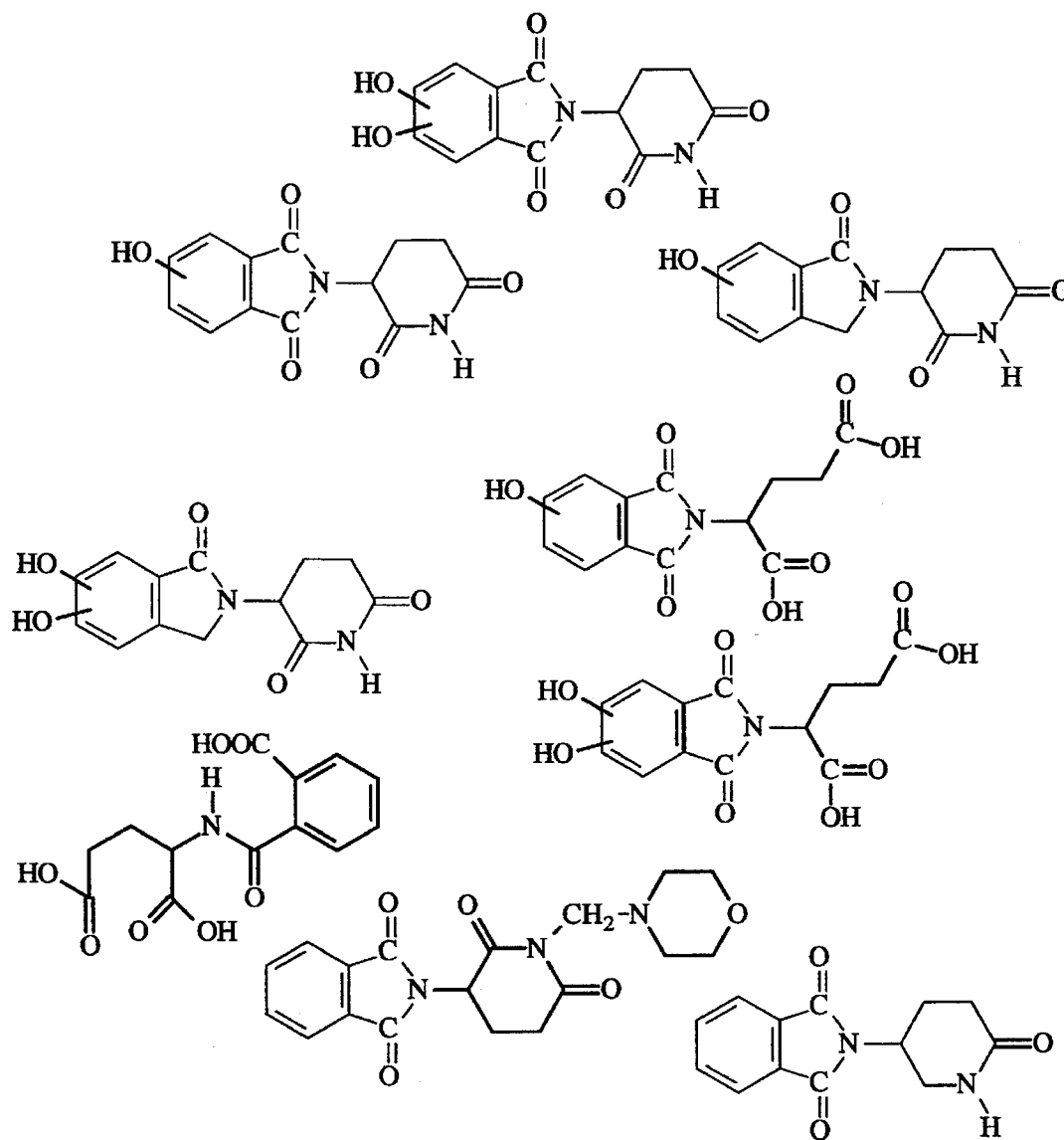


Figure 3

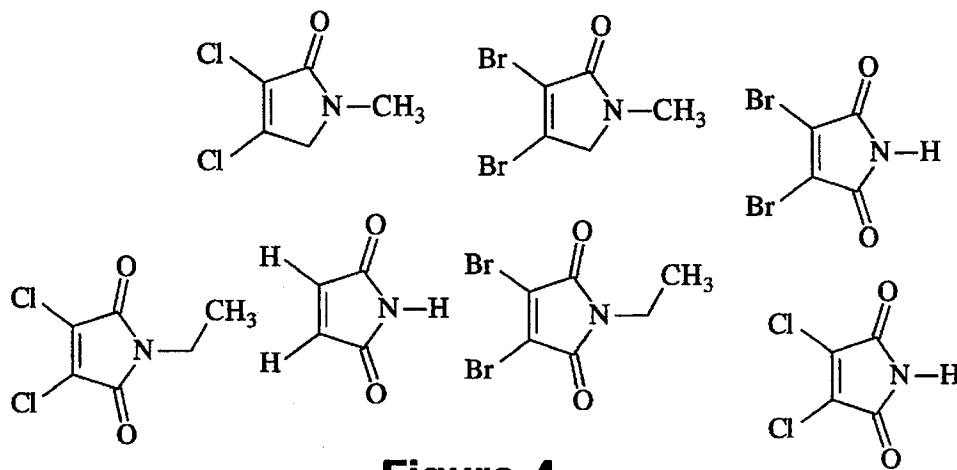


Figure 4

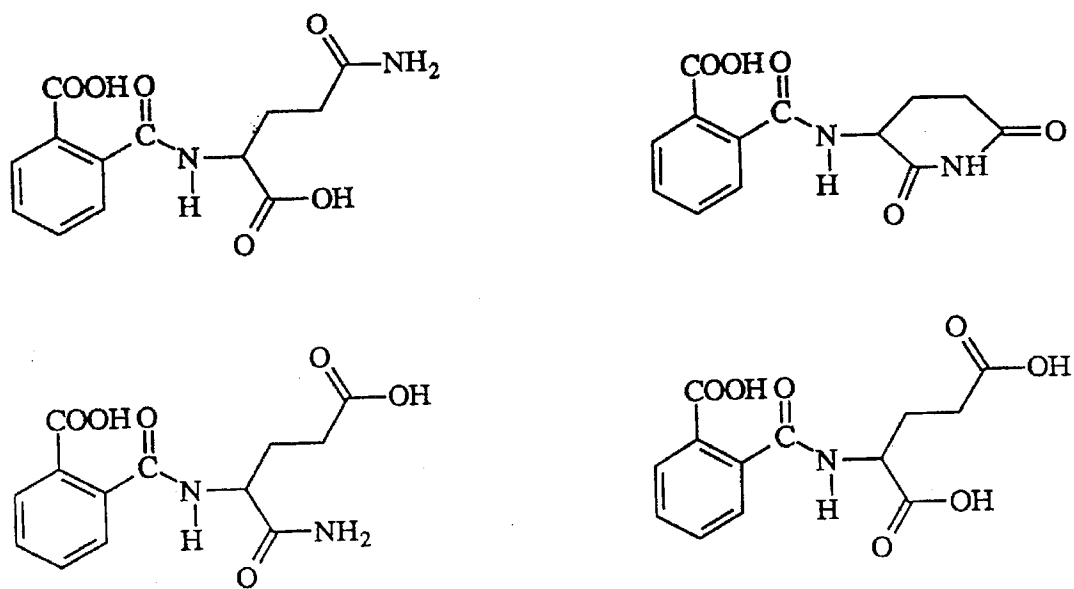


Figure 5

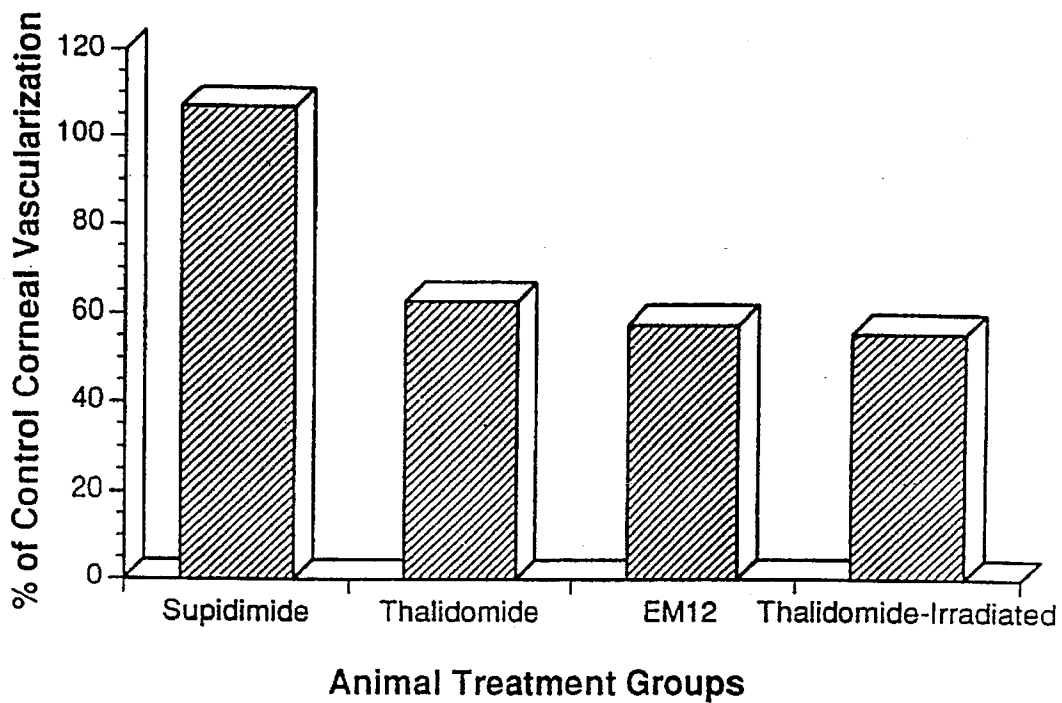


Figure 6

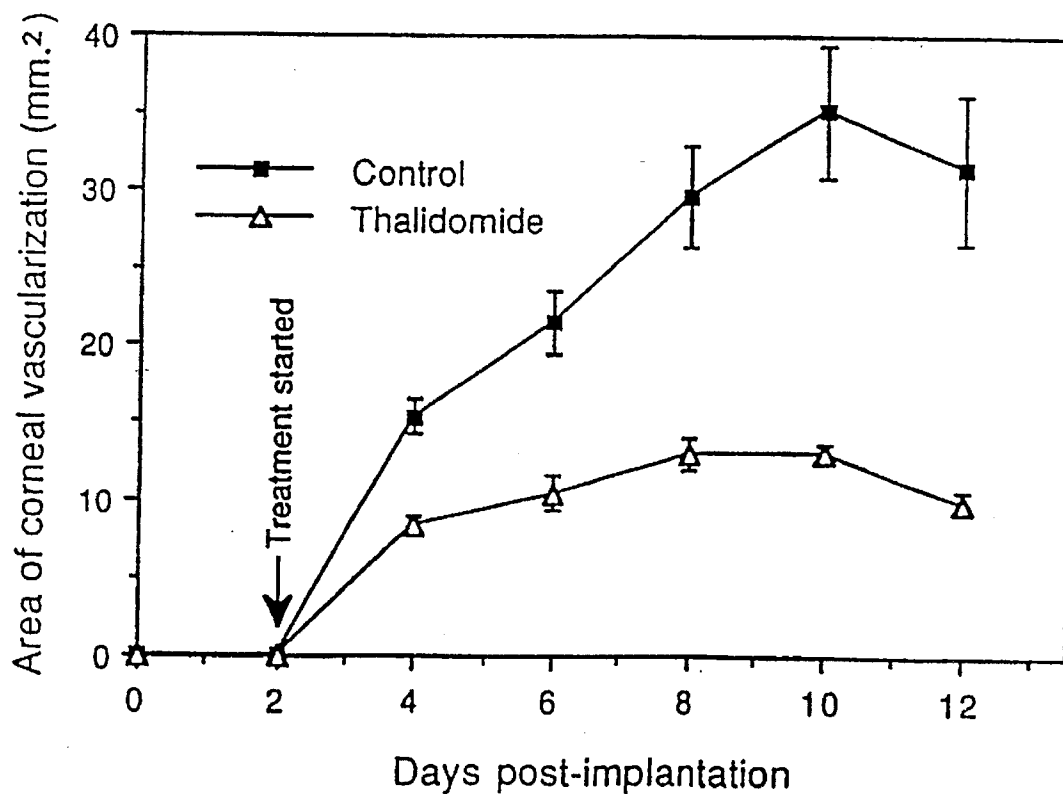


Figure 7

METHODS AND COMPOSITIONS FOR INHIBITION OF ANGIOGENESIS

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of U.S. patent application Ser. No. 08/025,046, filed Mar. 1, 1993, now abandoned.

TECHNICAL FIELD

The present invention relates to methods and compositions for preventing unwanted angiogenesis in a human or animal. More particularly, the present invention relates to a method for preventing unwanted angiogenesis, particularly in angiogenesis dependent or associated diseases, by administration of compounds such as thalidomide and related compounds.

BACKGROUND OF THE INVENTION

As used herein, the term "angiogenesis" means the generation of new blood vessels into a tissue or organ. Under normal physiological conditions, humans or animals only undergo angiogenesis in very specific restricted situations. For example, angiogenesis is normally observed in wound healing, fetal and embryonal development and formation of the corpus luteum, endometrium and placenta. The control of angiogenesis is a highly regulated system of angiogenic stimulators and inhibitors. The control of angiogenesis has been found to be altered in certain disease states and, in many cases, the pathological damage associated with the disease is related to the uncontrolled angiogenesis.

Both controlled and uncontrolled angiogenesis are thought to proceed in a similar manner. Endothelial cells and pericytes, surrounded by a basement membrane, form capillary blood vessels. Angiogenesis begins with the erosion of the basement membrane by enzymes released by endothelial cells and leukocytes. The endothelial cells, which line the lumen of blood vessels, then protrude through the basement membrane. Angiogenic stimulants induce the endothelial cells to migrate through the eroded basement membrane. The migrating cells form a "sprout" off the parent blood vessel, where the endothelial cells undergo mitosis and proliferate. The endothelial sprouts merge with each other to form capillary loops, creating the new blood vessel. In the disease state, prevention of angiogenesis could avert the damage caused by the invasion of the new microvascular system.

Persistent, unregulated angiogenesis occurs in a multiplicity of disease states, tumor metastasis and abnormal growth by endothelial cells and supports the pathological damage seen in these conditions. The diverse pathological states created due to unregulated angiogenesis have been grouped together as angiogenic dependent or angiogenic associated diseases. Therapies directed at control of the angiogenic processes could lead to the abrogation or mitigation of these diseases.

One example of a disease mediated by angiogenesis is ocular neovascular disease. This disease is characterized by invasion of new blood vessels into the structures of the eye such as the retina or cornea. It is the most common cause of blindness and is involved in approximately twenty eye diseases. In age-related macular degeneration, the associated visual problems are caused by an in growth of chorioidal capillaries through defects in Bruch's membrane with proliferation of fibrovascular tissue beneath the retinal pigment

epithelium. Angiogenic damage is also associated with diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, neovascular glaucoma and retrolental fibroplasia. Other diseases associated with corneal neovascularization include, but are not limited to, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sjogrens, acne rosacea, phlyctenulosis, syphilis, Mycobacteria infections, lipid degeneration, chemical burns, bacterial ulcers, fungal ulcers, Herpes simplex infections, Herpes zoster infections, protozoan infections, Kaposi sarcoma, Mooren ulcer, Terrien's marginal degeneration, mariginal keratolysis, rheumatoid arthritis, systemic lupus, polyarteritis, trauma, Wegeners sarcoidosis, Scleritis, Steven's Johnson disease, periphigoid radial keratotomy, and corneal graph rejection.

Diseases associated with retinal/choroidal neovascularization include, but are not limited to, diabetic retinopathy, macular degeneration, sickle cell anemia, sarcoid, syphilis, pseudoxanthoma elasticum, Pagets disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis/vitritis, mycobacterial infections, Lyme's disease, systemic lupus erythematosus, retinopathy of prematurity, Eales disease, Bechets disease, infections causing a retinitis or choroiditis, presumed ocular histoplasmosis, Bests disease, myopia, optic pits, Stargarts disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma and post-laser complications. Other diseases include, but are not limited to, diseases associated with rubeosis (neovasculariation of the angle) and diseases caused by the abnormal proliferation of fibrovascular or fibrous tissue including all forms of proliferative vitreoretinopathy.

Another disease in which angiogenesis is believed to be involved is rheumatoid arthritis. The blood vessels in the synovial lining of the joints undergo angiogenesis. In addition to forming new vascular networks, the endothelial cells release factors and reactive oxygen species that lead to pannus growth and cartilage destruction. The factors involved in angiogenesis may actively contribute to, and help maintain, the chronically inflamed state of rheumatoid arthritis.

Factors associated with angiogenesis may also have a role in osteoarthritis. The activation of the chondrocytes by angiogenic-related factors contributes to the destruction of the joint. At a later stage, the angiogenic factors would promote new bone formation. Therapeutic intervention that prevents the bone destruction could halt the progress of the disease and provide relief for persons suffering with arthritis.

Chronic inflammation may also involve pathological angiogenesis. Such disease states as ulcerative colitis and Crohn's disease show histological changes with the in growth of new blood vessels into the inflamed tissues. Bartonellosis, a bacterial infection found in South America, can result in a chronic stage that is characterized by proliferation of vascular endothelial cells. Another pathological role associated with angiogenesis is found in atherosclerosis. The plaques formed within the lumen of blood vessels have been shown to have angiogenic stimulatory activity.

One of the most frequent angiogenic diseases of childhood is the hemangioma. In most cases, the tumors are benign and regress without intervention. In more severe cases, the tumors progress to large cavernous and infiltrative forms and create clinical complications. Systemic forms of hemangiomas, the hemangiomatoses, have a high mortality rate. Therapy-resistant hemangiomas exist that cannot be treated with therapeutics currently in use.

Angiogenesis is also responsible for damage found in hereditary diseases such as Osler-Weber-Rendu disease, or hereditary hemorrhagic telangiectasia. This is an inherited disease characterized by multiple small angiomas, tumors of blood or lymph vessels. The angiomas are found in the skin and mucous membranes, often accompanied by epistaxis (nosebleeds) or gastrointestinal bleeding and sometimes with pulmonary or hepatic arteriovenous fistula.

Angiogenesis is prominent in solid tumor formation and metastasis. Angiogenic factors have been found associated with several solid tumors such as rhabdomyosarcomas, retinoblastoma, Ewing sarcoma, neuroblastoma, and osteosarcoma. A tumor cannot expand without a blood supply to provide nutrients and remove cellular wastes. Tumors in which angiogenesis is important include solid tumors, and benign tumors such as acoustic neuroma, neurofibroma, trachoma and pyogenic granulomas. Prevention of angiogenesis could halt the growth of these tumors and the resultant damage to the animal due to the presence of the tumor.

It should be noted that angiogenesis has been associated with blood-born tumors such as leukemias, any of various acute or chronic neoplastic diseases of the bone marrow in which unrestrained proliferation of white blood cells occurs, usually accompanied by anemia, impaired blood clotting, and enlargement of the lymph nodes, liver, and spleen. It is believed that angiogenesis plays a role in the abnormalities in the bone marrow that give rise to leukemia-like tumors.

Angiogenesis is important in two stages of tumor metastasis. The first stage where angiogenesis stimulation is important is in the vascularization of the tumor which allows tumor cells to enter the blood stream and to circulate throughout the body. After the tumor cells have left the primary site, and have settled into the secondary, metastasis site, angiogenesis must occur before the new tumor can grow and expand. Therefore, prevention of angiogenesis could lead to the prevention of metastasis of tumors and possibly contain the neoplastic growth at the primary site.

Knowledge of the role of angiogenesis in the maintenance and metastasis of tumors has led to a prognostic indicator for breast cancer. The amount of neovascularization found in the primary tumor was determined by counting the microvessel density in the area of the most intense neovascularization in invasive breast carcinoma. A high level of microvessel density was found to correlate with tumor recurrence. Control of angiogenesis by therapeutic means could possibly lead to cessation of the recurrence of the tumors.

Angiogenesis is also involved in normal physiological processes such as reproduction and wound healing. Angiogenesis is an important step in ovulation and also in implantation of the blastula after fertilization. Prevention of angiogenesis could be used to induce amenorrhea, to block ovulation or to prevent implantation by the blastula.

In wound healing, excessive repair or fibroplasia can be a detrimental side effect of surgical procedures and may be caused or exacerbated by angiogenesis. Adhesions are a frequent complication of surgery and lead to problems such as small bowel obstruction.

Several kinds of compounds have been used to prevent angiogenesis. Taylor et al. have used protamine to inhibit angiogenesis, see Taylor et al., *Nature* 297:307 (1982). The toxicity of protamine limits its practical use as a therapeutic. Folkman et al. have disclosed the use of heparin and steroids to control angiogenesis. See Folkman et al., *Science* 221:719 (1983) and U.S. Pat. Nos. 5,001,116 and 4,994,443. Steroids, such as tetrahydrocortisol, which lack glucocorticoid activity, have been found to be angiogenic inhibitors.

Other factors found endogenously in animals, such as a 4 kDa glycoprotein from bovine vitreous humor and a cartilage derived factor, have been used to inhibit angiogenesis. Cellular factors such as interferon inhibit angiogenesis. For example, interferon α or human interferon β has been shown to inhibit tumor-induced angiogenesis in mouse dermis stimulated by human neoplastic cells. Interferon β is also a potent inhibitor of angiogenesis induced by allogeneic spleen cells. See Sidky et al., *Cancer Research* 47:5155-5161 (1987). Human recombinant α interferon (alpha/A) was reported to be successfully used in the treatment of pulmonary hemangiomas, an angiogenesis-induced disease. See White et al., *New England J. Med.* 320:1197-1200 (1989).

Other agents which have been used to inhibit angiogenesis include ascorbic acid ethers and related compounds. See Japanese Kokai Tokkyo Koho No. 58-131978. Sulfated polysaccharide DS 4152 also shows angiogenic inhibition. See Japanese Kokai Tokkyo Koho No. 63-119500. A fungal product, fumagillin, is a potent angiostatic agent in vitro. The compound is toxic in vivo, but a synthetic derivative, AGM 12470, has been used in vivo to treat collagen II arthritis. Fumagillin and O-substituted fumagillin derivatives are disclosed in EPO Publication Nos. 0325199A2 and 0357061A1.

PCT Application No. WO 92/14455 to Kaplan et al. is directed to a method for controlling abnormal concentration of TNF- α by administering thalidomide or thalidomide derivatives to a patient with toxic concentrations of TNF- α .

The above compounds are either topical or injectable therapeutics. Therefore, there are drawbacks to their use as a general angiogenic inhibitor and lack adequate potency. For example, in prevention of excessive wound healing, surgery on internal body organs involves incisions in various structures contained within the body cavities. These wounds are not accessible to local applications of angiogenic inhibitors. Local delivery systems also involve frequent dressings which are impracticable for internal wounds, and increase the risk of infection or damage to delicate granulation tissue for surface wounds.

Thus, a method and composition are needed that are capable of inhibiting angiogenesis and which are easily administered. A simple and efficacious method of treatment would be through the oral route. If an angiogenic inhibitor could be given by an oral route, the many kinds of diseases discussed above, and other angiogenic dependent pathologies, could be treated easily. The optimal dosage could be distributed in a form that the patient could self-administer.

SUMMARY OF THE INVENTION

In accordance with the present invention, compositions and methods are provided that are effective in inhibiting unwanted angiogenesis. These compositions are easily administered by different routes including oral and can be given in dosages that are safe and provide angiogenic inhibition at internal sites. The present invention provides a method of treating mammalian diseases mediated by undesired and uncontrolled angiogenesis by administering a composition comprising an anti-angiogenic compound in a dosage sufficient to inhibit angiogenesis.

The present invention also includes angiogenic inhibiting compounds that contain an epoxide group. These angiogenic inhibiting compounds can be administered to a human or animal alone or with epoxide hydrolase inhibiting compounds.

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The present invention is especially useful for treating certain ocular neovascular diseases such as macular degeneration. The compounds which are contemplated as part of the present invention preferably can be given orally to the patient and thereby halt the progression of the disease. Other disease that can be treated using the present invention are diabetic retinopathy, neovascular glaucoma and retrolental fibroplasia.

Accordingly, it is an object of the present invention to provide a compound and method to inhibit unwanted angiogenesis in a human or animal.

It is yet another object of the present invention to provide a composition of inhibiting angiogenesis by oral administration of the composition.

It is another object of the present invention to provide a treatment for diseases mediated by angiogenesis.

It is yet another object of the present invention to provide a treatment for macular degeneration.

It is yet another object of the present invention to provide a treatment for all forms of proliferative vitreoretinopathy including those forms not associated with diabetes.

It is yet another object of the present invention to provide a treatment for solid tumors.

It is yet another object of the present invention to provide a method and composition for the treatment of blood-born tumors such as leukemia.

It is another object of the present invention to provide a method and composition for the treatment of hemangioma.

It is another object of the present invention to provide a method and composition for the treatment of retrolental fibroplasia.

It is another object of the present invention to provide a method and composition for the treatment of psoriasis.

It is another object of the present invention to provide a method and composition for the treatment of Kaposi's sarcoma.

It is another object of the present invention to provide a method and composition for the treatment of Crohn's diseases.

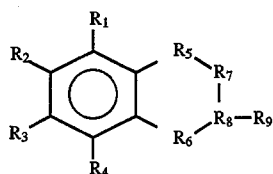
It is another object of the present invention to provide a method and composition for the treatment of diabetic retinopathy.

Other features and advantages of the invention will be apparent from the following description of preferred embodiments thereof.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiments and the appended claims.

BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1 through 3 are a listing of representative compounds in the genus represented by the following general formulas:



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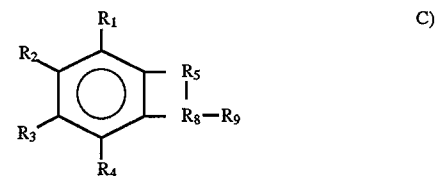
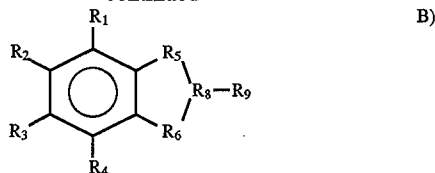


FIG. 4 is a listing of representative compounds in the genus represented by the following general formula:

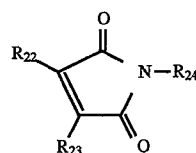


FIG. 5 is a listing of representative compounds in the genus represented by the following general formula:

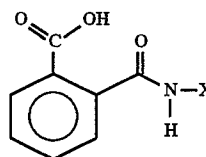


FIG. 6 shows the effect of thalidomide and EM12 on angiogenesis in a rabbit cornea model of angiogenesis.

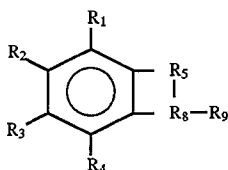
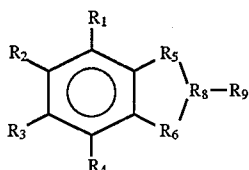
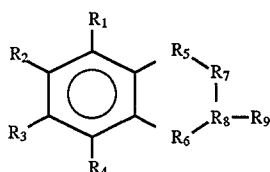
FIG. 7 shows the effect of thalidomide on the area of corneal vascularization in a rabbit cornea model of angiogenesis.

DETAILED DESCRIPTION

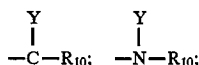
The present invention includes compositions and methods for the treatment of diseases that are mediated by angiogenesis. One embodiment of the present invention is the use of thalidomide or the metabolites of thalidomide as disclosed herein to inhibit unwanted angiogenesis. The present invention also includes compounds which cause dysmelia in the developing fetus and have anti-angiogenic activity. The present invention comprises a method of treating undesired angiogenesis in a human or animal comprising the steps of administering to the human or animal with the undesired angiogenesis a composition comprising an effective amount of a teratogenic compound that is anti-angiogenic.

Compounds that can be used in accordance with the present invention include compounds included in the following general formulae. Examples of compounds that have anti-angiogenic properties having one of the following three formulae (A), (B), or (C):

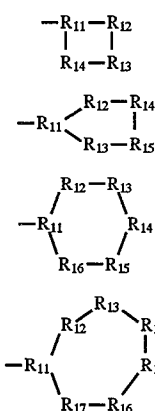
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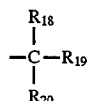
In the above formulae A), B), and C), R_1 , R_2 , R_3 and R_4 can be selected from: —H; —OH; =O, straight chained and branched alkanes, alkenes, alkynes; cyclic alkanes, alkenes, and alkynes; combinations of cyclic and acyclic alkanes, alkenes, and alkynes; alcohol, aldehyde, ketone, carboxylic acid, ester, or ether moieties in combination with acyclic, cyclic, or combination acyclic/cyclic moieties; aza; amino; — XO_n or —O— XO_n , [where $X=N$ and $n=2$; $X=S$ and $n=2$ or 3; or $X=P$ and $n=1-3$]; and halogens; R_5 , R_6 , R_7 , and R_8 are each independently selected from:



or —O— where Y is optional and is the same as defined above for R_1 ; and R_{10} is the same as defined above for R_1 , or (where Y is absent) R_{10} is =O; and R_9 is a moiety having formula D), E), F), G) or H):



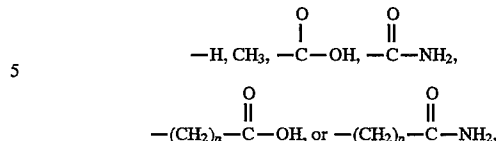
where each of $R_{11}-R_{17}$ is (independently) the same as defined above for R_5 ;



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where R_{18} , R_{19} and R_{20} are, independently selected from

A)



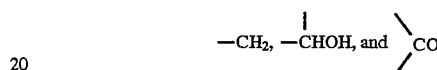
B)

and $n=1$ to 4.

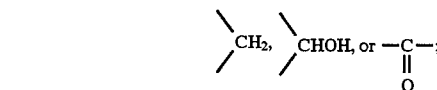
Accordingly, another aspect of the present invention features inhibiting angiogenesis in a mammal by administering a therapeutic composition comprising one of the above-described compounds in a dosage sufficient to inhibit angiogenesis

C)

In preferred embodiments, the compound has formula B), where R_5 and R_6 are selected from the group consisting of:



and R_9 has formula F) or H); and R_{14} and R_{16} are selected from the group consisting of:



and R_{15} and is —O—, or



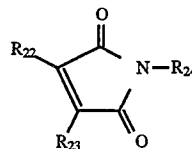
where R_{21} is —H, — CH_3 , or —OH. Specific preferred compounds according to this aspect of the present invention include thalidomide, its precursors, metabolites and analogs. Particular analogs include EM-12, N-phthaloyl-DL-glutamic acid (PGA) or N-phthaloyl-DL-glutamine anhydride. Examples of compounds that are members of this genus are listed in FIGS. 1 through 3. It is to be understood that the compounds included as part of the present invention are not to be limited to those compounds shown in FIGS. 1 through 3 and include all other compounds that are members of the genus described by the general formulas herein.

D)

E)

Compounds of the following formula that have anti-angiogenic properties:

F) 50



G) 55

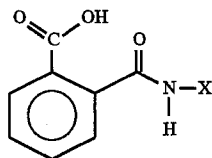
where R_{22} and R_{23} are (independently), —H, —F, —Cl, —Br, —I, — CH_3 , or — CH_2-CH_3 ; and R_{24} is —H, — CH_3 , or — CH_2-CH_3 .

H)

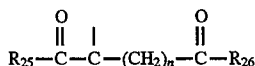
The present invention also features inhibiting angiogenesis in a mammal by administering a compound according to the above formulae in a dosage sufficient to inhibit angiogenesis. Examples of specific compounds that are members of this genus are listed in FIG. 4.

Angiogenesis inhibition hydrolysis products of thalidomide having the following general formula can be used in practicing the present invention:

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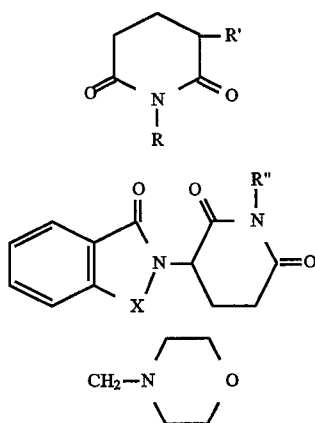


where X is R₆ as defined above, or



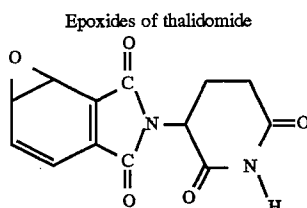
and R₂₅ and R₂₆ are, independently, —OH, —H, or NH₂, and n=1 through 4. Examples of such compounds are shown in FIG. 5.

Angiogenesis inhibition compounds having the following general formula can be used in practicing the present invention:



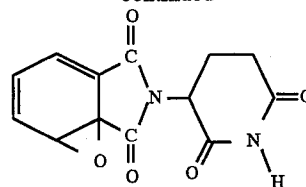
wherein compounds of structure (I), wherein R is selected from the group consisting of hydrogen, alkyl radicals of 1 to 6 carbon atoms, the phenyl radical, and the benzyl radical; and wherein R' is selected from the group consisting of the phthalimido radical and the succinimido radical and of structure (II), wherein X is CH₂ or C=O; R'' is H, —CH₂CH₃, —C₆H₅, —CH₂C₆H₅, —CH₂CH=CH₂, or (a) and hydrolysis products of the compounds wherein R'' is H and the piperidino ring or both the piperidino and the imido ring are hydrolyzed.

Another set of compounds that are considered part of the present invention are the epoxides of thalidomide, EM-12 and EM-138. Representative epoxide compounds are shown as follows:



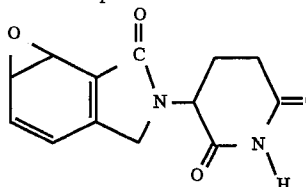
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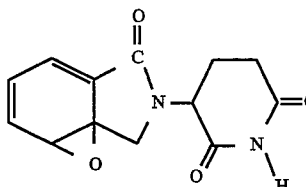
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Epoxides of EM 12



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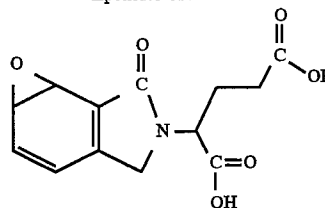
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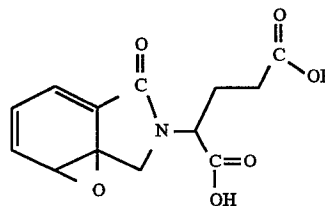
(I) 25

Epoxides of EM 138



(II) 30

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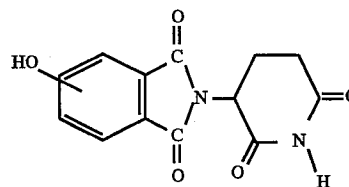


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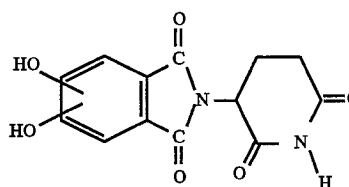
(a)

It should be understood that the epoxide can be attached at the 6,1 site on the benzene ring, the 1,2 site, the 2,3 site 3,4 or the 4,5 site. All of these compounds are contemplated as part of the present invention.

The epoxides of the thalidomide, EM-12, and EM138 can be hydrolyzed to the following compounds:



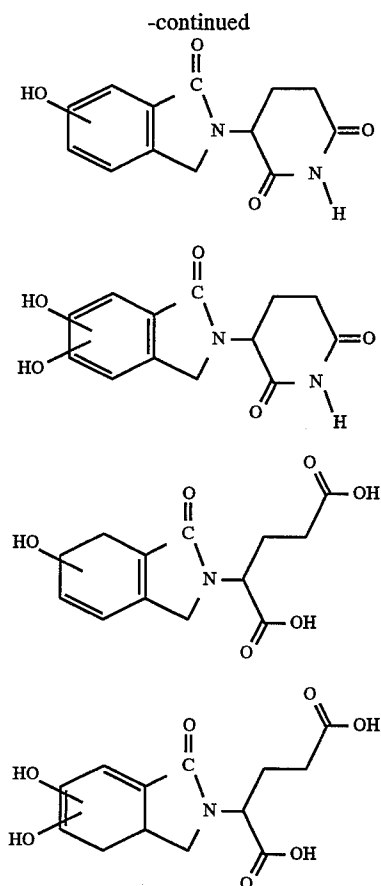
55



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65

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It is to be understood that the hydroxyl group can be on carbons 1, 2, 3, 4, 5 and 6 of the benzene ring. Also contemplated as part of the present invention are dihydroxyl compounds wherein the two hydroxyl groups are located bis to each other on carbons 1, 2, 3, 5 and 6 of the above compounds. The epoxides, the hydrolysis products of the epoxides, and the hydrolysis products of the thalidomide are all contemplated to be part of the present invention.

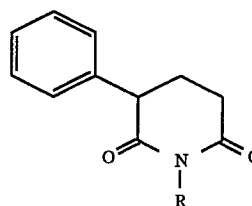
It is known that epoxides are hydrolyzed by a group of enzymes known as epoxide hydrolases. There is a class of compounds which are epoxide hydrolase inhibitors. Examples of these compounds are valpromide (2-propylpentanamide) and valproic acid (2-propylpentanoic acid). Because epoxides are important angiogenesis inhibitors, it is contemplated as part of the present invention, compositions comprising any of the angiogenesis inhibitors compounds recited herein in combination with epoxide hydrolase inhibitors. The epoxide hydrolase inhibitors can be administered to a human or animal together or sequentially. The epoxide group appears to be an important substituent common to several angiogenesis inhibitors. The use of epoxide hydrolase inhibitors to potentiate the activity of any angiogenesis inhibitor containing an epoxide is contemplated as part of the present invention. For example, the epoxide hydrolase inhibitors can be administered with the following epoxide-containing anti-angiogenesis compounds: AGM 1470, Eponimycin, microbial metabolites of *Scolecobasidiurn arenarium* designated f/2015, fr/111142 and fr/18487. See Oikawa, *Biochem Biophys. Res. Comm.*, Vol. 81:1070 (1971) and Otsuka, *J. Microbial. Biotech.*, Vol 1:163 (1991).

It is contemplated as an embodiment of the present invention the use of the epoxide containing angiogenesis

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inhibitors with or without epoxide hydrolase inhibitors as a treatment for diseases mediated by elevated or toxic levels of TNF- α . TNF- α has been recognized as manifesting a dose dependent toxicity. If present at low levels for a long period of time, TNF- α can result in cachexia. Cachexia is a general weight loss and wasting occurring in the course of some chronic diseases such as cancer, opportunistic infections of AIDS, inflammatory diseases, parasitic diseases, tuberculosis, and high dose IL-2 therapy. The epoxide containing angiogenesis inhibitors, with or without epoxide hydrolase inhibitors, are also effective in treating diseases such as septic shock, leprosy and graph vs. host disease.

Other embodiments are within the present invention. For example, other dysmelia-causing compounds can be used according to the present invention, e.g. 4-methylphthalic acid, pyridoxine, vasopressin, acetazolamide, or a compound having the following formula (where R=H, —OH, or —CH₃):



Other compounds which are teratogens, such as valproic acid (2-propylpentanoic acid), the retinoids, such as cis-retinoic acid, and rifampin may also be used in accordance with the invention.

In summary, the preferred compounds are thalidomide, as well as analogs, hydrolysis products, metabolites and precursors of thalidomide that are teratogenic, and, more specifically, that cause dismelia. However, it is to be understood that it is not necessary for a compound to have both teratogenic activity and angiogenesis inhibiting activity to be considered part of the present invention. Dismelia-causing compounds can be identified by the general procedures of Helm, *Arzneimittelforschung*, 31(i/6):941-949 (1981), in which rabbit pups are examined after exposure to the compound in utero. The compounds can generally be purchased, e.g., from Andralis Pharmaceuticals, Beltsville, Md., or synthesized according to known procedures. It is to be understood that the compounds of the present invention can exist as enantiomers and that the racemic mixture of enantiomers or the isolated enantiomers are all considered as within the scope of the present invention.

Many of the compounds that are contemplated as part of the present invention can be enriched in optically active enantiomers of the compounds specified above. Specifically, Blaschke has reported that the S enantiomers may be disproportionately responsible for the dismelia-producing effect of these compounds. See, generally Blaschke, *Arzneimittelforschung* 29:1640-1642 (1979). The above described articles generally describe procedures to obtain optically active preparations of the compounds of interest. See, e.g. Shealy et al., *Chem. Indus.* 1030 (1965); and Casini et al., *Farraaco Ed. Sci.* 19:563 (1964).

The compounds described above can be provided as pharmaceutically acceptable formulations using formulation methods known to those of ordinary skill in the art. These formulations can be administered by standard routes. In general, the combinations may be administered by the topical, transdermal, oral, rectal or parenteral (e.g., intravenous, subcutaneous or intramuscular) route. In addition, the combinations may be incorporated into biode-

gradable polymers allowing for sustained release of the compound, the polymers being implanted in the vicinity of where drug delivery is desired, for example, at the site of a tumor. The biodegradable polymers and their use are described, for example, in detail in Brem et al., *J. Neurosurg.* 74:441-446 (1991).

The dosage of the compound will depend on the condition being treated, the particular compound, and other clinical factors such as weight and condition of the human or animal and the route of administration of the compound. It is to be understood that the present invention has application for both human and veterinary use. For oral administration to humans, a dosage of between approximately 0.1 to 300 mg/kg/day, preferably between approximately 0.5 and 50 mg/kg/day, and most preferably between approximately 1 to 10 mg/kg/day, is generally sufficient.

The formulations include those suitable for oral, rectal, ophthalmic, (including intravitreal or intracameral) nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intratracheal, and epidural) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by conventional pharmaceutical techniques. Such techniques include the step of bringing into association the active ingredient and the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into associate the active ingredient 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, 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 liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil emulsion and as a bolus, etc.

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 ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface active or dispersing agent. Molded tablets may be made by molding, in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may be optionally coated or scored and may be formulated so as to provide a slow or controlled release of the active ingredient therein.

Formulations suitable for topical administration in the mouth include lozenges comprising the ingredients in a flavored basis, 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 ingredient to be administered in a suitable liquid carrier.

Formulations suitable for topical administration to the skin may be presented as ointments, creams, gels and pastes comprising the ingredient to be administered in a pharmaceutical acceptable carrier. A preferred topical delivery system is a transdermal patch containing the ingredient to be administered.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

Formulations suitable for nasal administration, wherein the carrier is a solid, include a coarse powder having a

particle size, for example, in the range of 20 to 500 microns which is administered in the manner in which snuff is administered, i.e., by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations, wherein the carrier is a liquid, for administration, as for example, a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient.

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 parenteral administration include aqueous and non-aqueous 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. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze-dried (lyophilized) conditions requiring only the addition of the sterile liquid carrier, for example, water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose, as herein above recited, or an appropriate fraction thereof, of the administered ingredient.

It should be understood that in addition to the ingredients, particularly mentioned above, the formulations of the present 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 flavoring agents.

Diseases associated with corneal neovascularization that can be treated according to the present invention include but are not limited to, diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, neovascular glaucoma and retrolental fibroplasia, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sjogrens, acne rosacea, phlyctenulosis, syphilis, Mycobacteria infections, lipid degeneration, chemical burns, bacterial ulcers, fungal ulcers, Herpes simplex infections, Herpes zoster infections, protozoan infections, Kaposi sarcoma, Mooren ulcer, Terrien's marginal degeneration, marginal keratolysis, trauma, rheumatoid arthritis, systemic lupus, polyarteritis, Wegeners sarcoidosis, Scleritis, Steven's Johnson disease, periphigoid radial keratotomy, and corneal graft rejection.

Diseases associated with retinal/choroidal neovascularization that can be treated according to the present invention include, but are not limited to, diabetic retinopathy, macular degeneration, sickle cell anemia, sarcoid, syphilis, pseudoxanthoma elasticum, Pagets disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis/vitritis, mycobacterial infections, Lyme's disease, systemic lupus erythematosus, retinopathy of prematurity, Eales disease, Bechets disease, infections causing a retinitis or choroiditis, presumed ocular histoplasmosis, Bests disease, myopia, optic pits, Stargarts disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma and post-laser complications. Other diseases include, but are not limited to, diseases associated

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with rubeosis (neovasculariation of the angle) and diseases caused by the abnormal proliferation of fibrovascular or fibrous tissue including all forms of proliferative vitreoretinopathy, whether or not associated with diabetes.

Another disease which can be treated according to the present invention is rheumatoid arthritis. It is believed that the blood vessels in the synovial lining of the joints undergo angiogenesis. In addition to forming new vascular networks, the endothelial cells release factors and reactive oxygen species that lead to pannus growth and cartilage destruction. The factors involved in angiogenesis may actively contribute to, and help maintain, the chronically inflamed state of rheumatoid arthritis.

Another disease that can be treated according to the present invention are hemangiomas, Osler-Weber-Rendu disease, or hereditary hemorrhagic telangiectasia, solid or blood borne tumors and acquired immune deficiency syndrome.

This invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

EXAMPLE I

The chick embryo chorioallantoic membrane assay described by Cram et al., *Science* 230:1375 et seq. (1985), is used to identify compounds that do not require further metabolic conversion. See also, U.S. Pat. No. 5,001,116, hereby incorporated by reference, which describes the CAM assay at col. 7 of the patent. Briefly, fertilized chick embryos are removed from their shell on day 3 or 4, and a methylcellulose disc containing the compound is implanted on the chorioallantoic membrane. The embryos are examined 48 hours later and, if a clear vascular zone appears around the methylcellulose disc, the diameter of that zone is measured.

EXAMPLE II

Rabbit cornea angiogenesis assay

Pellets for implantation into rabbit corneas were made by mixing 110 µl of saline containing 12 µg of recombinant bFGF (Takeda Pharmaceuticals-Japan) with 40 mg of sucralfate (Bukh Meditec-Denmark); this suspension was added to 80 µl of 12% hydron (Interferon Sciences) in ethanol. 10 µl aliquots of this mixture was then pipetted onto teflon pegs and allowed to dry producing approximately 17 pellets. A pellet was implanted into corneal micropockets of each eye of an anesthetized female New Zealand white rabbit, 2 mm from the limbus followed by topical application of erythromycin ointment onto the surface of the cornea. The animals were fed daily from 2 days post-implantation by gastric lavage with either drag suspended in 0.5% carboxymethyl cellulose or 0.5% carboxymethyl cellulose alone. Thalidomide was purchased from Andrus Pharmaceutal (Maryland) and the EM-12 and Supidimide were kindly provided by Gmnenthal GMBH (Germany). The animals were examined with a slit lamp every other day in a masked manner by the same corneal specialist. The area of corneal neovascularization was determined by measuring with a reticule the vessel length (L) from the limbus and the number of clock hours (C) of limbus involved. A formula was used to determine the area of a circular band segment:

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$C/12 * 3.1416 [r^2 - (r-L)^2]$ where $r=6$ mm the measured radius of the rabbit cornea. Various mathematical models were utilized to determine the amount of vascularized cornea and this formula was found to provide the most accurate approximation of the area of the band of neovascularization that grows towards the pellet.

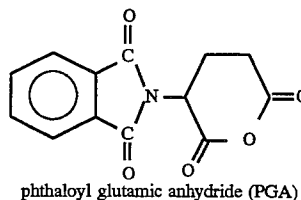
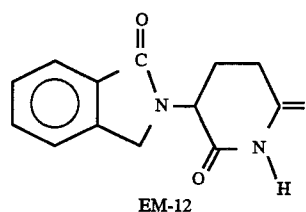
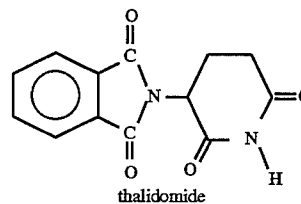
It is important to note that the rabbit cornea assay is preferable because it will generally recognize compounds that are inactive per se but are metabolized to yield active compounds. Thalidomide related compounds, as shown below in Example III, are known to be teratogens and are candidates for use in the present invention.

EXAMPLE III

Inhibition of bFGF induced corneal neovascularization by thalidomide and related analog expressed as percent of median control on day 8

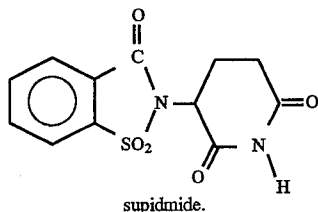
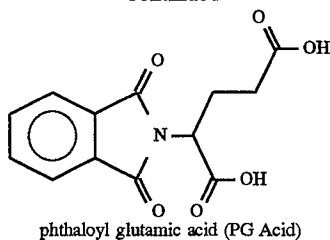
Pellets containing bFGF and sucralfate were implanted into micropockets of both corneas of rabbits according to Example II. Vessel in growth into clear cornea from the limbus was first noted on day 2 and treatments (200 mg/kg orally) were begun on this day. The area of corneal neovascularization was measured from day 4 through day 12. Day 8 measurements were used for comparison between groups. No regression of vessels and near maximal neovascularization was seen at this time point. Statistical analysis was performed with ANOVA with ranked data to account for interexperimental variation and to guard against a non-normal distribution of data (i.e. outliers) by utilizing a nonparametric method.

The compounds tested were as follows:



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-continued



Treatment with a dose of (200 mg/kg) of thalidomide resulted in an inhibition of the area of vascularized cornea that ranged from 30–51% in three experiments with a median inhibition of 36% (FIG. 6) (n=30 eyes, p=0.0001, 2 way ANOVA with ranked data). The inhibition of angiogenesis by thalidomide was seen after only two doses (FIG. 7). The rabbits did not demonstrate obvious sedation and there were no signs of toxicity or weight loss. The teratogenic analog EM-12, which shares the other properties of thalidomide was also inhibitory, with a median inhibition of 42% (n=10 eyes, p=0.002, 1-way ANOVA with ranked data). Supidimide, a nonteratogenic analog of thalidomide that retains the sedative properties of thalidomide, exhibited no activity (area 107% of control, n=10 eyes, not statistically different from control). Other analogs, PGA and PG acid displayed weaker inhibitory effects than thalidomide (data not shown). The density of vessel in growth in thalidomide-treated animals was also markedly reduced.

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EXAMPLE IV

EM-12 in rabbit cornea assay

EM-12 was tested in the rabbit cornea assay described in Example II at 100 mg/kg/day and showed 21% inhibition, and at 200 mg/kg/day the assay showed 43% inhibition.

EXAMPLE V

Phthaloyl glutamic acid in CAM

Phthaloyl glutamic acid was tested in the above described CAM assay and exhibit an vascular zone with a mild scar.

EXAMPLE VI

Phthaloyl glutamic acid in rabbit cornea assay

Phthaloyl glutamic acid described above at 200 mg/kg and exhibited 29% inhibition of angiogenesis.

EXAMPLE VII

Phthaloyl glutamic anhydride in CAM assay

Phthaloyl glutamic anhydride was test in the CAM assay described above and exhibited an vascular zone.

It should be understood, of course, that the foregoing relates only to preferred embodiments of the present invention and that numerous modifications or alterations may be made therein without departing from the spirit and the scope of the invention as set forth in the appended claims.

I claim:

1. A method of treating undesired angiogenesis in a human or animal comprising, administering to the human or animal with the undesired angiogenesis a composition comprising an angiogenesis-inhibiting amount of thalidomide.

2. The method of claim 1, wherein the composition further comprises an epoxide hydrolase inhibitor.

3. The method of claim 1, wherein the undesired angiogenesis is associated with corneal neovascularization.

* * * * *

EXHIBIT B



(12) **United States Patent**
D'Amato

(10) **Patent No.: US 6,235,756 B1**
 (45) **Date of Patent: *May 22, 2001**

(54) **METHODS AND COMPOSITIONS FOR INHIBITION OF ANGIOGENESIS BY THALIDOMIDE**

(75) Inventor: **Robert D'Amato**, Lancaster, PA (US)

(73) Assignee: **The Children's Medical Center Corporation**, Boston, MA (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.: **08/918,610**

(22) Filed: **Aug. 22, 1997**

Related U.S. Application Data

(63) Continuation of application No. 08/468,792, filed on Jun. 6, 1995, now Pat. No. 5,712,291, which is a continuation of application No. 08/168,817, filed on Dec. 15, 1993, now Pat. No. 5,629,327, which is a continuation-in-part of application No. 08/025,046, filed on Mar. 1, 1993, now abandoned.

(51) **Int. Cl.⁷ A61K 31/445**

(52) **U.S. Cl. 514/323**

(58) **Field of Search 514/323**

(56) **References Cited**

U.S. PATENT DOCUMENTS

2,830,991	4/1958	Keller et al.	546/200
3,560,495	2/1971	Frankus et al.	544/130
3,563,986	2/1971	Frankus et al.	544/130
3,625,946	12/1971	Koch et al.	546/200
3,705,162	12/1972	Graudums et al.	546/187
4,552,888	11/1985	Koppel et al.	514/474
4,808,402	2/1989	Leibovich et al.	424/423
4,994,443	2/1991	Folkman et al.	514/56
5,001,116	3/1991	Folkman et al.	514/56
5,021,404	6/1991	Folkman et al.	514/56
5,134,127	7/1992	Stella et al.	514/58
5,260,329	11/1993	Mongelli et al.	514/422
5,399,363	3/1995	Liversidge et al.	424/490
5,434,170	7/1995	Andrulis, Jr.	514/323
5,502,066	3/1996	Heinemann et al.	514/360
5,605,914	2/1997	Muller	514/339
5,635,517	6/1997	Muller et al.	514/323
5,643,915	7/1997	Andrulis, Jr. et al.	514/279
5,654,312	8/1997	Andrulis, Jr. et al.	514/279
5,679,696	10/1997	Fenton et al.	514/354
5,731,325	3/1998	Andrulis, Jr. et al.	514/323

FOREIGN PATENT DOCUMENTS

0325199	7/1989	(EP) .
0357061	3/1990	(EP) .
1182709	3/1970	(GB) .
WO 92/14455	9/1992	(WO) .
WO 92/18496	10/1992	(WO) .

OTHER PUBLICATIONS

Schulze-Osthoff, K. et al, *Am. J. Pathol.* 1990, 137(1), pp. 85-92.*

Allegri, A., "Confermata l'inefficacia della Talidomide Nella Terapia dei Tumori", *Gazzetta Medica Italiana*, pp. 124-127 (1964).

Aronson, et al., "Thalidomide-Induced Peripheral Neuropathy. Effect of Serum Factor on Nerve Cultures", *Arch. Dermatol.*, vol. 120, No. 11, pp. 1466-1470 (1984).

Avalos-Diaz, et al., "Inhibitory Effect on Endocytosis in Polymorphonuclear Cells Caused by Thalidomide", *Arch. Invest. Med.*, vol. 16, No. 2, pp. 139-143.

Bach, et al., "Thalidomide in Cancer Chemotherapy", *The Lancet*, vol. 1, p. 1271 (1963).

Balabanova, et al., "Lupus Erythematosus Hypertrophicus et Profundus", *Z. Hautkr.*, vol. 67, No. 9, pp. 812-815 (1992).

Barnhill, et al., "Thalidomide: Use and Possible Mode of Action in Reactional Lepromatous Leprosy and in Various Other Conditions", *J. Am. Acad. Dermatol.*, vol. 7, No. 3, pp. 317-323 (1982).

Beccerica, E., "L'approccio terapeutico al paziente anziano con artrite reumatoide", *Clin. Ter.*, vol. 122, pp. 289-298 (1987).

Bensinger, W.I., "Supportive Care in Marrow Transplantation", *Curr. Opin. Oncol.*, vol. 4, No. 4, pp. 614-623 (1992).

Bonnetblanc, et al., "Thalidomide and Recurrent Aphthous Stomatitis: a Follow-up Study", *Dermatology*, vol. 193, No. 4, pp. 321-323 (1996).

Boodman, S.G., "Questions About a Popular Prenatal Test", *The Washington Post* (1992).

Bowers, et al., "Effect of Thalidomide on Orogenital Ulceration", *Br. Med. Journ.*, vol. 287, No. 6395, pp. 799-800 (1983).

Braun, et al., "Thalidomide Metabolite Inhibits Tumor Cell Attachment to Concanavalin A Coated Surfaces", *Biochem. Biophys. Res. Comm.*, vol. 98, No. 4, pp. 1029-1034 (1981).

Brodthagen, H., "Significant Response of Oral Aphthosis to Thalidomide Treatment", *J. Am. Acad. Dermatol.*, vol. 13, No. 3, p. 509 (1985).

Brovarone, et al., "Occhio e gravidanza", *Minerva Ginecol.*, vol. 43, pp. 141-167 (1991).

Bubl, et al., "Dysphagia in Dermatologic Disease", *Dysphagia*, vol. 8, No. 2, pp. 85-90 (1993).

Bullock, W.E., "The Clinical Significance of Erythema Nodosum", *Hosp. Pract.*, vol. 21, No. 3, pp. 102E-2H, 102K-2L, 102Q-2R pas (1986).

(List continued on next page.)

Primary Examiner—Evelyn Huang

(74) *Attorney, Agent, or Firm*—Jones & Askew, LLP

(57) **ABSTRACT**

The present invention comprises a group of compounds that effectively inhibit angiogenesis. More specifically, thalidomide and various related compounds such as thalidomide precursors, analogs, metabolites and hydrolysis products have been shown to inhibit angiogenesis. Importantly, these compounds can be administered orally.

24 Claims, 5 Drawing Sheets

OTHER PUBLICATIONS

- Burger, et al., "Epidermolysis Bullosa Acquisita, a Rare Late Complication of Allogeneic Bone Marrow Transplantation?", *Bone Marrow Transplant*, vol. 9, No. 2, pp. 139–141 (1992).
- Calnan, et al., "Actinic Prurigo (Hutchinson's Summer Prurigo)", *Clin. Exp. Dermatol.*, vol. 2, No. 4, pp. 365–372 (1977).
- Cant, J.S., "Minor Ocular Abnormalities Associated With Thalidomide", *The Lancet*, p. 1134 (1966).
- Carmichael, et al., "Thalidomide: A Restricted Role", *Lancet*, vol. 339, No. 8805, p. 1362 (1992).
- Chapon, et al., "Neuropathies Caused by Thalidomide", *Rev. Neurol.*, vol. 141, No. 11, pp. 719–728 (1985).
- Chosidow, O, et al., "Sclerodermatous Chronic Graft–Versus–Host–Disease: Analysis of Seven Cases", *J. Am. Acad. Dermatol.*, vol. 26, No. 1, pp. 49–55 (1992).
- Claydon, et al., "Gastrointestinal Emergencies in HIV Infection", *Balliere's Clin Gastroenterol.*, vol. 5, No. 4, pp. 887–911 (1991).
- Clemmensen, et al., "Thalidomide Neurotoxicity", *Arch. Dermatol.*, vol. 120, No. 3, pp. 338–341 (1984).
- Congy, et al., "Plasma Zinc Levels in Elderly Patients Hospitalized in Long Stay Units. Correlations with Other Nutritional Markers, Immunological Tests and Survival", *Sem. Hop. Paris*, vol. 59, No. 45, pp. 3105–3108 (1983).
- Costa, et al., "Aseptic Adenitis in a Patient with Pyoderma Gangrenosum", *Ann. Dermatol.*, vol. 121, No. 8, pp. 550–552 (1994).
- Crawford, C.L., "Letter: Thalidomide in Erythema Nodosum Leprosum", *Lancet*, vol. 2, No. 839, pp. 1201–1202 (1973).
- Crawford, C.L., "Treatment of Erythema Nodosum Leprosum with Thalidomide", *Lancet*, vol. 2, No. 828, pp. 567–568 (1973).
- Crawford, C.L., "Use of Thalidomide in Leprosy [letter; comment]", *BMJ*, vol. 302, No. 6729, pp. 1603–1604 (1991).
- Current Bibliographics in Medicine, "Thalidomide: Potential Benefits and Risks", *National Inst. Health, National Library of Medicine*, pp. 1–72 (Jan. 1963–Jul. 1997).
- D'Amato, et al., "Angiogenesis Inhibition in Age-Related Macular Degeneration", *Ophthalmology*, vol. 102, No. 9, pp. 1261–1262 (1995).
- D'Amato, et al., "Microscopic Analysis of Retinal–Vessels Utilizing Fluorescein–Labeled High–Molecular–Weight Dextrans", *Invest. Ophthalmol. & Visual Science*, vol. 33, No. 4, p. 1082 (1992).
- Dark, et al., "Combretastin A–4, an Agent that Displays Potent and Selective Toxicity Toward Tumor Vasculature", *Cancer Research*, vol. 37, pp. 1829–1834 (1997).
- David–Bajar, K.M., "Subacute Cutaneous Lupus Erythematosus", *J. Invest. Dermatol.* vol. 100, No. 1, pp. 2s–8s (1993).
- DeKlerk, et al., "New Methods of Treatment for Renal Allografts Using the Baboon as a Primate Experimental Model", *J. Urol.* vol. 102, No. 5, pp. 532–540 (1969).
- Dhodapkar, et al., "A Phase II Pilot Study of Anti–Angiogenesis Therapy Using Thalidomide in Patients with Multiple Myeloma", *UARK 98–003*, pp. 1–15.
- DiPaolo, et al., "Thalidomide: Effects on Ehrlich Ascites Tumor Cells in vitro", *Science*, vol. 144, pp. 1583 (1964).
- DiPaolo, "Effect of Thalidomide on a Variety of Transplantable Tumors", *Cancer Chemo. Reports*, vol. 29, pp. 99–102 (1963).
- DiPaolo, "In Vitro Test Systems for Cancer Chemotherapy. II. Correlation of In Vitro Inhibition of Dehydrogenase and Growth with In Vivo Inhibition of Ehrlich Ascites Tumor", *P.S.E.B.M.*, vol. 114, pp. 384–387 (1963).
- DiPaolo et al., "Teratogenesis–oncogenesis: A Study of Possible Relationships", *Arch. Pathol.*, vol. 81, pp. 3–23 (1966).
- Derouaux, et al., "Le Traitement Actuel du Lupus Erythémateux Chronique", *Le Concours Med.* vol. 106, No. 31, pp. 2957–2961 (1984).
- Doutre, et al., "Pyoderma Gangrenosum and Hemopathies. A propos of 2 Cases", *Nouv Rev Fr Hematol*, vol. 29, No. 4, pp. 251–254 (1987).
- Dunn, et al., "Bone Marrow Transplantation and Cataract Development", *Arch. Ophthalmol.* vol. 111, No. 10, pp. 1367–1373 (1993).
- (Editorial) "Thalidomide in Dermatology and Leprosy", *Lancet*, vol. 2, No. 8446, pp. 80–81 (1985).
- (Editorial) "Thalidomide Tested for Treatment of AIDS", *U.S. Pharm.*, vol. 18, No. 8, p. 14 (1993).
- Elia, et al., "Giant Esophageal Ulcer Treated with Steroids in AIDS Patient(2)", *J. Acquired Immune Defic. Syndr.*, vol. 5, No. 8, pp. 848–849 (1992).
- Eriksson, et al., "Drug Exposure and Flow Cytometry Analyses in a Thalidomide Treatment Schedule that Prolongs Rat Cardiac Graft Survival", *Transplant Proc.*, vol. 24, No. 6, pp. 2560–2561 (1992).
- Fajardo, et al., "Dual Role of Tumor Necrosis Factor– α in Angiogenesis", *Am. J. Pathol.*, vol. 140, No. 3, pp. 539–544 (1992).
- Faure, et al., "PMN Leukocytes Chemotaxis: Inhibition by Thalidomide", *Pathol. Biol. (Paris)*, vol. 29, No. 10, pp. 601–604 (1981).
- Faure, et al., "Inhibition of PMN Leukocytes Chemotaxis by Thalidomide", *Arch. Dermatol. Res.*, vol. 269, No. 3, pp. 275–280 (1980).
- Francois, J., "Embryological Pigment Epithelial Dystrophies", *Ophthalmologica*, vol. 172, pp. 417–433 (1976).
- Fuller, et al., "Thalidomide, Peripheral Neuropathy and AIDS", *Int. J. STD AIDS*, vol. 2, No. 5, pp. 369–370 (1991).
- Furner, B.B., "Treatment of Subacute Cutaneous Lupus Erythematosus", *Int. J. Dermatol.*, vol. 29, No. 8, pp. 542–547 (1990).
- Gad, et al., "Thalidomide Induces Imbalances in T–Lymphocytes Sub–Populations in the Circulating Blood of Healthy Males", *Lepr. Rev.*, vol. 56, No. 1, pp. 35–39 (1985).
- Gaetani, M., "Studi Sull'Attività Antitumorale Della Thalidomide", *Giornale Italiano di Chemioterapia*, pp. 83–86.
- Gehanno, et al., "Mouth and Pharyngeal Hyperalgesic Syndromes in AIDS", *Ann. Otolaryngol Chir. Cervicofac.*, vol. 107, No. 5, pp. 311–313 (1990).
- Genovo, et al., "Treatment of Aphthosis with Thalidomide and with Colchicine", *Dermatologica*, vol. 168, No. 4, pp. 182–188 (1984).
- Geoghiou, et al., "HIV–Associated Oesophageal Ulcers Treated with Thalidomide", *Med. J. of Australia*, vol. 152, pp. 382–383 (1990).
- Gershbein, "Effect of Transplanted Tumor and Various Agents on Liver Regeneration During Pregnancy", *P.S.E.B.M.*, vol. 126, pp. 88–92 (1967).

- Goihman-Yahr, M., et al., "Autoimmune Diseases and Thalidomide II. Adjuvant Disease, Experimental Allergic Encephalomyelitis and Experimental Allergic Neuritis of the Rat", *Int. J. Leprosy*, vol. 42, No. 3, pp. 266-275 (1974).
- Gorin, I., et al., "Thalidomide May Cure AIDS Ulcers", *Nurs. Times*, vol. 86, No. 24, p. 10 (1990).
- Gorin, I., et al., "Thalidomide in Hyperalergic Pharyngeal Ulceration of AIDS", *Lancet*, vol. 335, p. 1343 (1990).
- Goulden, V., et al., "Linear Prurigo Simulating Dermatitis Artefacta in Dominant Dystrophic epidermolysis Bullosa", *Br. J. Dermatol.*, vol. 129, No. 4, pp. 443-446 (1993).
- Grinspan, et al., "Treatment of Aphthae with Thalidomide", *J. Am. Acad. Dermatol.*, vol. 20, No. 6, pp. 1060-1063 (1989).
- Grosshans, E., et al., "Thalidomide Therapy for Inflammatory Dermatoses", *Int. J. Dermatol.*, vol. 23, No. 9, pp. 598-602 (1984).
- Guidetti, E., et al., "Ricerche Sull'azione Immunodepressiva Della Thalidomide e del Prednisolone in Ratti Portatori Di Neoplasie Sperimentalmente Indotte", *Cancro*, vol. 22, pp. 503-512 (1969).
- Gunzler, V., "Thalidomide in Human Immunodeficiency Virus (HIV) Patients. A Review of Safety Considerations", *Drug Saf.*, vol. 7, No. 2, pp. 116-134 (1992).
- Hamza, M.H., "Treatment of Behcet's Disease with Thalidomide", *Clinical Rheumatol.*, vol. 5, No. 3, pp. 365-371 (1986).
- Harindra, et al., "Papulo-Pruritic Eruption and Giant Ulceration of the Mouth: A Difficult Clinical Feature to Treat in the Patient Infected with Human Immunodeficiency Virus(1)", *Arch. Intern. Med.*, vol. 152, No. 9, p. 1924 (1992).
- Hasper, M.F., "Chronic Cutaneous Lupus Erythematosus. Thalidomide Treatment of 11 Patients", *Arch. Dermatol.*, vol. 19, No. 10, pp. 812-815 (1983).
- Hasper, M.F., et al., "Thalidomide in the Treatment of Chronic Discoid Lupus", *Acta. Derm. Venereol.*, vol. 62, No. 4, pp. 321-324 (1982).
- Hastings, R.C., et al., "Thalidomide in the Treatment of Erythema Nodosum Leprosum. With a Note on Selected Laboratory Abnormalities in Erythema Nodosum Leprosum", *Clin. Pharmacol. Ther.*, vol. 11, No. 4, pp. 481-487 (1970).
- Heaton, et al., "Graft-versus-Host Disease Following Liver Transplantation", *J. R. Soc. Med.*, vol. 85, No. 6, pp. 313-314 (1992).
- Heney, D., et al., "Thalidomide in the Treatment of Graft-versus-Host Disease", *Biomed. Pharmacother.*, vol. 44, No. 4, pp. 199-204 (1990).
- Heney, D., et al., "Thalidomide for Chronic Graft-versus-Host Disease in Children", *Lancet*, vol. 2, No. 8623, p. 1317 (1988).
- Hojyo, et al., "Actinic Prurigo (9)", *Int. J. Dermatol.*, vol. 31, No. 5, pp. 372-373 (1992).
- Holm, et al., "Chronic Cutaneous Lupus Erythematosus Treated with Thalidomide", *Arch. Dermatol.*, vol. 129, No. 12, pp. 1548-1550 (1993).
- Hu, D.E., "Inhibition of Angiogenesis in Rats by IL-1 Receptor Antagonist and Selected Cytokine Antibodies", *Inflammation*, vol. 18, pp. 45-58 (1994).
- Ingber, D., "Drug News and Trial Developments", *AIDS Patient Care*, vol. 6, No. 6, p. 288 (1992).
- Randall, T., "Thalidomide's Back in the News, but in more Favorable Circumstances", *JAMA*, vol. 263, No. 11, pp. 1467-1468 (1990).
- Jacobson, et al., "The Diagnosis and Treatment of Leprosy", *South Med. J.*, vol. 69, No. 8, pp. 979-985 (1976).
- Jager, et al., "Clinical Observations in the Treatment of Leprosy Reaction with Cyclic Imides", *Int. J. Lepr. Other Mycobact Dis.*, vol. 39, No. 2, p. 589 (1971).
- Jenkins, et al., "Thalidomide, Orogenital Ulcers, and Risk of Teratogenicity", *The Lancet*, vol. 1, No. 8417-18, p. 511 (1985).
- Jenkins, et al., "Thalidomide in Severe Orogenital Ulceration", *The Lancet*, vol. 2, No. 8417-18, pp. 1424-1426 (1984).
- Jennings, et al., "Effect of Actinomycin D on the Production of Acute Phase Protein in the Rabbit", *Experientia*, vol. 25, pp. 305-306 (1969).
- Jew, et al., "Thalidomide in Erythema Nodosum Leprosum", *DICP*, vol. 24, No. 5, pp. 482-483 (1990).
- Jorizzo, et al., "Thalidomide Effects in Behcet's Syndrome and Pustular Vasculitis", *Arch. Intern. Med.*, vol. 146, No. 5, pp. 878-881 (1986).
- Juret, et al., "Absence d'effet Carcino-Frérateur du Thalidomide vis-à-vis de deux Tumeurs Greffées", *Société de Biologie*, vol. 23, pp. 246-249 (1963).
- Kaitin, K.I., "Graft-versus-Host Disease", *N. Engl. J. Med.*, vol. 325, No. 5, pp. 357-358 (1991).
- Katsuta, et al., "Carcinogenesis in Tissue Culture. 3. Effects of the Second Treatments on DAB-Induced Proliferating Liver Cells of Normal Rats in Culture", *Jpn. J. Exp. Med.*, vol. 35, No. 4, pp. 231-248 (1965).
- Kenyon, et al., "The Discovery of New Inhibitors of Angiogenesis Using an Improved Mouse Corneal Neovascularization Model", No. 459-367, p. S94 (Abstract only).
- Knop, et al., "Thalidomide in the Treatment of Sixty Cases of Chronic Discoid Lupus Erythematosus", *Br. J. Dermatol.*, vol. 108, No. 4, pp. 461-466 (1983).
- Korn, et al., "The Second International Workshop on Scleroderma Research", *Matrix*, vol. 13, No. 5, pp. 427-429 (1993).
- Kroger, et al., "Synergistic Effects of Thalidomide and Poly (ADP-ribose) Polymerase Inhibition on Type II Collagen-Induced Arthritis in Mice", *Inflammation*, vol. 20, No. 2, pp. 203-215 (1996).
- Kundu, et al., "Prurigo Nodularis in an HIV Positive Man (2)", *Genitourinary Medicine*, vol. 71, No. 2, pp. 129-130 (1995).
- Lane, et al., "Treatment of Actinic Prurigo with Intermittent Short-Course Topical 0.05% Clobetasol 17-Propionate. A Preliminary Report", *Arch. Dermatol.*, vol. 126, No. 9, pp. 1211-1213 (1990).
- Languillon, J., "The Effects of Thalidomide on Leprosy Reaction", *Int. J. Lepr. Other Mycobact. Dis.*, vol. 39, No. 2, pp. 590-592 (1971).
- Ledo, E., "Photodermatosis. Part I: Photobiology, Photoimmunology, and Idiopathic photodermatoses", *Int. J. Dermatol.*, vol. 32, No. 6, pp. 387-396 (1993).
- Lehner, T., et al., "Thalidomide, Orogenital Ulcers, and the Risk of Teratogenesis", *The Lancet*, vol. 8423, pp. 288-289 (1985).
- Levy, et al., "Treatment of Erythema Nodosum Leprosum with Thalidomide", *The Lancet*, vol. 2, No. 824, pp. 324-325 (1973).

- Lenicque, "Action of Thalidomide on the Induction of Tentacles in Regenerating Hydra Littoralis", *Acta. Zool.*, pp. 127-139 (1967).
- Lo, et al., "Treatment of Discoid Lupus Erythematosus", *Int. J. Dermatol.*, vol. 28, No. 8, pp. 497-507 (1989).
- Londono, F., "Thalidomide in the Treatment of Actinic Prurigo", *Int. J. Dermatol.*, vol. 12, No. 5, pp. 326-328 (1973).
- Lovell, et al., "Thalidomide in Actinic Prurigo", *Br. J. Dermatol.*, vol. 108, No. 4, pp. 467-471 (1983).
- Magana-Garcia, M., "Antimalarials for Children", *J. Am. Acad. Dermatol.*, vol. 30, No. 3, p. 510 (1994).
- Mascaro, et al., "Thalidomide in the Treatment of Recurrent, Necrotic, and Giant Mucocutaneous Aphthae and Aphthosis", *Arch. Derm.*, vol. 115, pp. 636-637 (1979).
- Matsubara, et al., "Inhibition of Human Endothelial Cell Proliferation by Gold Compounds", *J. Clin. Invest.*, vol. 79, pp. 1440-1446 (1987).
- Mauád, "Melhoras Clínicas Obtidas em Doentes Cancerosos Avançados com Tratamento Pela Talidomida Associada a Hormônios", *Anais Paulistas Medicina e Cirurgia*, pp. 15-39 (1963).
- Maurice, et al., "The Effect of Thalidomide on Arachidonic Acid Metabolism in Human Polymorphonuclear Leukocytes and Platelets", *Br. J. Dermatol.*, vol. 115, No. 6, pp. 677-680 (1986).
- McCarthy, et al., "Thalidomide for the Therapy of Graft-versus-Host Disease Following Allogeneic Bone Marrow Transplantation", *Biomed. Pharmacother.*, vol. 43, No. 9, pp. 693-697 (1989).
- McKenna, et al., "Linear IgA Disease, Oral Ulceration and Crohn's Disease", *Br. J. Dermatol.*, vol. 127, pp. 67-68 (1992).
- Miller, et al., "Zusammentreffen Einer Thalidomid-Induzierten Fehlbildung mit Einem Malignen Lymphom Hohen Malignitätsgrades", *Monatsschr. Kinderheilkd.*, vol. 128, pp. 27-29 (1980).
- Miller, et al., "Treatment of Chronic Erythema Nodosum Leprosum with Cyclosporine A Produces Clinical and Immunohistologic Remission", *Int. J. Lepr. Other Mycobact. Dis.*, vol. 55, No. 3, pp. 441-449 (1987).
- Miyachi, Y., "A Possible Mechanism of Action of Thalidomide on Rheumatoid Arthritis", *Arthritis and Rheuma.*, vol. 28, No. 7, p. 836 (1985).
- Miyachi, et al., "Effects of Thalidomide on the Generation of Oxygen Intermediates by Zymosan-Stimulated Normal Polymorphonuclear Leukocytes", *Arch. Dermatol. Res.*, vol. 274, Nos. 3-4, pp. 363-367 (1982).
- Mohri, et al., "Negative Effect of Thalidomide and Relative Substances on the Growth of HeLa Cells", *Chem. Pharm. Bull.*, vol. 16, pp. 2289-2292 (1968).
- Moncada, et al., "Thalidomide—Effect on T Cell Subsets as a Possible Mechanism of Action", *Int. J. Lepr. Other Mycobact. Dis.*, vol. 53, No. 2, pp. 201-205 (1985).
- Montrucchio, et al., "Tumor Necrosis Factor α -Induced Angiogenesis Depends on In Situ Platelet-Activating Factor Biosynthesis", *J. Exp. Med.*, vol. 180, pp. 377-382 (1994).
- Mshana, et al., "Thymus-Dependent Lymphocytes in Leprosy. II. Effect of Chemotherapy on T-Lymphocyte Subpopulations", *J. Clin. Immunol.*, vol. 2, No. 2, pp. 69-74 (1982).
- Mückter, et al., "Thalidomide and Tumor", *Antimicrobial Agents and Chemotherapy*, pp. 531-538 (1965).
- Munro, et al., "Pyoderma Gangrenosum Associated with Behcet's Syndrome—Response to Thalidomide", *Clin. Exp. Dermatol.*, vol. 13, No. 6, pp. 408-410 (1988).
- Moulin, et al., "Treatment of Jessner-Kanof Disease with Thalidomide", *Ann. Dermatol. Venerol.*, vol. 10, No. 8, pp. 611-614 (1983).
- Naafs, B., Bangkok Workshop on Leprosy Research. Treatment of Reactions and Nerve Damage. *Int. J. Lepr. Other Mycobact. Dis.*, vol. 64, No. 4, Suppl. S21-28 (1996).
- Neubert, "Teratogenicity: Any Relationship to Carcinogenicity?", *Institute for Toxicology and Embryopharmacology, Free University of Berlin, Berlin, Federal Rep. Of Germany*, pp. 169-178.
- Nicolas, J., et al., "Interferon Alfa Therapy in Severe Unresponsive Subacute Cutaneous Lupus Erythematosus", *New Engl. J. Med.*, vol. 321, No. 22, pp. 1550-1551 (1989).
- Nicolau, et al., "Thalidomide: Treatment of Severe Recurrent Aphthous Stomatitis in Patients with AIDS", *DICP*, vol. 24, No. 11, pp. 1054-1056 (1990).
- Nielsen, et al., "Thalidomide Enhances Superoxide Anion Release from Human Polymorphonuclear and Leukocytes", *Acta. Pathol. Microbiol. Immunol. Scand. [C]*, vol. 94, No. 6, pp. 233-237 (1986).
- Olson, et al., "Thalidomide (N-phthaloylglutamide) in the Treatment of Advanced Cancer", *Clin. Pharmacol. Therap.*, vol. 6, No. 3, pp. 292-297 (1965).
- Orzalesi, M., "Il Danno Iatrogeno in Neonatologica", *Ped. Med. Chir.*, vol. 14, pp. 105-112 (1992).
- Ostraat, et al., "Thalidomide Prolongs Graft Survival in Rat Cardiac Transplants", *Transplant Proc.*, vol. 24, No. 6, pp. 2624-2625 (1992).
- Paller, et al., "Proceedings of the Concurrent Sessions", *Pediatr. Dermatol.*, vol. 9, No. 4, pp. 397-406 (1992).
- Pearson, et al., "Treatment of Moderately Severe Erythema Nodosum Leprosum with Thalidomide—A Double-Blind Controlled Trial", *Lepr. Rev.*, vol. 40, No. 2, pp. 111-116 (1969).
- Peyron, et al., "The Pharmacological Basis for the Treatment of Photodermatoses", *Biochimie*, vol. 68, No. 6, pp. 899-904.
- Pfordte, "Über die Beeinflussung des Serumproperdinsystems Durch Verschiedene Arzneimittel", *Pharmazie*, vol. 26, pp. 301-302 (1971).
- Phillips, et al., "Tumor Necrosis Factor Alpha (rhTNF) Fails to Stimulate Angiogenesis in the Rabbit Cornea", *Anatomical Rec.*, vol. 245, pp. 53-56 (1996).
- Proenca, N.G., "Thalidomide: An Eclectic Medication in Dermatology", *Rev. Paul. Med.*, vol. 107, No. 1, pp. 41-46.
- Radeff, et al., "Recurrent Aphthous Ulcer in Patient Infected with Human Immunodeficiency Virus: Successful Treatment with Thalidomide", *J. Am. Acad. Dermatol.*, vol. 23, No. 3, Pt. 1, pp. 523-525 (1990).
- Rajan, et al., "A Clinical Study of Thalidomide Comparing Pre-Treatment and Post-Treatment Reactional Episodes and Corticosteroid Requirements", *Lepr. India*, vol. 55, No. 1, pp. 111-116 (1983).
- Randall, T., "Investigational New Drug (US) 'Orphan' Trials now Use Thalidomide from two Sources", *JAMA*, vol. 263, No. 11, p. 1474 (1990).
- Roe, et al., "Tumour-Incidence in Progeny of Thalidomide-Treated Mice", *British J. Cancer*, pp. 331-333, (1965).
- Ruggenini, et al., "Talidadomide e Tumori Sperimentali", *Cancro*, vol. 20, pp. 39-55 (1967).

- Rustin, et al., "Pyoderma Gangrenosum Associated with Behcet's Disease: Treatment with Thalidomide", *J. Am. Acad. Dermatol.*, vol. 23, No. 5, Pt. 1, pp. 941-944 (1990).
- Ryan, et al., "Thalidomide to Treat Esophageal Ulcer in AIDS (6)", *New Engl. J. Med.*, vol. 327, No. 3, pp. 208-209 (1992).
- Sampaio, et al., "Prolonged Treatment with Recombinant Interferon Gamma Induces Erythema Nodosum Lepromatous Leprosy Patients", *J. Exp. Med.*, vol. 175, No. 6, pp. 1729-1737 (1992).
- Sampaio, et al., "Thalidomide Selectively Inhibits Tumor Necrosis Factor Alpha Production by Stimulated Human Monocytes", *J. Exp. Med.*, vol. 173, No. 3, pp. 699-703 (1991).
- Santis, H.R., "Aphthous Stomatitis and its Management", *Curr. Opin. Dent.*, vol. 1, No. 6, pp. 763-768 (1991).
- Santos, et al., "In Vitro Tumor Necrosis Factor Production by Mononuclear Cells from Lepromatous Leprosy Patients and from Patients with Erythema Nodosumleprosum", *Clin. Immunol. Immunopathol.*, vol. 67, No. 3 I, pp. 199-203 (1993).
- Shannon, et al., "Inhibition of de Novo IgM Antibody Synthesis by Thalidomide as a Relevant Mechanism of Action in Leprosy", *Scand. J. Immunol.*, vol. 13, No. 6, pp. 553-562 (1981).
- Shannon, et al., "Thalidomide's Effectiveness in Erythema Nodosum Leprosum is Associated with a Decrease in CD4+ Cells in the Peripheral Blood", *Lepr. Rev.*, vol. 63, No. 1, pp. 5-11 (1992).
- Sheehan, N.J., "Thalidomide Neurotoxicity and Rheumatoid Arthritis", *Arthritis and Rheuma.*, vol. 29, No. 10, p. 1296 (1986).
- Sheskin, J., "The Treatment of Lepra Reaction in Lepromatous Leprosy. Fifteen Years' Experience with Thalidomide", *Int. J. Dermatol.*, vol. 19, No. 6, pp. 318-322 (1980).
- Sheskin, et al., "In Vivo Measurements of Iron, Copper and Zinc in the Skin of Prurigo Nodularis Patients Treated with Thalidomide", *Dermatologica*, vol. 162, No. 2, pp. 86-90 (1981).
- Silverman, W.A., "Medical Inflation", *Persp. Biol. Med.*, pp. 617-637 (1980).
- Smith, et al., "Studies on the Relationship Between the Chemical Structure and Embryotoxic Activity of Thalidomide and Related Compounds", *Chemical Structure and Embryopathy*, pp. 194-209.
- Style, A., "Early Diagnosis and Treatment of Leprosy in the United States", *American Family Physician*, vol. 52, No. 1, 172-178 (1995).
- Sugiura, et al., "Effect of Thalidomide on Transplantable Mouse, Rat, and Hamster Tumors", *GANN*, vol. 55, pp. 57-60 (1964).
- Suzuki, H., "The History of Iatrogenic Diseases in Japan", *First Department of Internal Medicinie, University of Envir. And Occup. Health*, Kitakyushu, Japan, pp. 35-40.
- Swift, T.R., "Thalidomide in Erythema Nodosum Leprosum", *Lancet*, vol. 2, No. 835, p. 966 (1973).
- Szydlowska, et al., "On the Application of Thalidomide as a Block of Functional Groups of Proteins in Histochemical Investigations", *Folia Histo. Cytochem.*, vol. 16, No. 3, pp. 233-240 (1978).
- Tamura, et al., "Combination Thalidomide and Cyclosporine for Cardiac Allograft Rejection. Comparison with Combination Methylprednisolone and Cyclosporine", *Transplantation*, vol. 49, No. 1, p. 20-25 (1990).
- Theophilus, S., "Treatment with Thalidomide in Steroid Dependency and Neuritis", *Lepr. India*, vol. 52, No. 3, pp. 423-428 (1980).
- Thomas, et al., "Effect of Thalidomide on Liver Regeneration in Rat", *Indian J. Exp. Biol.*, vol. 10, pp. 314-315 (1972).
- Thomas, et al., "Lack of Thalidomide Induced Aplasia in Regenerating Tail of Lizard, *Hemidactylus Flavivirdis*", *Indian J. Exp. Biol.*, vol. 10, pp. 316-317 (1972).
- Traldi, et al., "L'impiego Dell'imide Dell'Acido N'ftalilglutammico (Talidomide) Nella Terapia Sintomatica del Vomito di Molti Pazienti Affeti da Neoplasie Maligne o Causato Dalla Somministrazione di Cloridato di Mecloretamina", *Cancro*, vol. 18, No. 1, pp. 336-341 (1965).
- Trautman, J. R., "Treatment of Hansen's Disease", *Cutis*, vol. 18, No. 1, 62-65 (1976).
- Van den Broek, H., "Treatment of Prurigo Nodularis with Thalidomide", *Arch. Dermatol.*, vol. 116, No. 5, pp. 571-572 (1980).
- Vasilescu, et al., "Cercetari Privind Actiunea Talidomidei Asupra Celulelor Cultivate in Vitro", *Cerc. Fiziol.*, vol. 13, No. 4, pp. 293-300 (1968).
- Villa, et al., "Antimytotic Effect of Thalidomide and its Metabolites on the Chick Embryo Blood Cells", *Haematol. Latina*, vol. 6, pp. 217-221 (1963).
- Villa, et al., "Cytological Effects of Thalidomide", *The Lancet*, pp. 725 (1963).
- Vogelsang, et al., "Therapy of Chronic Graft-v-Host Disease in a Rat Model", *Blood*, vol. 74, No. 1, No. 1, pp. 507-511 (1989).
- Vogelsang, et al., "Thalidomide Induction of Bone Marrow Transplantation Tolerance", *Transplant Proc.*, vol. 19, No. 1, Pt. 3, pp. 2658-2661 (1987).
- Vogelsang, et al., "Treatment and Prevention of Acute Graft-versus-Host Disease with Thalidomide in a Rat Model", *Transplantation*, vol. 41, No. 5, pp. 644-647 (1986).
- Waters, M., "Use of Thalidomide in Leprosy", *BMJ*, vol. 303, No. 6800, p. 470 (1991).
- Waters, M.F., "An Internally-Controlled Double Blind Trial of Thalidomide in Severe Erythema Nodosum Leprosum", *Lepr. Rev.*, vol. 42, No. 1, pp. 26-42 (1971).
- Wesolowski, et al., "Effect of Light on a Murine Model of Retinopathy of Prematurity", *Invest. Opthamol. & Visual Science*, vol. 33, No. 4, p. 1281 (Abstract only).
- Williams, et al., "Thalidomide Hypersensitivity in AIDS", *Lancet*, vol. 337, pp. 436-437 (1991).
- Winkelmann, et al., "Thalidomide Treatment of Prurigo Nodularis", *Acta. Derm. Venereol.*, vol. 64, No. 5, pp. 412-417 (1984).
- Wood, et al., "The Potential use of Thalidomide in the Therapy of Graft-versus-Host Disease—A Review of Clinical and Laboratory Information", *Leuk. Res.*, vol. 14, No. 5, pp. 395-399 (1990).
- Woodyatt, P.B., "Thalidomide", *The Lancet*, p. 750. (1962).
- Wulff, et al., "Development of Polyneuropathy During Thalidomide Therapy", *Br. J. Dermatol.*, vol. 112, No. 4, pp. 475-480 (1985).
- Yazici, et al., "Practical Treatment Recommendations for Pharmacotherapy of Behcet's Syndrome", *Drugs*, vol. 42, No. 5, pp. 796-804 (1991).
- Youle, et al., "Treatment of Resistant Aphthous Ulceration with Thalidomide in Patients Positive for HIV Antibody", *BMJ*, vol. 298, No. 6671, p. 432 (1989).

- Youle, et al., "Thalidomide in Hyperalgetic Pharyngeal Ulceration of AIDS", *Lancet*, vol. 335, No. 8705, p. 1591 (1990).
- Young, et al., "Thalidomide Therapy on a Case of Prurigo Nodularis", *Ann. Dermatol.*, vol. 5, No. 2, pp. 117–120 (1993).
- Fazal, N. et al., "Effect of blocking TNF- α on intracellular BCG (*Bacillus Calmette Guerin*) growth in human monocyte-derived macrophages", *FEMS Microbiology Immunology*, vol. 105, pp. 337–346 (1992).
- Louzir, B. et al., "Érythroleucémie chez un patient ayant une maladie de Behcet et traité au long cours par thalidomide", *Annales De Medecine Interne*, vol. 143, pp. 479–480, (1992).
- Hatfill, S.J., et al., "Induction of Morphological Differentiation in the Human Leukemic Cell Line K562 by Exposure to Thalidomide Metabolites", *Leukemia Research*, vol. 15, No. 2/3, pp. 129–136 (1991).
- "Thalidomide: 20 Years On", *The Lancet*, vol. II, No. 8245, pp. 510–511 (1981).
- Revuz, J., "Actualité du Thalidomide", *Annales De Dermatologie*, vol. 117, pp. 313–321 (1990).
- Vladutiu, A., "Another Chance for Thalidomide?", *The Lancet*, vol. I, No. 7444, pp. 981–982 (1966).
- Fabro, S. et al., "The Metabolism of Thalidomide: Some Biological Effects of Thalidomide and its Metabolites", *British Journal of Pharmacology and Chemotherapy*, vol. 25, pp. 352–362 (1965).
- Fabro, S., "Biochemical Basis of Thalidomide Teratogenicity", *The Biochemical Basis of Chemical Teratogenesis*, Chapter 5, pp. 159–178 (1981).
- Eisenbud, L. et al., "Recurrent aphthous stomatitis of the Behcet's type: Successful treatment with thalidomide", *Oral Surgery, Oral Medicine, Oral Pathology*, vol. 64, No. 3, pp. 289–292 (1987).
- Buckley, C. et al., "Pyoderma Gangrenosum with Severe Pharyngeal Ulceration", *Journal of the Royal Society of Medicine*, vol. 83, pp. 290–291 (1990).
- Barnes, P.F. et al., "Tumor Necrosis Factor Production in Patients with Leprosy", *Infection and Immunity*, vol. 60, No. 4, pp. 1441–1446 (1992).
- Barnhill, R.L. et al., "Studies on the anti-inflammatory properties of thalidomide: Effects on polymorphonuclear leukocytes and monocytes", *Journal of the American Academy of Dermatology*, vol. 11, No. 5, Part 1, pp. 814–819 (1984).
- Dicken, C.H., "Malignant Pyoderma", *Journal of the American Academy of Dermatology*, vol. 13, No. 6, pp. 1021–1025 (1985).
- O'Patey, et al., "Thalidomide et colite ulcéreuse dans la maladie de Behcet", *Gastroenterol Clinique Biologique*, vol. 13, pp. 104–110 (1989).
- Naafs, B. et al., "Thalidomide Therapy", *International Journal of Dermatology*, vol. 24, No. 2, pp. 131–134 (1985).
- Blaschke, V.G. et al., "Chromatographische Racemattrennung von Thalidomide and teratogene Wirkung der Enantiomere", *Arzneimittel Forschung Drug Research*, vol. II, No. 10, 1640–1642 (1979).
- Kaplan, G. et al., "TNF α regulation of HIV1: biology and therapy", *Research in Immunology*, vol. 145, No. 8–9, pp. 685–690 (1994).
- Bazzoli, A.S. et al., "The effects of thalidomide and two analogues on the regenerating forelimb of the newt", *Journal of Embryology and Experimental Morphology*, vol. 41, pp. 125–135 (1977).
- Bernal, J.E. et al., "Cellular Immune Effects of Thalidomide in Actinic Prurigo", *International Journal of Dermatology*, vol. 31, No. 8, pp. 599–600 (1992).
- Chaudhry, A. P. et al., "Effects of Prednisolone and Thalidomide on Induced Submandibular Gland Tumors in Hamsters", *Cancer Research*, vol. 26, Part 1, pp. 1884–1886 (1996).
- Gershbein, L. L., "The thalidomide analog, EM 12, enhances 1,2-dimethylhydrazine-induction of rat colon adenocarcinomas", *Cancer Letters*, vol. 60, pp. 129–133 (1991).
- Rainsford, K.D., "Disease-modifying antirheumatic and immunoregulatory agents", *Baillière's Clinical Rheumatology International Practice and Research*, vol. 4, No. 3, pp. 405–433 (1990).
- Hamza, M., "Behcet's disease, palmoplantar pustulosis and HLA-B27 treatment with thalidomide", *Clinical and Experimental Rheumatology*, vol. 8, No. 4, pp. 427 (1990).
- Ehrlich, G.E., "Behcet's Disease: Current Concepts", *Comprehensive Therapy*, vol. 15, No. 1, pp. 27–30 (1989).
- Barriere, H., "Sarcoides Cutanées. Traitement par la thalidomide", *La Presse Médicale*, vol. 15, No. 12, pp. 963 (1983).
- Haffner, M.E., "Studies Involving Orphan Products for Treating/Diagnosing Women's Diseases", *Food and Drug Law Journal*, vol. 48, pp. 205–211 (1993).
- Sherman, et al., "Thalidomide: A Twenty-Five Year Perspective", *Food Drug Cosmetic Law Journal*, vol. 41, pp. 458–466 (1986).
- Burrows, N. P., "Diagnosis and Management of Systemic Lupus Erythematosus Thalidomide Modifies Disease", *British Medical Journal*, vol. 307, No. 6909, pp. 939–940 (Oct. 1993).
- Gutiérrez-Rodríguez, O. "Thalidomide A Promising New Treatment for Rheumatoid Arthritis", *Arthritis and Rheumatism*, vol. 27, No. 10, pp. 1118–1121 (Oct. 1984).
- Gutiérrez-Rodríguez, O., et al., "Treatment of Refractory Rheumatoid Arthritis—The Thalidomide Experience", *Journal of Rheumatology*, vol. 16, No. 2, pp. 1158–1163 (Feb. 1989).
- Williams, K.M., "Enantiomers in Arthritic Disorders", *Pharmacology & Therapeutics*, vol. 46, No. 2, pp. 273–295 (1990).
- De Cock, K. M., "Treatment of Ulcerative Colitis", letter, *British Medical Journal*, vol. 1, p. 1356 (May 1979).
- Larsson, H., Treatment of Severe Colitis in Behcet's Syndrome with Thalidomide (CG-217), *Journal of Internal Medicine*, vol. 228, No. 4, pp. 405–407 (Oct. 1990) [letter].
- Waters, M.F. et al., "Treatment of Ulcerative Colitis with Thalidomide", *British Medical Journal*, vol. 1, No. 6166, p. 792 (1979).
- Crain, E. et al., "The Effect of Thalidomide on Experimental Autoimmune Myasthenia Gravis", *Journal of Autoimmunity*, vol. 2, pp. 197–202 (1989).
- Gunzler, V. "Thalidomide—A Therapy for the immunological Consequences of HIV Infection?", vol. 30, No. 2, pp. 105–109 (Oct. 1989).
- Handley, J. et al., "Chronic Bullous Disease of Childhood and Ulcerative Colitis", *Pediatric Dermatology*, vol. 10, No. 3, pp. 256–258 (Sep. 1993).
- Handley, J. et al., "Chronic Bullous Disease of Childhood and Ulcerative Colitis", *Pediatric Dermatology*, vol. 127, Supplement 40, pp. 67–68 (Jul. 1992).

- Field, E.O. et al., "Effect of Thalidomide on the Graft Versus Host Reaction", *Nature A Weekly Journal of Science*, vol. 211, No. 5055, p. 1308-1310 (Sep. 1966).
- Hellmann, K. et al., "Prolongation of Skin Homograft Survival by Thalidomide", *British Medical Journal*, vol. II, pp. 687-689 (Sep. 1965).
- Heney, D. et al., "Thalidomide Treatment for Chronic Graft-Versus-Host Disease", *British Journal of Haematology*, vol. 78, pp. 23-27 (1991).
- Keenan, R. J. et al., "Immunosuppressive Properties of Thalidomide Inhibition on In Vitro Lymphocyte Proliferation Alone and in Combination with Cyclosporine or FK506¹", *Transplantation*, vol. 52, No. 5, pp. 908-910 (Nov. 1991).
- Lopez, J. et al., "Thalidomide as Therapy for Intestinal Chronic GVHD", *Bone Marrow Transplantation*, vol. 11, No. 3, pp. 251-252 (1993).
- Bach, A. et al., "Studies on the Possible Anti-Neoplastic Effect of Thalidomide", *ACTA Pathologica et Microbiologica Scandinavica*, vol. 59, pp. 491-499 (1963).
- Bahmer, F. A., Therapy of Lymphocytic Infiltration, *Der Hautarzt*, vol. 43, p. 663 (1992).
- Gershbein L. L., "Effect of Various Agents on Liver Regeneration and Walker Tumor Growth in Partially Hepatectomized Rats", *Cancer Research*, vol. 26 Part 1, pp. 1905-1908 (Sep. 1966).
- Roe, F. J. et al., "Thalidomide and Neoplasia", *Nature*, vol. 200, pp. 1016-1017 (Dec. 1963).
- Makonkawkeyoon, S. et al., "Thalidomide Inhibits the Replication of Human Immunodeficiency Virus Type 1", *Proceedings of the National Academy of Sciences*, vol. 90, No. 13, pp. 5974-5978 (Jul. 1993).
- Meza, A.D. et al., "Managing the Gastrointestinal Complications of AIDS", *Drug Therapy*, vol. 23, No. 11, pp. 74-83 (1993).
- Ghigliotti, G. R. et al., "Thalidomide: Treatment of Choice of Aphthous Ulcers in Patients Seropositive for Human Immunodeficiency Virus", *Journal of the American Academy of Dermatology*, vol. 28, No. 2, Part 1, pp. 271-272 (Feb. 1993).
- Grinspan, D., "Significant Response of Oral Aphthosis to Thalidomide Treatment", *American Academy of Dermatology*, vol. 12, No. 1, Part 1, pp. 85-90 (Jan. 1985).
- Kürkcüoğlu, N. et al., "Thalidomide in the Treatment of Recurrent Necrotic Mucocutaneous Aphthae", *British Journal of Dermatology*, vol. 112, No. 5, p. 632 (May 1985).
- Powell, R.J. et al., "Investigation and Treatment of Oro-genital Ulceration; Studies on a Possible Mode of Action of Thalidomide", *British Journal of Dermatology*, vol. 113, Supplement 28, pp. 141-144 (1985).
- Revuz, J. et al., "Crossover Study of Thalidomide vs. Placebo in Severe Recurrent Aphthous Stomatitis", *Archives of Dermatology*, vol. 126, No. 7, pp. 923-927 (Jul. 1990).
- Apt, W., "Effect of Thalidomide on the Course of Experimental Chagas' Disease", *BOI Chili Parasitol*, vol. 20, No. 3, pp. 84-86 (Sep. 1965).
- Vicente, T. et al., "In Vitro Activity of Thalidomide against mycobacterium Avium Complex", *Archives of Internal Medicine*, vol. 153, p. 534 (Feb. 1993).
- Eravelly, J. et al., "Thalidomide in Weber-Christian Disease", *Lancet*, vol. 1, No. 8005, p. 251 (1977) [letter].
- Lueprasitakul, W. et al., "Effect of Thalidomide on the incidence of Iodine-Induced and Spontaneous Lymphocytic Thyroiditis and Spontaneous Diabetes Mellitus in the BB/W or Rat", *Acta Endocrinologica*, vol. 123, No. 1, pp. 79-83 (Jul. 1990).
- Misery, L. et al., "Remission of Langerhans Cell Histiocytosis with Thalidomide Treatment", *Clinical and Experimental Dermatology*, vol. 18, No. 5, p. 487 (Sep. 1993).
- Rhoton, A.J., "Role for Thalidomide in Primary Biliary Cirrhosis Treatment", *Gastroenterology*, vol. 105, No. 3, p. 956 (Sep. 1993).
- Thomas, L. et al., "Successful treatment of Adult's Langerhans Cell Histiocytosis with Thalidomide, Report of Two cases and Literature Review", *Archives of Dermatology*, vol. 129, pp. 1261-1264 (Oct. 1993).
- Prigent F. et al., "Cutaneous Sarcoidosis. Treatment with Thalidomide", *Press Med.*, vol. 12, No. 47, p. 3007 (1983) [French].
- Fabro, S. et al., "Teratogenic Activity of Thalidomide and Related Compounds," *Life Sciences*, 3:987-992 (1964).
- Shealy et al., "D- and L-Thalidomide," *Chem. and Indus.*, pp. 1030-1031 (Jun. 12, 1965).
- Folkman, "Tumor Angiogenesis: Therapeutic Implications," *The New England Journal of Medicine*, 285(21):1182-1186 (Nov. 18, 1971).
- Jonsson, N. Ake, et al., "Chemical Structure and Teratogenic Properties," *Acta Pharm. Succica*, 9:521-542 (1972).
- Jonsson, N. Ake, et al., "Chemical Structure and Teratogenic Properties," *Acta Pharm. Succica*, 9:431-446 (1972).
- Jonsson, N. Ake, et al., "Chemical Structure and Teratogenic Properties," *Acta Pharm. Succica*, 9:543-562 (1972).
- Eriksson, Sven O., "Synthesis and Alkaline Hydrolysis of some N-substituted Phthalimides," *Acta Pharm. Succica*, 10:63-74 (1973).
- Gimbrone et al., "Tumor Growth and Neovascularization: An Experimental Model Using the Rabbit Cornea," *J. Nat'l Cancer Institute*, 52(2):413-419 (1974).
- Fickentscher, K., "Stereochemical Properties and Teratogenic Activity of Some Tetrahydrophthalimides," *Mol. Pharmacology*, 13:133-141 (1977).
- Knighton et al., "Avascular And Vascular Phases Of Tumor Growth In The Chick Embryo," *Br. J. Cancer*, 35:347-356 (1977).
- Blaschke, "Chromatographic Separation of Racemic Thalidomide and Teratogenic Activity of its Enantiomers (Chromatographische Racemattrennung von Thalidomid und teratogene Wirkung der Enantiomere)," *Drug Research*, 29 (II/10): 1640-1642 (1979). (English lang. summary on first page).
- Muthukkaruppan et al., "Angiogenesis in the Mouse Cornea," *Science*, 205:1416-1418 (Sep. 28, 1979).
- Waters, et al., "Treatment of Ulcerative Colitis with Thalidomide," *British Medical Journal*, p. 792 (Mar. 24, 1979).
- Flohe et al., "Studies on the Hypothetical Relationship of Thalidomide-induced Embryopathy and Collagen Biosynthesis," *Arzneimittelforschung (Germany, West)*, 31(2):315-20 (1981).
- Helm, *Drug Research*, 31 (I/6): 941-949 (1981).
- Taylor et al., *Nature*, 297: p. 307 (1982).
- Belaube et al., "Should Thalidomide be Rehabilitated?," *Sem. Hôp. Paris*, 59(45):3101-3104 (Dec. 8, 1983).
- Folkman et al., *Science*, 221: p. 719 (1983).
- Hendler, Sheldon S. et al., "Thalidomide for Autoimmune Disease," *Medical Hypotheses*, 10:437-443 (1983).

- Crum et al., *Science*, 230: 1375 et seq. (1985).
- Koch, H.P. et al., "Thalidomide and Cogeners as Anti-inflammatory Agents," *Progress in Medicinal Chemistry*, 22:166-242 (1985).
- Sidky et al., *Cancer Research*, 47: pp 5155-5161 (1987).
- Srivastava et al., "The Prognostic Significance of Tumor Vascularity in Intermediate-Thickness Skin Melanoma," *Am. J. Pathol.*, 133:419-423 (1988). (abstract from Medline: Accession No. 06745734).
- Chen et al., "Plasam Pharmacokinetics and Urinary Excretion of Thalidomide After Oral Dosing in Healthy Male Volunteers," *Drug Metabolism and Disposition*, 17(4):402-405 (1989).
- White et al., *New England J. Med.*, 320: pp. 1197-1200 (1989).
- Folkman et al., "Induction of Angiogenesis During the Transition from Hyperplasia to Neoplasia," *Nature*, 339:58-61 (May 4, 1989).
- "Thalidomide," *The Merck Index*, Eleventh Edition, Budavari et al., eds., Merck & Co., New Jersey, p. 9182 (1989).
- Eger, K. et al., "Synthesis, Central Nervous System Activity and Teratogenicity of a Homothalidomide," *Arzneim.-Forsch/Drug Res.* 40 (II. Nr. 10):1073-1075 (1990).
- Ingber et al., "Synthetic Analogs Of Fumagillin That Inhibit Angiogenesis And Suppress Tumour Growth," *Nature*, 348:555-557 (Dec. 6, 1990).
- Brem et al., *Neurosurg.*, 74: 441-446 (1991).
- Matsuyama, Toshifumi, "Cytokines and HIV infection: is AIDS a Tumor Necrosis Factor Disease?," *AIDS*, 5(12):1405-1417 (1991).
- Weidner et al., "Tumor Angiogenesis and Metastasis—Correlation in Invasive Breast Carcinoma," *N.E. J. Med.*, 324(1):1-8 (1991).
- Colville-Nash et al., "Angiogenesis and Rheumatoid Arthritis: Pathogenic and Therapeutic Implications," *Ann. Rheum. Dis.*, 51:919-925 (1992).
- Folkman and Shing, "Angiogenesis," *J. Biol. Chem.*, 267(16):10931-10934 (1992).
- Schweigerer and Fotsis, "Angiogenesis and angiogenesis inhibitors in paediatric diseases," *Eur. J. Pediatr.* 151:472-476 (1992).
- Torry and Rongish, "Angiogenesis in the Uterus: Potential Regulation and Relation to Tumor Angiogenesis," *Am. J. Reprod. Immunol.*, 27:171-179 (1992).
- Vogelsang et al., "Thalidomide for the Treatment of Chronic Graft-Versus-Host Disease," *New England Journal of Medicine*, 326:1055-1058 (1992).
- Weidner et al., "Angiogenesis in Prostate Carcinoma," *Am. J. Pathol.*, 143(2):401-409 (1993).
- D'Amato et al., "Thalidomide is an Inhibitor Angiogenesis," *PNAS USA*, 91:4082-4085 (1994).
- Oikawa et al., "Eponemycin, A Novel Antibiotic, is a Highly Powerful Angiogenesis Inhibitor," *Biochem. Biophys. Res. Comm.*, 181(3):1070-1076 (Dec. 31, 1991).
- Otsuka et al., "A New Potent Angiogenesis Inhibitor, FR-118487," *J. Microb. Biotech.*, 1(3):163-168 (1991).

* cited by examiner

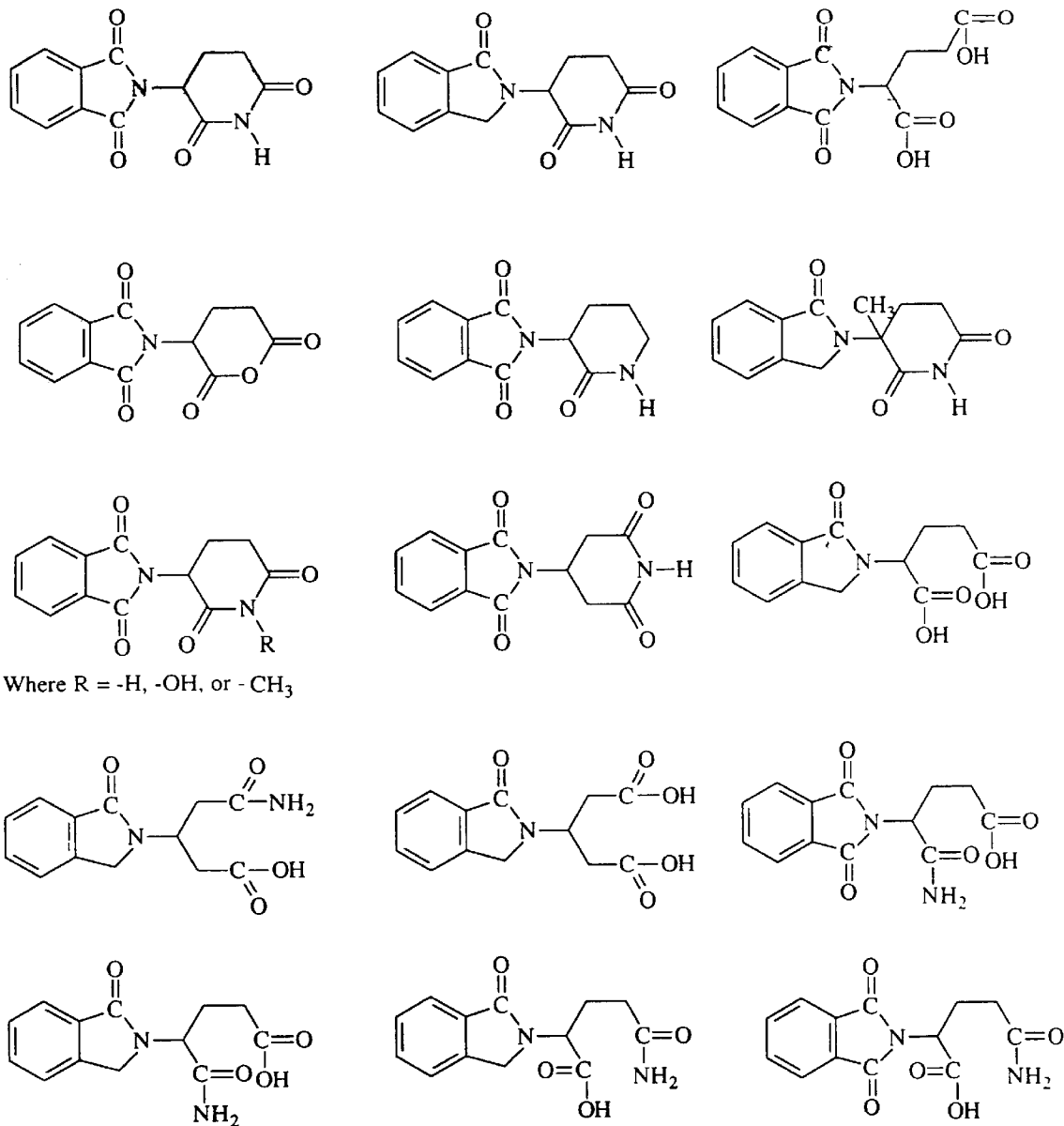


Figure 1

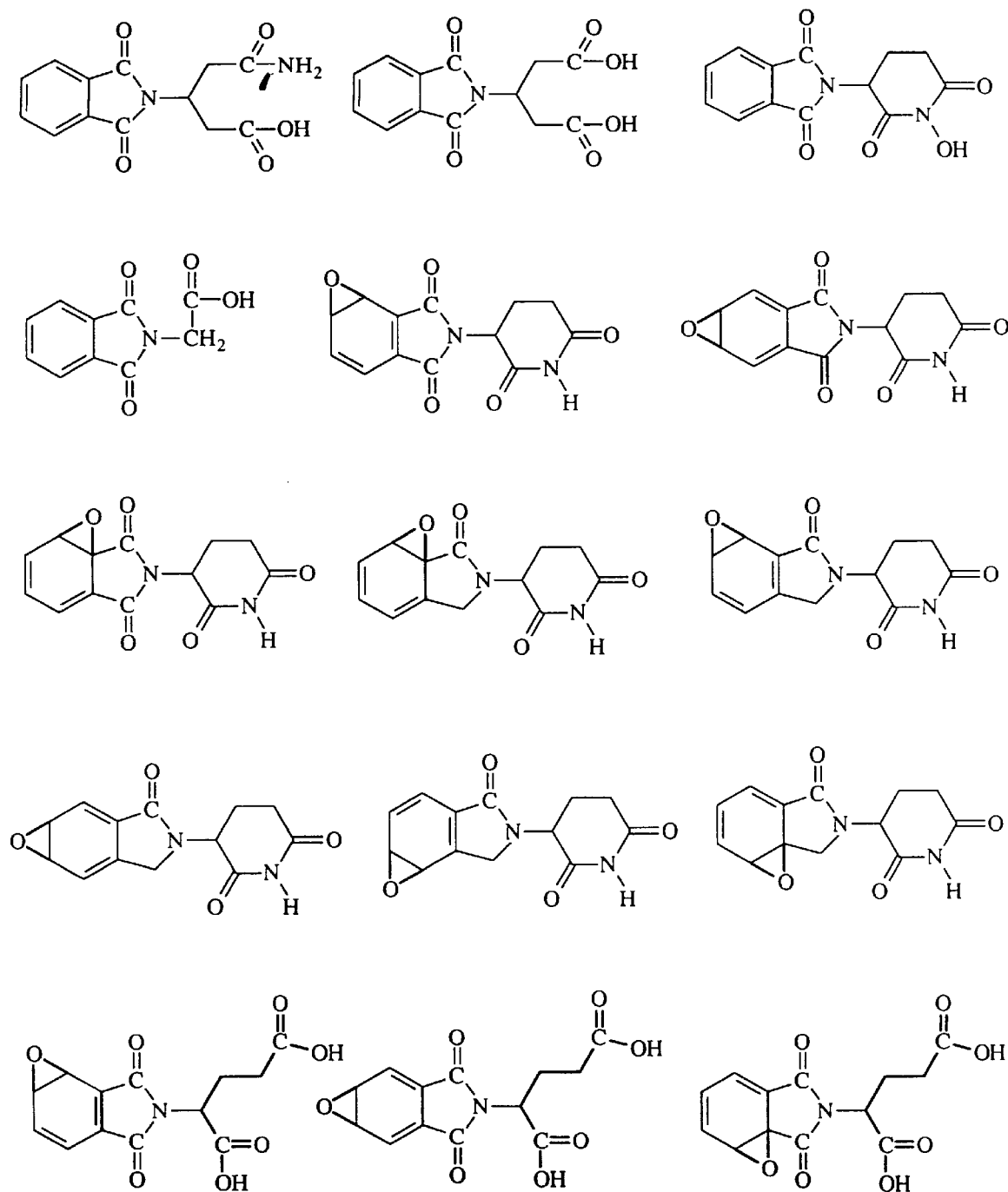


Figure 2

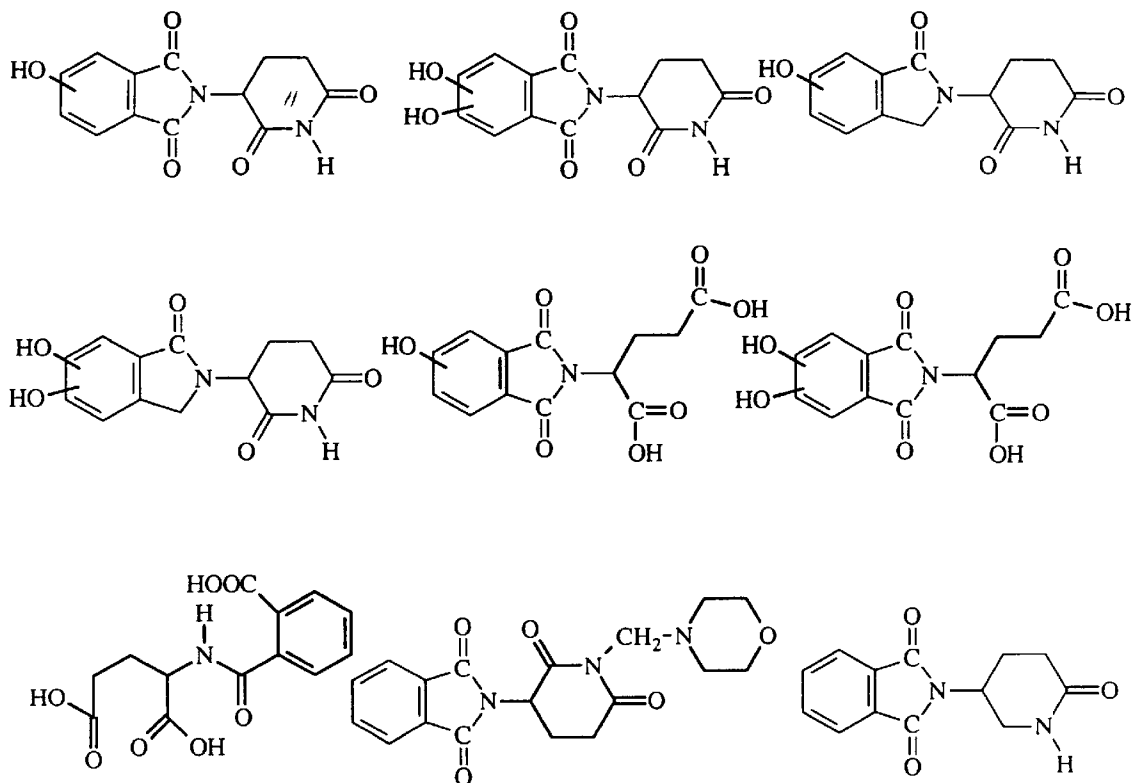


Figure 3

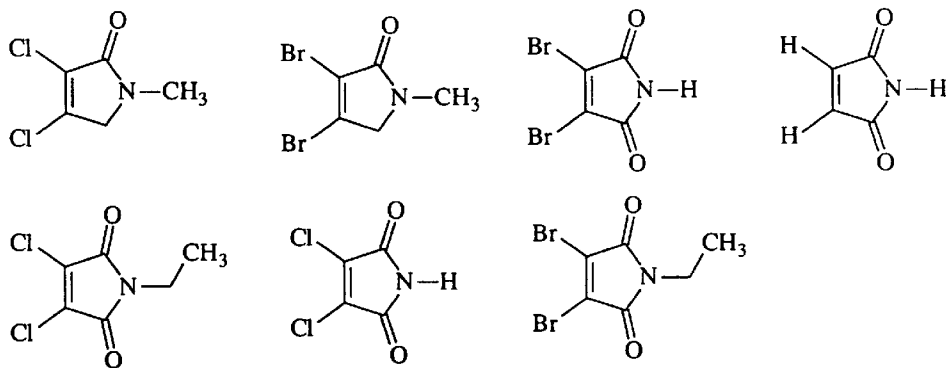


Figure 4

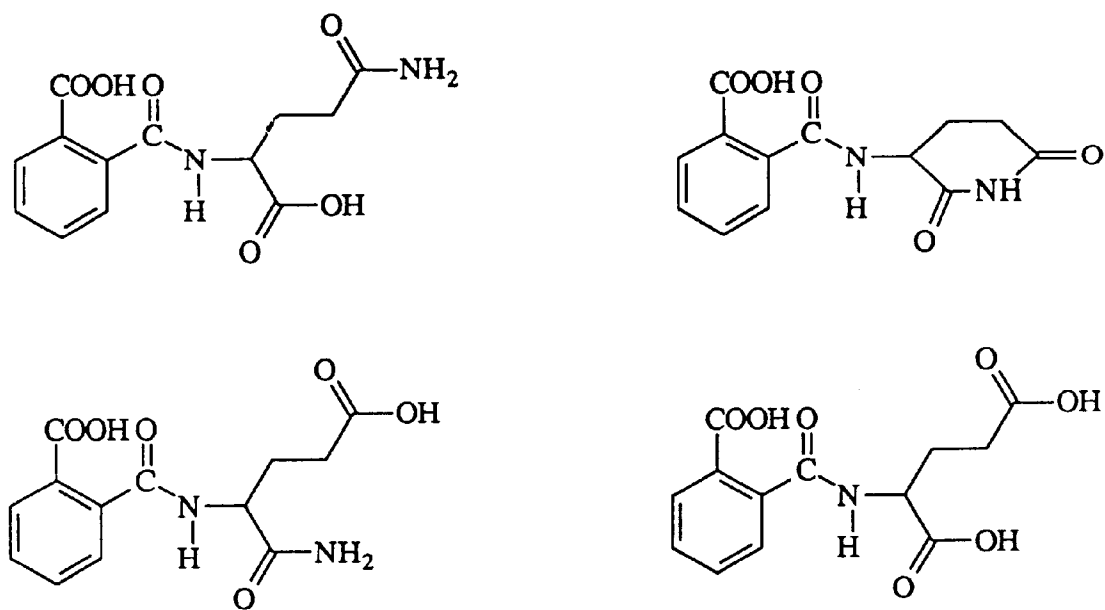


Figure 5

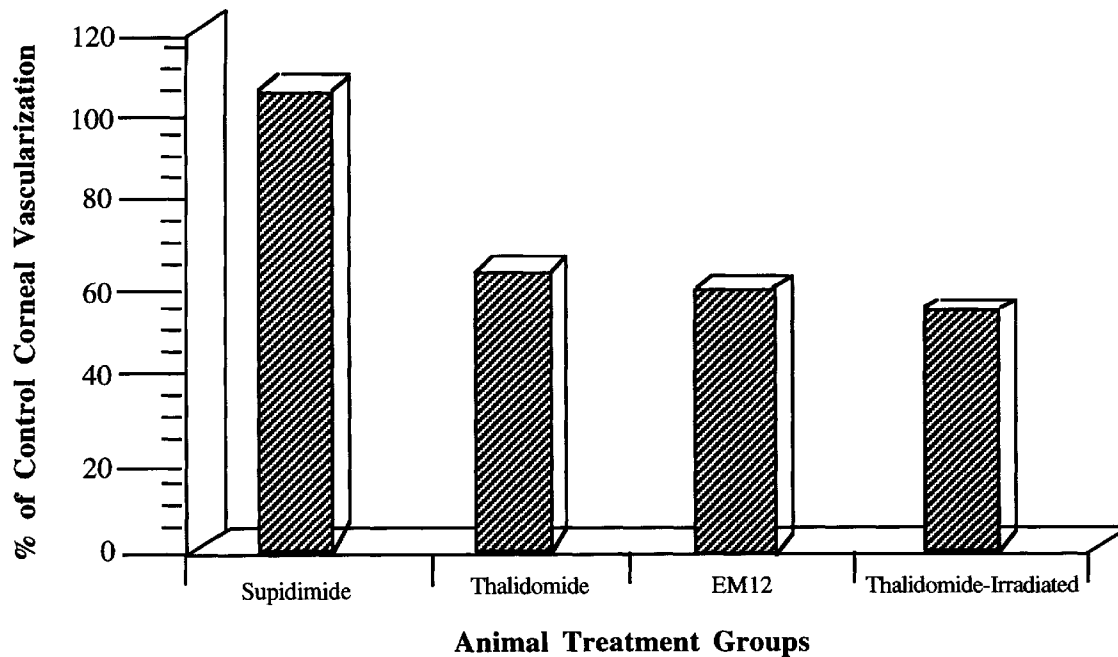


Figure 6

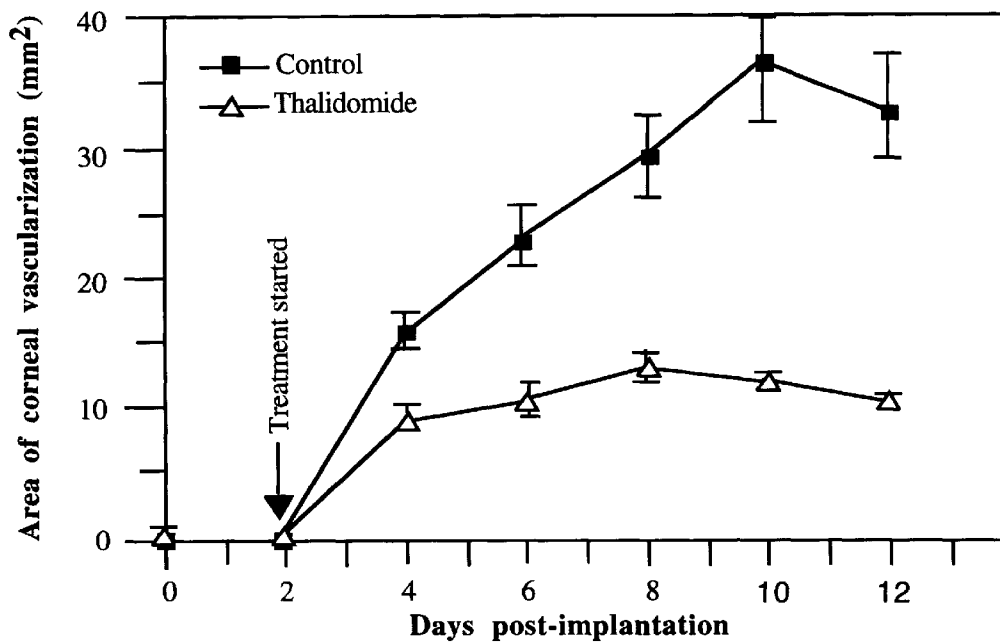


Figure 7

**METHODS AND COMPOSITIONS FOR
INHIBITION OF ANGIOGENESIS BY
THALIDOMIDE**

**CROSS REFERENCE TO RELATED
APPLICATION**

This application is a continuation of U.S. patent application Ser. No. 08/468,792, filed Jun. 6, 1995, now U.S. Pat. No. 5,712,291; which is a continuation of U.S. patent application Ser. No. 08/168,817, filed Dec. 15, 1993, now U.S. Pat. No. 5,629,327; which is a continuation-in-part of U.S. patent application Ser. No. 08/025,046, filed Mar. 1, 1993, now abandoned.

TECHNICAL FIELD

The present invention relates to methods and compositions for preventing unwanted angiogenesis in a human or animal. More particularly, the present invention relates to a method for preventing unwanted angiogenesis, particularly in angiogenesis dependent or associated diseases, by administration of compounds such as thalidomide and related compounds.

BACKGROUND OF THE INVENTION

As used herein, the term "angiogenesis" means the generation of new blood vessels into a tissue or organ. Under normal physiological conditions, humans or animals only undergo angiogenesis in very specific restricted situations. For example, angiogenesis is normally observed in wound healing, fetal and embryonal development and formation of the corpus luteum, endometrium and placenta. The control of angiogenesis is a highly regulated system of angiogenic stimulators and inhibitors. The control of angiogenesis has been found to be altered in certain disease states and, in many cases, the pathological damage associated with the disease is related to the uncontrolled angiogenesis.

Both controlled and uncontrolled angiogenesis are thought to proceed in a similar manner. Endothelial cells and pericytes, surrounded by a basement membrane, form capillary blood vessels. Angiogenesis begins with the erosion of the basement membrane by enzymes released by endothelial cells and leukocytes. The endothelial cells, which line the lumen of blood vessels, then protrude through the basement membrane. Angiogenic stimulants induce the endothelial cells to migrate through the eroded basement membrane. The migrating cells form a "sprout" off the parent blood vessel, where the endothelial cells undergo mitosis and proliferate. The endothelial sprouts merge with each other to form capillary loops, creating the new blood vessel. In the disease state, prevention of angiogenesis could avert the damage caused by the invasion of the new microvascular system.

Persistent, unregulated angiogenesis occurs in a multiplicity of disease states, tumor metastasis and abnormal growth by endothelial cells and supports the pathological damage seen in these conditions. The diverse pathological states created due to unregulated angiogenesis have been grouped together as angiogenic dependent or angiogenic associated diseases. Therapies directed at control of the angiogenic processes could lead to the abrogation or mitigation of these diseases.

One example of a disease mediated by angiogenesis is ocular neovascular disease. This disease is characterized by invasion of new blood vessels into the structures of the eye such as the retina or cornea. It is the most common cause of

blindness and is involved in approximately twenty eye diseases. In age-related macular degeneration, the associated visual problems are caused by an ingrowth of chorioidal capillaries through defects in Bruch's membrane with proliferation of fibrovascular tissue beneath the retinal pigment epithelium. Angiogenic damage is also associated with diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, neovascular glaucoma and retrolental fibroplasia. Other diseases associated with corneal neovascularization include, but are not limited to, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sjogrens, acne rosacea, phlyctenulosis, syphilis, Mycobacteria infections, lipid degeneration, chemical burns, bacterial ulcers, fungal ulcers, *Herpes simplex* infections, *Herpes zoster* infections, protozoan infections, Kaposi sarcoma, Mooren ulcer, Terrien's marginal degeneration, marginal keratolysis, rheumatoid arthritis, systemic lupus, polyarteritis, trauma, Wegeners sarcoidosis, Scleritis, Steven's Johnson disease, periphigoid radial keratotomy, and corneal graph rejection.

Diseases associated with retinal/choroidal neovascularization include, but are not limited to, diabetic retinopathy, macular degeneration, sickle cell anemia, sarcoid, syphilis, pseudoxanthoma elasticum, Pagets disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis/vitritis, mycobacterial infections, Lyme's disease, systemic lupus erythematosus, retinopathy of prematurity, Eales disease, Bechets disease, infections causing a retinitis or choroiditis, presumed ocular histoplasmosis, Bests disease, myopia, optic pits, Stargarts disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma and post-laser complications. Other diseases include, but are not limited to, diseases associated with rubeosis (neovasculariation of the angle) and diseases caused by the abnormal proliferation of fibrovascular or fibrous tissue including all forms of proliferative vitreoretinopathy.

Another disease in which angiogenesis is believed to be involved is rheumatoid arthritis. The blood vessels in the synovial lining of the joints undergo angiogenesis. In addition to forming new vascular networks, the endothelial cells release factors and reactive oxygen species that lead to pannus growth and cartilage destruction. The factors involved in angiogenesis may actively contribute to, and help maintain, the chronically inflamed state of rheumatoid arthritis.

Factors associated with angiogenesis may also have a role in osteoarthritis. The activation of the chondrocytes by angiogenic-related factors contributes to the destruction of the joint. At a later stage, the angiogenic factors would promote new bone formation. Therapeutic intervention that prevents the bone destruction could halt the progress of the disease and provide relief for persons suffering with arthritis.

Chronic inflammation may also involve pathological angiogenesis. Such disease states as ulcerative colitis and Crohn's disease show histological changes with the ingrowth of new blood vessels into the inflamed tissues. Bartonellosis, a bacterial infection found in South America, can result in a chronic stage that is characterized by proliferation of vascular endothelial cells. Another pathological role associated with angiogenesis is found in atherosclerosis. The plaques formed within the lumen of blood vessels have been shown to have angiogenic stimulatory activity.

One of the most frequent angiogenic diseases of childhood is the hemangioma. In most cases, the tumors are

benign and regress without intervention. In more severe cases, the tumors progress to large cavernous and infiltrative forms and create clinical complications. Systemic forms of hemangiomas, the hemangiomatoses, have a high mortality rate. Therapy-resistant hemangiomas exist that cannot be treated with therapeutics currently in use.

Angiogenesis is also responsible for damage found in hereditary diseases such as Osler-Weber-Rendu disease, or hereditary hemorrhagic telangiectasia. This is an inherited disease characterized by multiple small angiomas, tumors of blood or lymph vessels. The angiomas are found in the skin and mucous membranes, often accompanied by epistaxis (nosebleeds) or gastrointestinal bleeding and sometimes with pulmonary or hepatic arteriovenous fistula.

Angiogenesis is prominent in solid tumor formation and metastasis. Angiogenic factors have been found associated with several solid tumors such as rhabdomyosarcomas, retinoblastoma, Ewing sarcoma, neuroblastoma, and osteosarcoma. A tumor cannot expand without a blood supply to provide nutrients and remove cellular wastes. Tumors in which angiogenesis is important include solid tumors, and benign tumors such as acoustic neuroma, neurofibroma, trachoma and pyogenic granulomas. Prevention of angiogenesis could halt the growth of these tumors and the resultant damage to the animal due to the presence of the tumor.

It should be noted that angiogenesis has been associated with blood-born tumors such as leukemias, any of various acute or chronic neoplastic diseases of the bone marrow in which unrestrained proliferation of white blood cells occurs, usually accompanied by anemia, impaired blood clotting, and enlargement of the lymph nodes, liver, and spleen. It is believed that angiogenesis plays a role in the abnormalities in the bone marrow that give rise to leukemia-like tumors.

Angiogenesis is important in two stages of tumor metastasis. The first stage where angiogenesis stimulation is important is in the vascularization of the tumor which allows tumor cells to enter the blood stream and to circulate throughout the body. After the tumor cells have left the primary site, and have settled into the secondary, metastasis site, angiogenesis must occur before the new tumor can grow and expand. Therefore, prevention of angiogenesis could lead to the prevention of metastasis of tumors and possibly contain the neoplastic growth at the primary site.

Knowledge of the role of angiogenesis in the maintenance and metastasis of tumors has led to a prognostic indicator for breast cancer. The amount of neovascularization found in the primary tumor was determined by counting the microvessel density in the area of the most intense neovascularization in invasive breast carcinoma. A high level of microvessel density was found to correlate with tumor recurrence. Control of angiogenesis by therapeutic means could possibly lead to cessation of the recurrence of the tumors.

Angiogenesis is also involved in normal physiological processes such as reproduction and wound healing. Angiogenesis is an important step in ovulation and also in implantation of the blastula after fertilization. Prevention of angiogenesis could be used to induce amenorrhea, to block ovulation or to prevent implantation by the blastula.

In wound healing, excessive repair or fibroplasia can be a detrimental side effect of surgical procedures and may be caused or exacerbated by angiogenesis. Adhesions are a frequent complication of surgery and lead to problems such as small bowel obstruction.

Several kinds of compounds have been used to prevent angiogenesis. Taylor et al. have used protamine to inhibit

angiogenesis, see Taylor et al., *Nature* 297:307 (1982). The toxicity of protamine limits its practical use as a therapeutic. Folkman et al. have disclosed the use of heparin and steroids to control angiogenesis. See Folkman et al., *Science* 221:719 (1983) and U.S. Pat. Nos. 5,001,116 and 4,994,443. Steroids, such as tetrahydrocortisol, which lack gluco and mineral corticoid activity, have been found to be angiogenic inhibitors.

Other factors found endogenously in animals, such as a 4 kDa glycoprotein from bovine vitreous humor and a cartilage derived factor, have been used to inhibit angiogenesis. Cellular factors such as interferon inhibit angiogenesis. For example, interferon α or human interferon β has been shown to inhibit tumor-induced angiogenesis in mouse dermis stimulated by human neoplastic cells. Interferon β is also a potent inhibitor of angiogenesis induced by allogeneic spleen cells. See Sidky et al., *Cancer Research* 47:5155-5161 (1987). Human recombinant α interferon (alpha/A) was reported to be successfully used in the treatment of pulmonary hemangiomatosis, an angiogenesis-induced disease. See White et al., *New England J. Med.* 320:1197-1200 (1989).

Other agents which have been used to inhibit angiogenesis include ascorbic acid ethers and related compounds. See Japanese Kokai Tokkyo Koho No. 58-131978. Sulfated polysaccharide DS 4152 also shows angiogenic inhibition. See Japanese Kokai Tokkyo Koho No. 63-119500. A fungal product, fumagillin, is a potent angiostatic agent in vitro. The compound is toxic in vivo, but a synthetic derivative, AGM 12470, has been used in vivo to treat collagen II arthritis. Fumagillin and O-substituted fumagillin derivatives are disclosed in EPO Publication Nos. 0325199A2 and 0357061A1.

PCT Application No. WO 92/14455 to Kaplan et al. is directed to a method for controlling abnormal concentration of TNF- α by administering thalidomide or thalidomide derivatives to a patient with toxic concentrations of TNF- α .

The above compounds are either topical or injectable therapeutics. Therefore, there are drawbacks to their use as a general angiogenic inhibitor and lack adequate potency. For example, in prevention of excessive wound healing, surgery on internal body organs involves incisions in various structures contained within the body cavities. These wounds are not accessible to local applications of angiogenic inhibitors. Local delivery systems also involve frequent dressings which are impracticable for internal wounds, and increase the risk of infection or damage to delicate granulation tissue for surface wounds.

Thus, a method and composition are needed that are capable of inhibiting angiogenesis and which are easily administered. A simple and efficacious method of treatment would be through the oral route. If an angiogenic inhibitor could be given by an oral route, the many kinds of diseases discussed above, and other angiogenic dependent pathologies, could be treated easily. The optimal dosage could be distributed in a form that the patient could self-administer.

SUMMARY OF THE INVENTION

In accordance with the present invention, compositions and methods are provided that are effective in inhibiting unwanted angiogenesis. These compositions are easily administered by different routes including oral and can be given in dosages that are safe and provide angiogenic inhibition at internal sites. The present invention provides a method of treating mammalian diseases mediated by undes-

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ired and uncontrolled angiogenesis by administering a composition comprising an anti-angiogenic compound in a dosage sufficient to inhibit angiogenesis.

The present invention also includes angiogenic inhibiting compounds that contain an epoxide group. These angiogenic inhibiting compounds can be administered to a human or animal alone or with epoxide hydrolase inhibiting compounds.

The present invention is especially useful for treating certain ocular neovascular diseases such as macular degeneration. The compounds which are contemplated as part of the present invention preferably can be given orally to the patient and thereby halt the progression of the disease. Other disease that can be treated using the present invention are diabetic retinopathy, neovascular glaucoma and retrolental fibroplasia.

Accordingly, it is an object of the present invention to provide a compound and method to inhibit unwanted angiogenesis in a human or animal.

It is yet another object of the present invention to provide a composition of inhibiting angiogenesis by oral administration of the composition.

It is another object of the present invention to provide a treatment for diseases mediated by angiogenesis.

It is yet another object of the present invention to provide a treatment for macular degeneration.

It is yet another object of the present invention to provide a treatment for all forms of proliferative.

It is yet another object of the present invention to provide a treatment for all forms of proliferative vitreoretinopathy including those forms not associated with diabetes.

It is yet another object of the present invention to provide a treatment for solid tumors.

It is yet another object of the present invention to provide a method and composition for the treatment of blood-born tumors such as leukemia.

It is another object of the present invention to provide a method and composition for the treatment of hemangioma.

It is another object of the present invention to provide a method and composition for the treatment of retrolental fibroplasia.

It is another object of the present invention to provide a method and composition for the treatment of psoriasis.

It is another object of the present invention to provide a method and composition for the treatment of Kaposi's sarcoma.

It is another object of the present invention to provide a method and composition for the treatment of Crohn's diseases.

It is another object of the present invention to provide a method and composition for the treatment of diabetic retinopathy.

Other features and advantages of the invention will be apparent from the following description of preferred embodiments thereof.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiments and the appended claims.

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BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1 through 3 are a listing of representative compounds in the genus represented by the following general formulas:

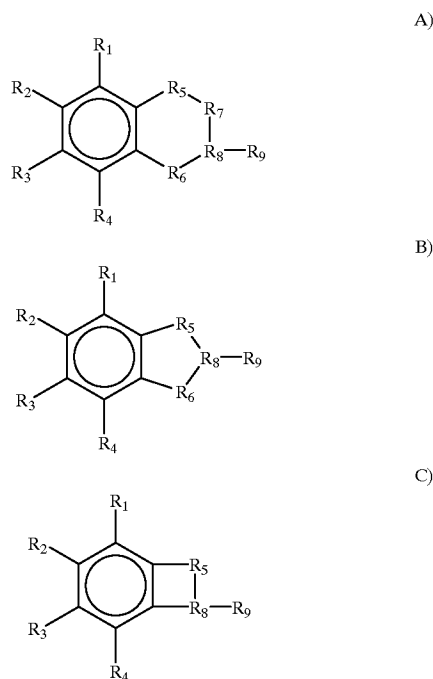


FIG. 4 is a listing of representative compounds in the genus represented by the following general formula:

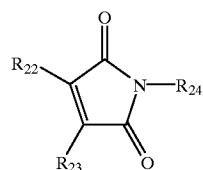


FIG. 5 is a listing of representative compounds in the genus represented by the following general formula:

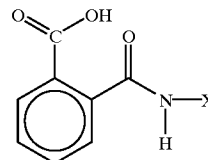


FIG. 6 shows the effect of thalidomide and EM12 on angiogenesis in a rabbit cornea model of angiogenesis.

FIG. 7 shows the effect of thalidomide on the area of corneal vascularization in a rabbit cornea model of angiogenesis.

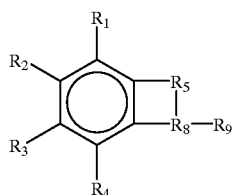
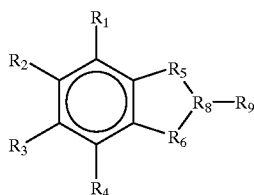
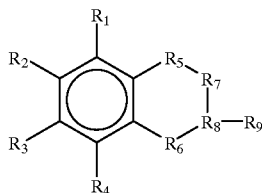
DETAILED DESCRIPTION

The present invention includes compositions and methods for the treatment of diseases that are mediated by angiogenesis. One embodiment of the present invention is the use of thalidomide or the metabolites of thalidomide as disclosed

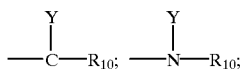
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herein to inhibit unwanted angiogenesis. The present invention also includes compounds which cause dysmelia in the developing fetus and have anti-angiogenic activity. The present invention comprises a method of treating undesired angiogenesis in a human or animal comprising the steps of administering to the human or animal with the undesired angiogenesis a composition comprising an effective amount of a teratogenic compound that is anti-angiogenic.

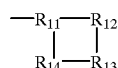
Compounds that can be used in accordance with the present invention include compounds included in the following general formulae. Examples of compounds that have anti-angiogenic properties having one of the following three formulae (A), (B), or (C):



In the above formulae A), B), and C), R₁, R₂, R₃ and R₄ can be selected from: —H; —OH; =O, straight chained and branched alkanes, alkenes, alkynes; cyclic alkanes, alkenes, and alkynes; combinations of cyclic and acyclic alkanes, alkenes, and alkynes; alcohol, aldehyde, ketone, carboxylic acid, ester, or other moieties in combination with acyclic, cyclic, or combination acyclic/cyclic moieties; aza; amino; —XO_n or —O—XO_n, [where X=N and n=2; X=S and n=2 or 3; or X=P and n=1-3]; and halogens; R₅, R₆, R₇, and R₈ are each independently selected from:



or —O— where Y is optional and is the same as defined above for R₁; and R₁₀ is the same as defined above for R₁, or (where Y is absent) R₁₀ is =O; and R₉ is a moiety having formula D), E), F), G) or H):



A) 15

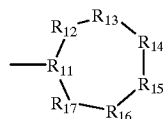
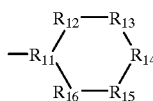
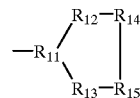
B) 25

C) 35

D) 65

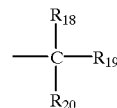
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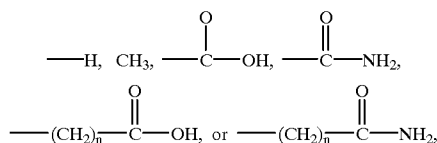
where each of R₁₁–R₁₇ is (independently) the same as defined above for R₅;

H) 30



where R₁₈, R₁₉ and R₂₀ are, independently selected from

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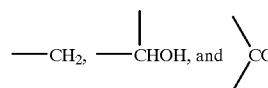


and n=1 to 4.

Accordingly, another aspect of the present invention features inhibiting angiogenesis in a mammal by administering a therapeutic composition comprising one of the above-described compounds in a dosage sufficient to inhibit angiogenesis.

In preferred embodiments, the compound has formula B), where R₅ and R₆ are selected from the group consisting of:

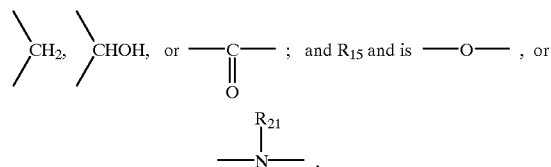
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and R₉ has formula F) or H); and R₁₄ and R₁₆ are selected from the group consisting of:

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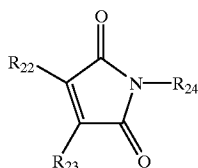
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where R₂₁ is —H, —CH₃, or —OH. Specific preferred compounds according to this aspect of the present invention include thalidomide, its precursors, metabolites and analogs. Particular analogs include EM-12, N-phthaloyl-DL-glutamic acid (PGA) or N-phthaloyl-DL-glutamine anhy-

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dride. Examples of compounds that are members of this genus are listed in FIGS. 1 through 3. It is to be understood that the compounds included as part of the present invention are not to be limited to those compounds shown in FIGS. 1 through 3 and include all other compounds that are members of the genus described by the general formulas herein.

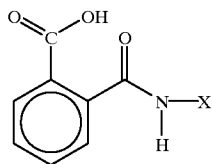
Compounds of the following formula that have anti-angiogenic properties:



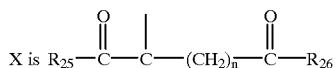
where R_{22} and R_{23} are (independently), —H, —F, —Cl, —Br, —I, —CH₃, or —CH₂—CH₃; and R_{24} is —H, —CH₃, or —CH₂—CH₃.

The present invention also features inhibiting angiogenesis in a mammal by administering a compound according to the above formulae in a dosage sufficient to inhibit angiogenesis. Examples of specific compounds that are members of this genus are listed in FIG. 4.

Angiogenesis inhibition hydrolysis products of thalidomide having the following general formula can be used in practicing the present invention:



where X is R_6 as defined above, or

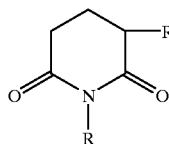


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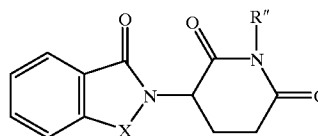
and R_{25} and R_{26} are, independently, —OH, —H, or NH_2 , and $n=1$ through 4. Examples of such compounds are shown in FIG. 5.

Angiogenesis inhibition compounds having the following general formula can be used in practicing the present invention:

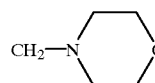
10 (I)



15 (II)



(a)



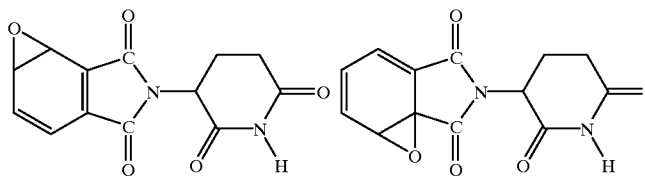
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wherein compounds of structure (I), wherein R is selected from the group consisting of hydrogen, alkyl radicals of 1 to 6 carbon atoms, the phenyl radical, and the benzyl radical; and wherein R' is selected from the group consisting of the phthalimido radical and the succinimido radical and of structure (II), wherein X is CH_2 or $C=O$; R'' is H, —CH₂CH₃, —C₆H₅, —CH₂C₆H₅, —CH₂CH=CH₂, or (a) and hydrolysis products of the compounds wherein R'' is H and the piperidino ring or both the piperidino and the imido ring are hydrolyzed.

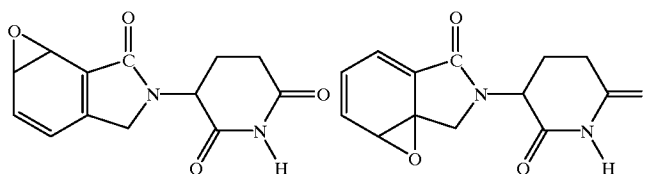
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Another set of compounds that are considered part of the present invention are the epoxides of thalidomide, EM-12 and EM-138. Representative epoxide compounds are shown as follows:



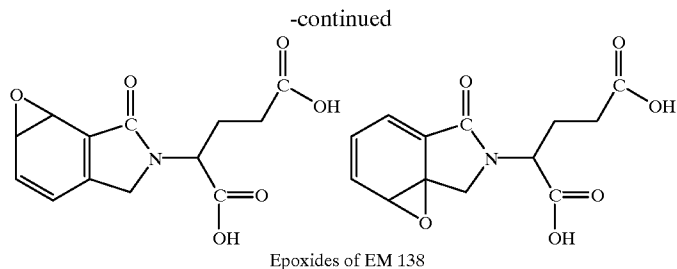
Epoxides of thalidomide



Epoxides of EM 12

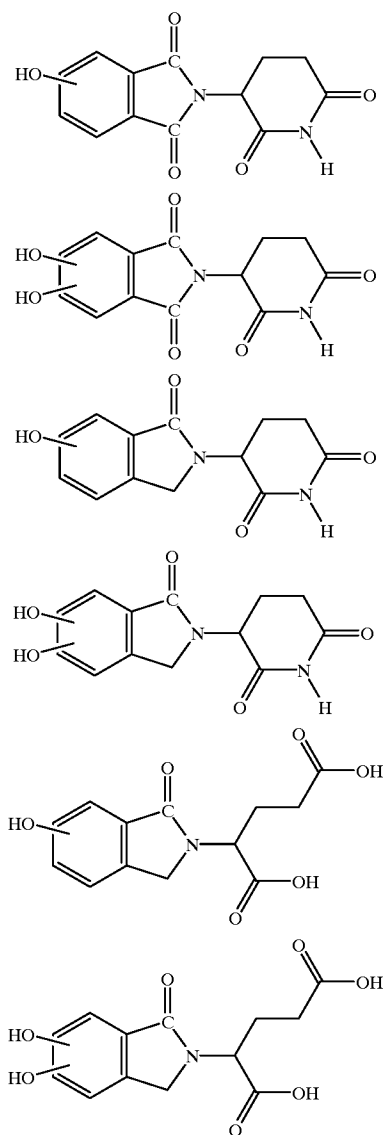
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It should be understood that the epoxide can be attached at the 6,1 site on the benzene ring, the 1,2 site, the 2,3 site, 3,4 or the 4,5 site. All of these compounds are contemplated as part of the present invention.

The epoxides of the thalidomide, EM-12, and EM 138 can be hydrolyzed to the following compounds:



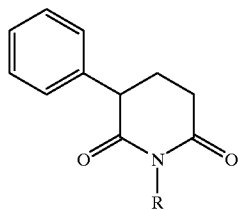
15 It is to be understood that the hydroxyl group can be on carbons 1, 2, 3, 4, 5 and 6 of the benzene ring. Also contemplated as part of the present invention are dihydroxyl compounds wherein the two hydroxyl groups are located bis to each other on carbons 1, 2, 3, 5 and 6 of the above compounds. The epoxides, the hydrolysis products of the epoxides, and the hydrolysis products of the thalidomide are all contemplated to be part of the present invention.

20 It is known that epoxides are hydrolyzed by a group of enzymes known as epoxide hydrolases. There is a class of compounds which are epoxide hydrolase inhibitors. 25 Examples of these compounds are valpromide (2-propylpentanamide) and valproic acid (2-propylpentanoic acid). Because epoxides are important angiogenesis inhibitors, it is contemplated as part of the present invention, compositions comprising any of the angiogenesis inhibitors compounds recited herein in combination with epoxide hydrolase inhibitors. The epoxide hydrolase inhibitors can be administered to a human or animal together or sequentially. The epoxide group appears 35 to be an important substituent common to several angiogenesis inhibitors. The use of epoxide hydrolase inhibitors to potentiate the activity of any angiogenesis inhibitor containing an epoxide is contemplated as part of the present invention. For example, the epoxide hydrolase inhibitors can be administered with the following epoxide-containing anti-angiogenesis compounds: AGM 1470, Eponimycin, microbial metabolites of *Scolecobasidium arenarium* designated 40 f/2015, fr/111142 and fr/18487. See Oikawa, *Biochem Biophys. Res. Comm.*, Vol. 81:1070 (1971) and Otsuka, *J. Microbial. Biotech.*, Vol 1:163 (1991).

45 It is contemplated as an embodiment of the present invention the use of the epoxide containing angiogenesis inhibitors with or without epoxide hydrolase inhibitors as a treatment for diseases mediated by elevated or toxic levels of TNF- α . TNF- α has been recognized as manifesting a dose dependent toxicity. If present at low levels for a long period of time, TNF- α can result in cachexia. Cachexia is a general weight loss and wasting occurring in the course of some chronic diseases such as cancer, opportunistic infections of AIDS, inflammatory diseases, parasitic diseases, tuberculosis, and high dose IL-2 therapy. The epoxide containing angiogenesis inhibitors, with or without epoxide hydrolase inhibitors, are also effective in treating diseases such as septic shock, leprosy and graph vs. host disease.

60 Other embodiments are within the present invention. For example, other dysmelia-causing compounds can be used according to the present invention, e.g. 4-methylphthalic acid, pyridoxine, vasopressin, acetazolamide, or a compound having the following formula (where R=H, —OH, or —CH₃):

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Other compounds which are teratogens, such as valproic acid (2-propylpentanoic acid), the retinoids, such as cis-retinoic acid, and rifampin may also be used in accordance with the invention.

In summary, the preferred compounds are thalidomide, as well as analogs, hydrolysis products, metabolites and precursors of thalidomide that are teratogenic, and, more specifically, that cause dismelia. However, it is to be understood that it is not necessary for a compound to have both teratogenic activity and angiogenesis inhibiting activity to be considered part of the present invention. Dismelia-causing compounds can be identified by the general procedures of Helm, *Arzneimittelforschung*, 31(i/6):941-949 (1981), in which rabbit pups are examined after exposure to the compound in utero. The compounds can generally be purchased, e.g., from Andrulis Pharmaceuticals, Beltsville, Md., or synthesized according to known procedures. It is to be understood that the compounds of the present invention can exist as enantiomers and that the racemic mixture of enantiomers or the isolated enantiomers are all considered as within the scope of the present invention.

Many of the compounds that are contemplated as part of the present invention can be enriched in optically active enantiomers of the compounds specified above. Specifically, Blaschke has reported that the S enantiomers may be disproportionately responsible for the dismelia-producing effect of these compounds. See, generally Blaschke, *Arzneimittelforschung* 29:1640-1642 (1979). The above described articles generally describe procedures to obtain optically active preparations of the compounds of interest. See, e.g. Shealy et al., *Chem. Indus.* 1030 (1965); and Casini et al., *Farmaco Ed. Sci.* 19:563 (1964).

The compounds described above can be provided as pharmaceutically acceptable formulations using formulation methods known to those of ordinary skill in the art. These formulations can be administered by standard routes. In general, the combinations may be administered by the topical, transdermal, oral, rectal or parenteral (e.g., intravenous, subcutaneous or intramuscular) route. In addition, the combinations may be incorporated into biodegradable polymers allowing for sustained release of the compound, the polymers being implanted in the vicinity of where drug delivery is desired, for example, at the site of a tumor. The biodegradable polymers and their use are described, for example, in detail in Brem et al., *J. Neurosurg.* 74:441-446 (1991).

The dosage of the compound will depend on the condition being treated, the particular compound, and other clinical factors such as weight and condition of the human or animal and the route of administration of the compound. It is to be understood that the present invention has application for both human and veterinary use. For oral administration to humans, a dosage of between approximately 0.1 to 300 mg/kg/day, preferably between approximately 0.5 and 50 mg/kg/day, and most preferably between approximately 1 to 10 mg/kg/day, is generally sufficient.

The formulations include those suitable for oral, rectal, ophthalmic, (including intravitreal or intracameral) nasal,

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topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intratracheal, and epidural) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by conventional pharmaceutical techniques. Such techniques include the step of bringing into association the active ingredient and the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into associate the active ingredient 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, 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 liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil emulsion and as a bolus, etc.

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 ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface active or dispersing agent. Molded tablets may be made by molding, in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may be optionally coated or scored and may be formulated so as to provide a slow or controlled release of the active ingredient therein.

Formulations suitable for topical administration in the mouth include lozenges comprising the ingredients in a flavored basis, 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 ingredient to be administered in a suitable liquid carrier.

Formulations suitable for topical administration to the skin may be presented as ointments, creams, gels and pastes comprising the ingredient to be administered in a pharmaceutical acceptable carrier. A preferred topical delivery system is a transdermal patch containing the ingredient to be administered.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

Formulations suitable for nasal administration, wherein the carrier is a solid, include a coarse powder having a particle size, for example, in the range of 20 to 500 microns which is administered in the manner in which snuff is administered, i.e., by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations, wherein the carrier is a liquid, for administration, as for example, a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient.

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 parenteral administration include aqueous and non-aqueous 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. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze-dried (lyophilized) conditions requiring only the addition of the sterile liquid carrier, for example, water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose, as herein above recited, or an appropriate fraction thereof, of the administered ingredient.

It should be understood that in addition to the ingredients, particularly mentioned above, the formulations of the present 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 flavoring agents.

Diseases associated with corneal neovascularization that can be treated according to the present invention include but are not limited to, diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, neovascular glaucoma and retrolental fibroplasia, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sjogrens, acne rosacea, phlyctenulosis, syphilis, Mycobacteria infections, lipid degeneration, chemical burns, bacterial ulcers, fungal ulcers, *Herpes simplex* infections, *Herpes zoster* infections, protozoan infections, Kaposi sarcoma, Mooren ulcer, Terrien's marginal degeneration, marginal keratolysis, trauma, rheumatoid arthritis, systemic lupus, polyarteritis, Wegeners sarcoidosis, Scleritis, Steven's Johnson disease, pemphigoid radial keratotomy, and corneal graft rejection.

Diseases associated with retinal/choroidal neovascularization that can be treated according to the present invention include, but are not limited to, diabetic retinopathy, macular degeneration, sickle cell anemia, sarcoid, syphilis, pseudoxanthoma elasticum, Pagets disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis/vitritis, mycobacterial infections, Lyme's disease, systemic lupus erythematosus, retinopathy of prematurity, Eales disease, Bechets disease, infections causing a retinitis or choroiditis, presumed ocular histoplasmosis, Bests disease, myopia, optic pits, Stargarts disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma and post-laser complications. Other diseases include, but are not limited to, diseases associated with rubeosis (neovasculariation of the angle) and diseases caused by the abnormal proliferation of fibrovascular or fibrous tissue including all forms of proliferative vitreoretinopathy, whether or not associated with diabetes.

Another disease which can be treated according to the present invention is rheumatoid arthritis. It is believed that the blood vessels in the synovial lining of the joints undergo angiogenesis. In addition to forming new vascular networks, the endothelial cells release factors and reactive oxygen species that lead to pannus growth and cartilage destruction. The factors involved in angiogenesis may actively contribute to, and help maintain, the chronically inflamed state of rheumatoid arthritis.

Another disease that can be treated according to the present invention are hemangiomas, Osler-Weber-Rendu disease, or hereditary hemorrhagic telangiectasia, solid or blood borne tumors and acquired immune deficiency syndrome.

This invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

EXAMPLE I

The chick embryo chorioallantoic membrane assay described by Crum et al., *Science* 230:1375 et seq. (1985), is used to identify compounds that do not require further metabolic conversion. See also, U.S. Pat. No. 5,001,116, hereby incorporated by reference, which describes the CAM assay at col. 7 of the patent. Briefly, fertilized chick embryos are removed from their shell on day 3 or 4, and a methylcellulose disc containing the compound is implanted on the chorioallantoic membrane. The embryos are examined 48 hours later and, if a clear avascular zone appears around the methylcellulose disc, the diameter of that zone is measured.

EXAMPLE II

Rabbit Cornea Angiogenesis Assay

Pellets for implantation into rabbit corneas were made by mixing 110 μ l of saline containing 12 μ g of recombinant bFGF (Takeda Pharmaceuticals-Japan) with 40 mg of sucralfate (Bukh Meditec-Denmark); this suspension was added to 80 μ l of 12% hydron (Interferon Sciences) in ethanol. 10 μ l aliquots of this mixture was then pipetted onto teflon pegs and allowed to dry producing approximately 17 pellets. A pellet was implanted into corneal micropockets of each eye of an anesthetized female New Zealand white rabbit, 2 mm from the limbus followed by topical application of erythromycin ointment onto the surface of the cornea. The animals were fed daily from 2 days post-implantation by gastric lavage with either drug suspended in 0.5% carboxymethyl cellulose or 0.5% carboxymethyl cellulose alone. Thalidomide was purchased from Andrus Pharmatec (Maryland) and the EM-12 and Supidimide were kindly provided by Grunenthal GMBH (Germany). The animals were examined with a slit lamp every other day in a masked manner by the same corneal specialist. The area of corneal neovascularization was determined by measuring with a reticule the vessel length (L) from the limbus and the number of clock hours (C) of limbus involved. A formula was used to determine the area of a circular band segment: $C/12 * 3.1416 [r^2 - (r-L)^2]$ where $r=6$ mm the measured radius of the rabbit cornea. Various mathematical models were utilized to determine the amount of vascularized cornea and this formula was found to provide the most accurate approximation of the area of the band of neovascularization that grows towards the pellet.

It is important to note that the rabbit cornea assay is preferable because it will generally recognize compounds that are inactive per se but are metabolized to yield active compounds. Thalidomide related compounds, as shown below in Example III, are known to be teratogens and are candidates for use in the present invention.

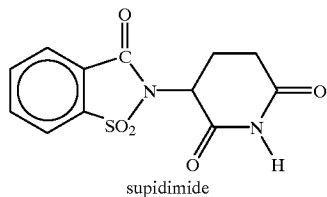
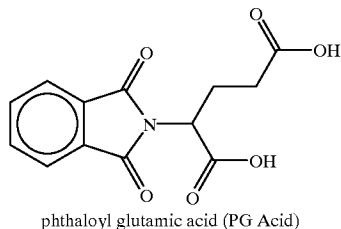
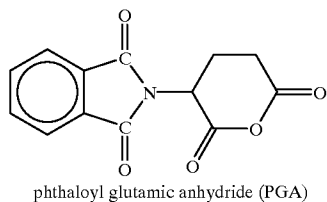
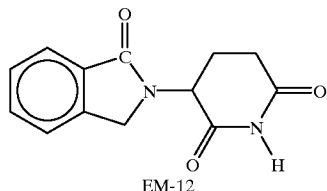
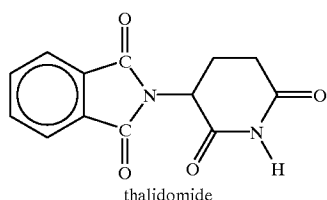
EXAMPLE III

Inhibition of bFGF Induced Corneal Neovascularization By Thalidomide and Related Analog Expressed as Percent of Median Control on Day 8

Pellets containing bFGF and sucralfate were implanted into micropockets of both corneas of rabbits according to

Example II. Vessel ingrowth into clear cornea from the limbus was first noted on day 2 and treatments (200 mg/kg orally) were begun on this day. The area of corneal neovascularization was measured from day 4 through day 12. Day 8 measurements were used for comparison between groups. No regression of vessels and near maximal neovascularization was seen at this time point. Statistical analysis was performed with ANOVA with ranked data to account for interexperimental variation and to guard against a non-normal distribution of data (i.e. outliers) by utilizing a nonparametric method.

The compounds tested were as follows:



Treatment with a dose of (200 mg/kg) of thalidomide resulted in an inhibition of the area of vascularized cornea that ranged from 30–51% in three experiments with a median inhibition of 36% (FIG. 6) (n=30 eyes, p=0.0001, 2 way ANOVA with ranked data). The inhibition of angiogenesis by thalidomide was seen after only two doses (FIG. 7). The rabbits did not demonstrate obvious sedation and there were no signs of toxicity or weight loss. The teratogenic analog EM-12, which shares the other properties of thalidomide was also inhibitory, with a median inhibition of 42% (n=10 eyes, p=0.002, 1-way ANOVA with ranked data). Supidimide, a nonteratogenic analog of thalidomide that retains the sedative properties of thalidomide, exhibited no activity (area 107% of control, n=10 eyes, not statistically

different from control). Other analogs, PGA and PG acid displayed weaker inhibitory effects than thalidomide (data not shown). The density of vessel ingrowth in thalidomide-treated animals was also markedly reduced.

EXAMPLE IV

EM-12 in Rabbit Cornea Assay

EM-12 was tested in the rabbit cornea assay described in Example II at 100 mg/kg/day and showed 21% inhibition, and at 200 mg/kg/day the assay showed 43% inhibition.

EXAMPLE V

Phthaloyl Glutamic Acid in CAM

Phthaloyl glutamic acid was tested in the above described CAM assay and exhibit an avascular zone with a mild scar.

EXAMPLE VI

Phthaloyl Glutamic Acid in Rabbit Cornea Assay

Phthaloyl glutamic acid described above at 200 mg/kg and exhibited 29% inhibition of angiogenesis.

EXAMPLE VII

Phthaloyl Glutamic Anhydride in CAM Assay

Phthaloyl glutamic anhydride was test in the CAM assay described above and exhibited an avascular zone.

It should be understood, of course, that the foregoing relates only to preferred embodiments of the present invention and that numerous modifications or alterations may be made therein without departing from the spirit and the scope of the invention as set forth in the appended claims.

What is claimed is:

1. A method of treating undesired angiogenesis in a human or animal having a tumor comprising administering to the human or animal in need thereof an angiogenesis inhibiting amount of thalidomide.
2. The method of claim 1 wherein the tumor is Kaposi's sarcoma.
3. The method of claim 1 wherein the tumor is heman-gioma.
4. The method of claim 1 wherein the tumor is a solid tumor.
5. The method of claim 1 wherein the tumor is a blood borne tumors.
6. The method of claim 1 wherein the tumor is rhab-domyosarcoma.
7. The method of claim 1 wherein the tumor is retino-blastoma.
8. The method of claim 1 wherein the tumor is Ewing's sarcoma.
9. The method of claim 1 wherein the tumor is neuro-blastoma.
10. The method of claim 1 wherein the tumor is osteosa-roma.
11. The method of claim 1 wherein the tumor is neurofi-broma.
12. The method of claim 1 wherein the tumor is pyogenic granulomas.
13. The method of claim 1 wherein the tumor is breast cancer.
14. The method of claim 1 wherein the amount adminis-tered is between approximately 0.1 and approximately 300 mg/kg/day.
15. The method of claim 14 wherein the amount adminis-tered is between approximately 0.5 and approximately 50 mg/kg/day.
16. The method of claim 15 wherein the amount admin-istered is between approximately 1 and approximately 10 mg/kg/day.

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17. The method of claim 1 wherein the thalidomide is administered in the form of a tablet.

18. The method of claim 1 wherein the thalidomide is administered in the form of a capsule.

19. The method of claim 1 wherein the thalidomide is administered in the form of a lozenge, a cachet, a solution, a suspension, an emulsion, a powder, an aerosol, a suppository, a spray, a pastille, an ointment, a cream, a paste, a foam, a gel, a tamport, or a pessary.

20. The method of claim 1, 4, 5, or 14 wherein the administration is oral.

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21. The method of claim 1, 4, 5, or 14 wherein the administration is sublingual, buccal, rectal, vaginal, or nasal.

22. The method of claim 1, 4, 5, or 14 wherein the administration is parenteral.

23. The method of claim 1, 4, 5, or 14 wherein the administration is transdermal or topical.

24. A method of treating undesired angiogenesis in a human or animal having leukemia comprising administering to the human or animal in need thereof an angiogenesis inhibiting amount of thalidomide.

* * * * *

EXHIBIT C



US007435745B2

(12) **United States Patent**
D'Amato

(10) **Patent No.:** **US 7,435,745 B2**
(45) **Date of Patent:** **Oct. 14, 2008**

(54) **METHODS AND COMPOSITIONS FOR INHIBITION OF ANGIOGENESIS**

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

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

(21) Appl. No.: **11/411,230**

(22) Filed: **Apr. 26, 2006**

(65) **Prior Publication Data**

US 2007/0049566 A1 Mar. 1, 2007

Related U.S. Application Data

(63) Continuation of application No. 09/287,377, filed on Apr. 7, 1999, now abandoned, which is a continuation of application No. 08/963,058, filed on Nov. 3, 1997, now abandoned.

(60) Provisional application No. 60/028,708, filed on Nov. 5, 1996.

(51) **Int. Cl.**
A61K 31/445 (2006.01)

(52) **U.S. Cl.** **514/323**

(58) **Field of Classification Search** None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

2,830,991 A	4/1958	Keller et al.	260/281
3,560,495 A	2/1971	Frankus	260/247.1
3,563,986 A	2/1971	Frankus	260/247.1
3,625,946 A	12/1971	Heinrich et al.	260/281
3,705,162 A	12/1972	Graudums et al.	260/281
4,552,888 A	11/1985	Koppel et al.	514/474
4,994,443 A	2/1991	Folkman et al.	514/56
5,001,116 A	3/1991	Folkman et al.	514/56
5,021,404 A	6/1991	Folkman et al.	514/26
5,134,127 A	7/1992	Stella et al.	514/58
5,385,901 A	1/1995	Kaplan et al.	514/231.5
5,399,363 A	3/1995	Liversidge et al.	424/490
5,405,855 A	4/1995	Andrulis, Jr. et al.	514/323
5,434,170 A	7/1995	Andrulis, Jr. et al.	514/323
5,443,824 A	8/1995	Piacquadio	424/78.02
5,502,066 A	3/1996	Heinemann	514/360
5,605,684 A	2/1997	Piacquadio	424/78.02
5,605,914 A	2/1997	Muller	514/339
5,629,327 A *	5/1997	D'Amato	514/323
5,643,915 A	7/1997	Andrulis, Jr. et al.	514/279
5,654,312 A	8/1997	Andrulis, Jr. et al.	514/279
5,679,696 A	10/1997	Fenton et al.	514/354
5,731,325 A	3/1998	Andrulis, Jr. et al.	514/323
6,235,756 B1 *	5/2001	D'Amato	514/323

FOREIGN PATENT DOCUMENTS

EP	1182709	3/1970
EP	0325199	7/1989
EP	0357061	3/1990

JP	58-131978	8/1983
JP	63-119500	5/1988
WO	WO 91/10424	7/1991
WO	WO 92/14455	9/1992
WO	WO 92/18496	10/1992
WO	WO 94/20085	9/1994
WO	WO 95/04533	2/1995

OTHER PUBLICATIONS

Gutman et al. Failure of Thalidomide to inhibit tumor growth and angiogenesis in vivo. *Anticancer Research*, 1996, 16:3673-3677.*
 Thomas et al. Current role of thalidomide in cancer treatment. *Current Opinion in Oncology*, 2000, 12:564-573.*
 Alexanian et al. Primary dexamethasone treatment of multiple myeloma. *Blood*, 1992, vol. 80, No. 4, pp. 887-890.*
 Hendler, S.S. et., "Immune Modulators Thalidomide", *The Oxygen Breakthrough 30 Days to an Illness-Free Life*, William Morrow and Company, Inc., New York, pp. 217-219 (1989).
 Kenyon, et al., "The Discovery of New Inhibitors of Angiogenesis Using an Improved Mouse Corneal Neovascularization Model", *Association for Research in Vision and Ophthalmology*, 1995 Annual Meeting, Fort Lauderdale, Florida, No. 459-367, p. S94 (Abstract Only) (1995).
 Neubert, "Teratogenicity: Any Relationship to Carcinogenicity?"; *International Agency for Research on Cancer*, Cedex, France, Institute for Toxicology and Embryopharmacology, Free University of Berlin, Berlin Federal Rep. of Germany, pp. 169-178, (1979).
 Peyron, et al., "The Pharmacological Basis for the Treatment of Phodermatoses"; *Biochimie*, vol. 68, No. 6, pp. 899-904 (1986).
 Proenca, N.G., "Thalidomide: An Eclectic Medication in Dermatology"; *Rev. Paul. Med.*, vol. 107, No. 1, pp. 41-46. (Abstract Only) (1989).
 Robbins, K.C. "The Plasminogen-Plasmin Enzyme System"; *Fibrinolysis, Hemostasis and Thrombosis Basic Principles and Clinical Practice*, 2nd Ed., Chapter 21, pp. 340-351 (1987).
 Smith, et al., "Studies on the Relationship Between the Chemical Structure and Embryotoxic Activity of Thalidomide and Related Compounds"; *Chemical Structure and Embryopathy*, London, pp. 194-209 (1965).
 Wesolowski, et al., "Effect of Light on a Murine Model of Retinopathy of Prematurity"; *Assn. for Research in Vision and Ophthalmology*, Annual Meeting, Sarasota, Florida (1992).
 Algire, G. H., "Vascular Reactions of Normal and Malignant tissues In Vivo. I. Vascular Reactions of Mice to Wounds and to Normal and Neoplastic Transplants"; *J. Nat'l Canc. Inst.*, pp. 73-85 (1945).

(Continued)

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(57) **ABSTRACT**

The present invention comprises a group of compounds that effectively inhibit angiogenesis. More specifically, thalidomide and various related compounds such as thalidomide precursors, analogs, metabolites and hydrolysis products have been shown to inhibit angiogenesis and to treat disease states resulting from angiogenesis. Additionally, antiinflammatory drugs, such as steroids and NSAIDs can inhibit angiogenesis dependent diseases either alone or in combination with thalidomide and related compounds. Importantly, these compounds can be administered orally.

US 7,435,745 B2

Page 2

OTHER PUBLICATIONS

- Allegri, A., "Confermata l'inefficacia della Talidomide Nella Terapia dei Tumori", *Gazzetta Medica Italiana*, pp. 124-127 (1964).
- Amb, S. et al., "Interactive Effects of Nitric Oxide and the p53 Tumor Suppressor Gene in Carcinogenesis and Tumor Progression", *The FASEB Journal*, vol. 11, pp. 443-448 (1997).
- Apt, W. "Effect of Thalidomide on the Course of Experimental Chagas' Disease", *BOI. Chil. Parasitol.*, vol. 20, No. 3, pp. 84-86 (1965).
- Aronson, et al., "Thalidomide-Induced Peripheral Neuropathy. Effect of Serum Factor on Nerve Cultures", *Arch. Dermatol.*, vol. 120, No. 11, pp. 1466-1470 (1984).
- Bach, et al., "Studies on the Possible Anti-Neoplastic Effect of Thalidomide", *ACTA Pathologica ET Microbiologica scandinavica*, vol. 59, pp. 491-499 (1963).
- Bach, et al., "Thalidomide in Cancer Chemotherapy", *The Lancet*, vol. 1, p. 1271 (1963).
- Bahmer, F.A., "Therapie bei lymphozytischer Infiltration", *Der Hautarzt*, vol. 43, p. 663 (1992).
- Balabanova, et al., "Lupus Erythematosus Hypertrophicus et Profundus", *Z. Hautkr.*, vol. 67, No. 9, pp. 812-815 (1992).
- Barnes, et al., "Tumor Necrosis Factor Production in Patients with Leprosy", *Infection and Immunity*, vol. 60, No. 4, pp. 1441-1446 (1992).
- Barnhill, et al., "Thalidomide: Use and Possible Mode of Action in Reactional Lepromatous Leprosy and in Various Other Conditions", *J. Am. Acad. Dermatol.*, vol. 7, No. 3, pp. 317-323 (1982).
- Barnhill, R. et al., "Studies on the Anti-inflammatory Properties of Thalidomide: Effects on Polymorphonuclear Leukocytes and Monocytes", *J. Am. Acad. Derm.*, vol. 11, No. 5, Part 1, pp. 814-819 (1984).
- Barriere, H., "Traitement par la thalidomide", *La Presse Médicale*, vol. 12, No. 15, pp. 963 (1983).
- Bazzoli, et al., "The Effects of Thalidomide and Two Analogues on the Regenerating Forelimb of the Newt", *J. Embryol. Exp. Morph.*, vol. 41, pp. 125-135 (1977).
- Beck, Jr. et al. "Vascular Development: Cellular and Molecular Regulation", *The FASEB Journal*, vol. 11, pp. 365-374 (1997).
- Beccerica, E., "L'approccio terapeutico al paziente anziano con artrite reumatoide", *Clin. Ter.*, vol. 122, pp. 289-298 (1987).
- Belaube, P. et al., "Should Thalidomide be Rehabilitated?", *Sem. Hop. Paris*, vol. 59, No. 45, pp. 3101-3104 (1983).
- Bensinger, W.L., "Supportive Care in Marrow Transplantation", *Curr. Opin. Oncol.*, vol. 4, No. 4, pp. 614-623 (1992).
- Bernal, et al., "Cellular Immune Effects of Thalidomide in Actinic Prurigo", *Int'l. J. of Dermat.*, vol. 31, No. 8, pp. 599-600 (1992).
- Blair, R. et al., "Human Mast Cells Stimulate Vascular Tube Formation", *Journal of Clinical Investigation*, vol. 99, No. 11, pp. 2691-2700 (1997).
- Blaschke, V. et al., "Chromatographische Racemattrennung von Thalidomid und teratogene Wirkung der Enantiomere, *Arzneimittel Forsch./Drug Res.*, vol. 29, No. II, pp. 1640-1642 (1979).
- Bonifacino, J. et al., "A Peptide Sequence Confers Retention and Rapid Degradation in the Endoplasmic Reticulum", *Science*, vol. 2, pp. 79-80 (1990).
- Bonnetblanc, et al., "Thalidomide and Recurrent Aphthous Stomatitis: a Follow-up Study", *Dermatology*, vol. 193, No. 4, pp. 321-323 (1996).
- Boodman, S.G., "Questions About a Popular Prenatal Test", *The Washington Post* (1992).
- Bowers, et al., "Effect of Thalidomide on Orogenital Ulceration", *Br. Med. Journ.*, vol. 287, No. 6395, pp. 799-800 (1983).
- Braun, et al., "Thalidomide Metabolite Inhibits Tumor Cell Attachment to Concanavalin A Coated Surfaces", *Biochem. Biophys. Res. Comm.*, vol. 98, No. 4, pp. 1029-1034 (1981).
- Brem, H. et al., "Interstitial Chemotherapy with Drug Polymer Implants for the Treatment of Recurrent Gliomas", *J. Neurosurg.*, vol. 74, pp. 441-446 (1991).
- Bressler, S. et al., "Clinicopathologic Correlation of Occult Choroidal Neovascularization in Age-Related Macular Degeneration", *Arch. Ophthalmol.*, vol. 110, p. 827 (1992).
- Brodthagen, H., "Significant Response of Oral Aphthosis to Thalidomide Treatment", *J. Am. Acad. Dermatol.*, vol. 13, No. 3, p. 509 (1985).
- Brovarone, et al., "Occhio e gravidanza", *Minerva Ginecol.*, vol. 43, pp. 141-167 (1991).
- Browne, M.J. et al., Expression of Recombinant Human Plasminogen and Aglycoplasminogen in HeLa Cells, *Fibrinolysis*, vol. 5, pp. 257-260 (1991).
- Bubl, et al., "Dysphagia in Dermatologic Disease", *Dysphagia*, vol. 8, No. 2, pp. 85-90 (1993).
- Buckley, C. et al., "Pyoderma Gangrenosum with Severe Pharyngeal Ulceration", *J. of Royal Society of Medicine*, vol. 83, pp. 590, 591 (1990).
- Buelens, I., Treatment of a Grade II Astrocytoma with Thalidomide (Phthalylglutamic Acid Imide) [Behandlung eines Astrocytoms II. Grades mit Thaliodmid (N-Phthalylglutaminsäureimid)], *Arzneim.-Forsch.*, vol. 17, No. 5, pp. 646-648 (1967).
- Bullock, W.E., "The Clinical Significance of Erythema Nodosum", *Hosp. Pract.*, vol. 21, No. 3, pp. 102E-2H, 102K-2L, 102Q-2R pas (1986).
- Burger, et al., "Epidermolysis Bullosa Acquisita, a Rare Late Complication of Allogeneic Bone Marrow Transplantation?", *Bone Marrow Transplant*, vol. 9, No. 2, pp. 139-141 (1992).
- Burrows, N.P., "Thalidomide Modifies Disease", *Brit. Med. J.*, vol. 307, No. 6909, pp. 939-940 (1993).
- Buckley, C. et al., "Pyoderma Gangrenosum with Severe Pharyngeal Ulceration", *J. of the Royal Soc. of Med.*, vol. 83, pp. 590-591 (1990).
- Calnan, et al., "Actinic Prurigo (Hutchinson's Summer Prurigo)", *Clin. Exp. Dermatol.*, vol. 2, No. 4, pp. 365-372 (1977).
- Cant, J.S., "Minor Ocular Abnormalities Associated With Thalidomide", *The Lancet*, p. 1134 (1966).
- Carmichael, et al., "Thalidomide: A Restricted Role", *Lancet*, vol. 339, No. 8805, p. 1362 (1992).
- Cashin, C.H. et al., "Angiogenesis and Chronic Inflammation", *Agents and Actions*, vol. 34, No. 3/4, pp. 332-338 (1991).
- Casini, G. et al., "Preparazione Di Uno Degli Antipodi Ottici Della 2-Ftalimmidoglutarimide", *Il Pharmaco, Ed. Sc.*, vol. XIX, Fasc. 6, pp. 563-565 (1964).
- Chapon, et al., "Neuropathies Caused by Thalidomide", *Rev. Neurol.*, vol. 141, No. 11, pp. 719-728 (1985).
- Chaudhry, A. et al., "Effects of Prednisolone and Thalidomide on Induced Submandibular Gland tumors in Hampsters", *Canc. Res.*, vol. 26, No. 9, Part 1, pp. 1884-1886 (1966).
- Chen, T. et al., "Plasma Pharmacokinetics and Urinary Excretion of Thalidomide after Oral Dosing in Healthy Male Volunteers", *The Am. Society for Pharma. and Experi. Therapeutics*, vol. 17, No. 4, pp. 402-405 (1988).
- Chosidow, O. et al., "Sclerodermatous Chronic Graft-Versus-Host Disease: Analysis of Sevencases", *J. Am. Acad. Dermatol.*, vol. 26, No. 1, pp. 49-55 (1992).
- Claydon, et al., "Gastrointestinal Emergencies in HIV Infection", *Balliere's Clin Gastroenterol.*, vol. 5, No. 4, pp. 887-911 (1991).
- Clemmensen, et al., "Thalidomide Neurotoxicity", *Arch. Dermatol.*, vol. 120, No. 3, pp. 338-341 (1984).
- Colville-Nash, P.R. et al., "Angiogenesis and rheumatoid arthritis: Pathogenic and Therapeutic Implications", *Ann. Rheum. Dis.*, vol. 51, pp. 919-925 (1992).
- Congy, et al., "Plasma Zinc Levels in Elderly Patients Hospitalized in Long Stay Units. Correlations with Other Nutritional Markers, Immunological Tests and Survival", *Sem. Hop. Paris*, vol. 59, No. 45, pp. 3105-3108 (1983).
- Costa, et al., "Aseptic Adenitis in a Patient with Pyoderma Gangrenosum", *Ann. Dermatol.*, vol. 121, No. 8, pp. 550-552 (1994) (Abstract Only).
- Crain, E. et al., "The Effect of Thalidomide on Experimental Autoimmune Myasthenia Gravis", *J. of Autoimmunity*, vol. 2, pp. 197-202 (1989).
- Crawford, C.L., "Letter: Thalidomide in Erythema Nodosum Leprosum", *Lancet*, vol. 2, No. 839, pp. 1201-1202 (1973).
- Crawford, C.L., "Treatment of Erythema Nodosum Leprosum with Thalidomide", *Lancet*, vol. 2, No. 828, pp. 567-568 (1973).
- Crawford, C.L., "Use of Thalidomide in Leprosy [letter; comment]", *BMJ*, vol. 302, No. 6729, pp. 1603-1604 (1991).

US 7,435,745 B2

Page 3

- Crum, et al., "A New Class of Steroids Inhibits Angiogenesis in the Presence of Heparin or a Heparin Fragment", *Science*, vol. 230, pp. 1375-1378 (1985).
- Current Bibliographics in Medicine, "Thalidomide: Potential Benefits and Risks", *National Inst. Health, National Library of Medicine*, pp. 1-72 (Jan. 1963-Jul. 1997).
- Dark, et al., "Combretastin A-4, an Agent that Displays Potent and Selective Toxicity Toward Tumor Vasculature", *Cancer Research*, vol. 37, pp. 1829-1834 (1997).
- David-Bajar, K.M., "Subacute Cutaneous Lupus Erythematosus", *J. Invest. Dermatol.* vol. 100, No. 1, pp. 2s-8s (1993).
- D'Amato, R. J. et al., "Thalidomide is an Inhibitor of Angiogenesis", *Proc. Natl. Acad. Sci.*, vol. 91, pp. 4082-4085 (1994).
- D'Amato, et al., "Angiogenesis Inhibition in Age-Related Macular Degeneration", *Ophthalmology*, vol. 102, No. 9, pp. 1261-1262 (1995).
- D'Amato, et al., "Microscopic Analysis of Retinal-Vessels Utilizing Fluorescein-Labeled High-Molecular-Weight Dextran", *Invest. Ophthalmol. & Visual Science*, vol. 33, No. 4, p. 1082 (1992).
- De, A.U. et al., "Possible Antineoplastic Agents I", *J. of Pharma. Sci.*, vol. 64, No. 2, pp. 262-266 (1975).
- De Cock, K., "Treatment of Ulcerative Colitis", *Brit. Med. J.*, vol. 1, pp. 1356 (1979).
- DeKlerk, et al., "New Methods of Treatment for Renal Allografts Using the Baboon as a Primate Experimental Model", *J. Urol.* vol. 102, No. 5, pp. 532-540 (1969).
- Dhodapkar, et al., "A Phase II Pilot Study of Anti-Angiogenesis Therapy Using Thalidomide in Patients with Multiple Myeloma", *UARK 98-003*, pp. 1-15, 1995.
- Dicken, C. H., "Malignant pyoderma", *J. of the Am. Acad. of Dermatol.*, vol. 13, No. 6, pp. 1021-1025 (1985).
- DiPaolo, et al., "Thalidomide: Effects on Ehrlich Ascites Tumor Cells in vitro", *Science*, vol. 144, pp. 1583 (1964).
- DiPaolo, "Effect of Thalidomide on a Variety of Transplantable Tumors", *Cancer Chemo. Reports*, vol. 29, pp. 99-102 (1963).
- DiPaolo, "In Vitro Test Systems for Cancer Chemotherapy. II. Correlation of In Vitro Inhibition of Dehydrogenase and Growth with In Vivo Inhibition of Ehrlich Ascites Tumor", *P.S.E.B.M.*, vol. 114, pp. 384-387 (1963).
- DiPaolo et al., "Teratogenesis-oncogenesis: A Study of Possible Relationships", *Arch. Path.*, vol. 81, pp. 3-23 (1966).
- Dorveaux, et al., "Le Traitement Actuel du Lupus Erythemateux Chronique", *Le Concours Med.* vol. 106, No. 31, pp. 2957-2961 (1984).
- Doutre, et al., "Pyoderma Gangrenosum and Hemopathies. A propos of 2 Cases", *Nouv Rev Fr Hematol*, vol. 29, No. 4, pp. 251-254 (1987).
- Dunn, et al., "Bone Marrow Transplantation and Cataract Development", *Arch. Ophthalmol.* vol. 111, No. 10, pp. 1367-1373 (1993).
- (Editorial) "Thalidomide in Dermatology and Leprosy", *Lancet*, vol. 2, No. 8446, pp. 80-81 (1985).
- (Editorial) "Thalidomide Tested for Treatment of AIDS", *U.S. Pharm.*, vol. 18, No. 8, p. 14 (1993).
- Eger, K. et al., "Synthesis, Central Nervous System Activity and Teratogenicity of a Homothalidomide", *Arzheim. Forsch/Drug Res.*, vol. 40 (II), No. 10, pp. 1073-1075 (1990).
- Ehrlich, G.E., "Behcet's Disease: Current Concepts", *Comprehensive Therapy*, vol. 15, No. 1, pp. 27-30 (1989).
- Elia, et al., "Giant Esophageal Ulcer Treated with Steroids in AIDS Patient(2)", *J. Acquired Immune Defic. Syndr.*, vol. 5, No. 8, pp. 848-849 (1992).
- Eisenbud, L. et al., "Recurrent Aphthous Stomatitis of the Behcet's Type: Successful Treatment with Thalidomide", *Oral Surgery, Oral Medicine, Oral Pathology*, vol. 64, No. 3, pp. 289-292 (1987).
- Eravelly, J. et al., "Thalidomide in Weber-Christian Disease", *The Lancet*, vol. 1, No. 8005, p. 251 (1977).
- Eriksson, et al., "Drug Exposure and Flow Cytometry Analyses in a Thalidomide Treatment Schedule that Prolongs Rat Cardiac Graft Survival", *Transplant Proc.*, vol. 24, No. 6, pp. 2560-2561 (1992).
- Eriksson, S. O. et al., "Synthesis and Alkaline Hydrolysis of Some N-substituted Phthalimides", *Acta Pharm. Suecica*, vol. 10, pp. 63-74 (1973).
- Fabro, S. et al., "The Metabolism of Thalidomide: Some Biological Effects of Thalidomide and its Metabolites", *Brit. J. Pharmacol.*, vol. 25, pp. 352-362 (1965).
- Fabro, S. et al., "Teratogenic Activity of Thalidomide and Related Compounds", *Life Sci.*, vol. 3, pp. 987-992 (1964).
- Fabro, S. M.D., "Biochemical Basis of Thalidomide Teratogenicity", *The Biochemical Basis of Chemical Teratogenesis*, Chapter 5, pp. 159-178 (1981).
- Fajardo, et al., "Dual Role of Tumor Necrosis Factor- α in Angiogenesis", *Am. J. Pathol.*, vol. 140, No. 3, pp. 539-544 (1992).
- Faure, et al., "PMN Leukocytes Chemotaxis: Inhibition by Thalidomide", *Pathol. Biol. (Paris)*, vol. 29, No. 10, pp. 601-604 (1981).
- Faure, et al., "Inhibition of PMN Leukocytes Chemotaxis by Thalidomide", *Arch. Dermatol. Res.*, vol. 269, No. 3, pp. 275-280 (1980).
- Fazal, N. et al., "Effect of Blocking TNF- α on Intracellular BCG (*Bacillus Calmette Guerin*) Growth in Human Monocyte-Derived Macrophages", *FEMS Microbiology Immunology*, vol. 105, pp. 337-346 (1992).
- Fickentscher, K. et al., "Stereochemical Properties and Teratogenic Activity of Some Tetrahydrophthalimides", *Mol. Pharmacol.*, vol. 13, pp. 133-141 (1977).
- Field, E. O. et al., "Effect of Thalidomide on Splenomegaly (GVH Reaction) in the Chick Embryo. Treatment of Donor Cells Only", *Nature*, vol. 211, No. 5055, pp. 1309-1310 (1966).
- Flohe, L. et al., "Studies on the Hypothetical Relationship of Thalidomide-induced Embryopathy and Collagen Biosynthesis", *Arzneimittel/Forschung (Germany West)*, vol. 31, No. 2, pp. 315-320 (1981).
- Folkman, J. et al., "Angiogenesis", *J. Biol. Chem.*, vol. 267, No. 16, pp. 10931-10934 (1992).
- Folkman, J. et al., "Angiogenesis Inhibition and Tumor Regression Caused by Heparin or a Heparin Fragment in the Presence of Cortisone", *Science*, vol. 221, pp. 719-725 (1983).
- Folkman, J. et al., "Induction of Angiogenesis During the Transition from Hyperplasia to Neoplasia", *Nature*, vol. 339, pp. 58-61 (1989).
- Folkman, J. et al., "Tumor Behavior in Isolated Perfused Organs: In Vitro Growth and Metastases of Biopsy material in Rabbit Thyroid and Canine Intestinal Segment", *Anal. of Surgery*, pp. 491-502 (1966).
- Folkman, J., "Tumor Angiogenesis: Therapeutic Implications", *The New England J. of Med.*, vol. 285, No. 21, pp. 1182-1186, (1971).
- Folkman, J., "Angiogenesis and Its Inhibitors", *Important Advances in Oncology*, J.B. Lippincott H. Company, pp. 42-62 (1985).
- Folkman, J., "Angiogenesis in Cancer, Vascular, Rheumatoid and Other Disease", *Nature Medicine*, vol. 1, No. 1, pp. 27-30 (1995).
- Folkman, J., "What is the Evidence that Tumors are Angiogenesis Dependent?", *J. Natl' Cancer Inst.*, vol. 82, No. 1, (1990).
- Francois, J., "Embryological Pigment Epithelial Dystrophies", *Ophthalmologica*, vol. 172, pp. 417-433 (1976).
- Fuller, et al., "Thalidomide, Peripheral Neuropathy and AIDS", *Int. J. STD AIDS*, vol. 2, No. 5, pp. 369-370 (1991).
- Furner, B.B., "Treatment of Subacute Cutaneous Lupus Erythematosus", *Int. J. Dermatol.*, vol. 29, No. 8, pp. 542-547 (1990).
- Gad, et al., "Thalidomide Induces Imbalances in T-Lymphocyte Sub-Populations in the Circulating Blood of Healthy Males", *Lepr. Rev.*, vol. 56, No. 1, pp. 35-39 (1985).
- Gaetani, M., "Study Sull' Attivita Antitumorale Della Talidomide", *Giornale Italiano diu Chemioterapia*, pp. 83-86, 1994.
- Gehanno, et al., "Mouth and Pharyngeal Hyperalgesic Syndromes in AIDS", *Ann. Otolaryngol Chir. Cervicofac*, vol. 107, No. 5, pp. 311-313 (1990).
- Genvo, et al., "Treatment of Aphthosis with Thalidomide and with Colchicine", *Dermatologica*, vol. 168, No. 4, pp. 182-188 (1984).
- Geoghiou, et al., "HIV-Associated Oesophageal Ulcers Treated with Thalidomide", *Med. J. of Australia*, vol. 152, pp. 382-383 (1990).
- Gershbein, "Effect of Transplanted Tumor and Various Agents on Liver Regeneration During Pregnancy", *P.S.E.B.M.*, vol. 126, pp. 88-92 (1967).
- Gershbein, L. L., "The Thalidomide Analog, EM 12, Enhances 1,2-dimethylhydrazine-induction of Rat Colon Adenocarcinomas", *Canc. Ltrs.*, vol. 60, pp. 129-133 (1991).

US 7,435,745 B2

Page 4

- Gershbein, L. L., "Effect of Various Agents on Liver Regeneration and Walker Tumor Growth in Partially Hepatectomized Rats", *Canc. Res.*, vol. 26, No. 9, pp. 1905-1908 (1966).
- Ghigliotti, G. et al., "Thalidomide: Treatment of Choice for Aphthous Ulcers in Patients Seropositive for Human Immunodeficiency Virus", *J. of the Am. Acad. of Dermatol.*, vol. 28, No. 2, Part I, pp. 271-272 (1993).
- Gimbrone, M.A. et al., "Tumor Dormancy in Vivo by Prevention of Neovascularization", *Journal of Experimental Medicine*, vol. 136, pp. 261-276 (1972).
- Gimbrone, M. A. et al., "Tumor Growth and Neovascularization: An Experimental Model Using the Rabbit Cornea", *J. Nat. Canc. Instit.*, vol. 52, No. 2, pp. 413-419 (1974).
- Goihman-Yahr, Mauricio et al., "Significance of Neutrophil Activation in Reactional Lepromatous Leprosy: Effects of Thalidomide in vivo and in vitro. Activation in Adjuvant Disease", *Int. Archs. Allergy Appl. Immun.*, vol. 57, pp. 317-332 (1978).
- Goihman-Yahr, M., et al., "Autoimmune Diseases and Thalidomide II. Adjuvant Disease, Experimental Allergic Encephalomyelitis and Experimental Allergic Neuritis of the Rat", *Int. J. Leprosy*, vol. 42, No. 3, pp. 266-275 (1974).
- Gorin, I. et al., "Thalidomide May Cure AIDS Ulcers", *Nurs. Times*, vol. 86, No. 24, p. 10 (1990).
- Gorin, I., et al., "Thalidomide in Hyperalergic Pharyngeal Ulceration of AIDS", *Lancet*, vol. 335, p. 1343 (1990).
- Goulden, V., et al., "Linear Prurigo Simulating Dermatitis Artefacta in Dominant Dystrophic epidermolysis Bullosa", *Br. J. Dermatol.*, vol. 129, No. 4, pp. 443-446 (1993).
- Grabstald, H. et al., "Clinical Experiences with Thalidomide in Patients with Cancer", *Clinical Pharmacol. and Therapeutics*, vol. 6, pp. 298-302 (1965).
- Grant, D.S. et al., "Scatter Factor Induces Blood Vessel Formation in vivo", *Proc. Natl. Acad. Sci.*, vol. 90, pp. 1937-1941 (1993).
- Grinspan, et al., "Treatment of Aphthae with Thalidomide", *J. Am. Acad. Dermatol.*, vol. 20, No. 6, pp. 1060-1063 (1989).
- Grinspan, D., "Significant Response of Oral Aphthosis to Thalidomide Treatment", *Am. Acad. of Dermatol.*, vol. 12, No. 1, Part I, pp. 85-90 (1985).
- Grosshans, E., et al., "Thalidomide Therapy for Inflammatory Dermatoses", *Int. J. Dermatol.*, vol. 23, No. 9, pp. 598-602 (1984).
- Guidetti, E., et al., "Ricerche Sull'azione Immunodepressiva Della Talidomide e del Prednisolone in Ratti Portatori Di Neoplasie Sperimentalmente Indotte", *Cancro*, vol. 22, pp. 503-512 (1969).
- Günzler, V., "Thalidomide in Human Immunodeficiency Virus (HIV) Patients. A Review of Safety Considerations", *Drug Saf.*, vol. 7, No. 2, pp. 116-134 (1992).
- Günzler, V., "Thalidomide—A Therapy for the Immunological Consequences of HIV Infection?", *Medical Hypotheses*, vol. 30, No. 2, pp. 105-109 (1989).
- Gutiérrez-Rodríguez, O. et al., "Treatment of Refractory Rheumatoid Arthritis—The Thalidomide Experience", *The J. of Rheumatology*, vol. 16, No. 2, pp. 158-163 (1989).
- Gutiérrez-Rodríguez, O. et al., "Thalidomide A Promising New Treatment for Rheumatoid Arthritis", *Arthritis and Rheumatism*, vol. 27, No. 10, pp. 1118-1121 (1984).
- Haffner, M.E. M.D., "Studies Involving Orphan Products for Treating/ Diagnosing Women's Diseases", *Food and Drug Law Journal*, vol. 48, pp. 205-211 (1992).
- Hamza, M. M.D., "Behcet's Disease, Palmoplantar Pustulosis and HLA-B27 Treatment with Thalidomide", *Clinical and Experimental Rheumatology*, vol. 8, No. 4, p. 427 (1990).
- Hamza, M.H., "Treatment of Behcet's Disease with Thalidomide", *Clinical Rheumatol.*, vol. 5, No. 3, pp. 365-371 (1986).
- Handley, J. et al., "Chronic Bullous Disease of Childhood and Ulcerative Colitis", *Pediatric Dermatol.*, vol. 10, No. 3, pp. 256-258, (1993).
- Handley, J. et al., "Chronic Bullous Disease of Childhood and Ulcerative Colitis", *Brit. J. of Dermatol.*, vol. 127, Supp. 40, pp. 67-68 (1992).
- Harindra, et al., "Papulo-Pruritic Eruption and Giant Ulceration of the Mouth: A Difficult Clinical Feature to Treat in the Patient Infected with Humanimmunodeficiency Virus(1)", *Arch. Intern. Med.*, vol. 152, No. 9, p. 1924 (1992).
- Harris, A., "Antiangiogenesis for Cancer Therapy", *Lancet*, vol. 349 (Suppl. II), pp. 13-15 (1997).
- Hasper, M.F., "Chronic Cutaneous Lupus Erythematosus. Thalidomide Treatment of 11 Patients", *Arch. Dermatol.*, vol. 19, No. 10, pp. 812-815 (1983).
- Hasper, M.F., et al., "Thalidomide in the Treatment of Chronic Discoid Lupus", *Acta. Derm. Venereol.*, vol. 62, No. 4, pp. 321-324 (1982).
- Hastings, R.C., et al., "Thalidomide in the Treatment of Erythema Nodosum Leprosum. With a Note on Selected Laboratory Abnormalities in Erythema Nodosum Leprosum", *Clin. Pharmacol. Ther.*, vol. 11, No. 4, pp. 481-487 (1970).
- Hatfill, S.J. et al., "Induction of Morphological Differentiation in the Human Leukemic Cell Line K562 by Exposure to Thalidomide Metabolites", *Leukemia Res.*, vol. 15, No. 2/3, pp. 129-136 (1991).
- Hayashi, S. et al., "A Synthetic Peptide for α Chemokines Inhibits the Growth of Melanoma Cell Lines", *J. Clin. Invest.*, vol. 99, No. 11, pp. 2581-2587 (1997).
- Heaton, et al., "Graft-versus-Host Disease Following Liver Transplantation", *J. R. Soc. Med.*, vol. 85, No. 6, pp. 313-314 (1992).
- Hellman, K. et al., "Prolongation of Skin Homograft Survival by Thalidomide", *Brit. Med. J.*, vol. 2, pp. 687-689 (1965).
- Helm, F. -Ch. et al., "Comparative Teratological Investigation of Compounds Structurally and Pharmacologically Related to Thalidomide", *Arzneim.-Forsch./Drug Res.*, vol. 31 (I), No. 6, pp. 941-949 (1981).
- Hendler, S. S. et al., "Thalidomide for Autoimmune Disease", *Medical Hypotheses*, vol. 10, pp. 437-443 (1983).
- Heny, D. et al., "Thalidomide Treatment for Chronic Graft-Versus-Host Disease", *Brit. J. of Haematol.* vol. 78, pp. 23-27 (1991).
- Heny, D., et al., "Thalidomide in the Treatment of Graft-versus-Host Disease", *Biomed. Pharmacother.*, vol. 44, No. 4, pp. 199-204 (1990).
- Heny, D., et al., "Thalidomide for Chronic Graft-versus-Host Disease in Children", *Lancet*, vol. 2, No. 8623, p. 1317 (1988).
- Hojyo, et al., "Actinic Prurigo (9)", *Int. J. Dermatol.*, vol. 31, No. 5, pp. 372-373 (1992).
- Holm, et al., "Chronic Cutaneous Lupus Erythematosus Treated with Thalidomide", *Arch. Dermatol.*, vol. 129, No. 12, pp. 1548-1550 (1993).
- Hori, A. et al., "Suppression of Solid Tumor Growth by Immunoneutralizing Monoclonal Antibody against Human Basic Fibroblast Growth Factor", *Canc. Res.*, vol. 51, pp. 6180-6184 (1991).
- Hu, D.E., "Inhibition of Angiogenesis in Rats by IL-1 Receptor Antagonist and Selected Cytokine Antibodies", *Inflammation*, vol. 18, pp. 45-58 (1994).
- Hu, J. et al., "A Novel Regulatory Function of Proteolytically Cleaved VEGF-2 for Vascular Endothelial and Smooth Muscle Cells", *The FASEB Journal*, vol. 11, pp. 498-504 (1997).
- Ingber, D. et al., "Synthetic Analogues of Fumagillin that Inhibit Angiogenesis and Suppress Tumor Growth", *Ltrs. to Nat.*, vol. 348, pp. 555-557 (1990).
- Ingber, D., "Drug News and Trial Developments", *AIDS Patient Care*, vol. 6, No. 6, p. 288 (1992).
- Randall, T., "Thalidomide's Back in the News, but in more Favorable Circumstances", *JAMA*, vol. 263, No. 11, pp. 1467-1468 (1990).
- Jacobson, J. et al., "Thalidomide for the Treatment of Oral Aphthous Ulcers in Patients with Human Immunodeficiency Virus Infection", *The New England Journal of Medicine*, vol. 336, No. 21, pp. 1487-1493 (1997).
- Jacobson, et al., "The Diagnosis and Treatment of Leprosy", *South Med. J.*, vol. 69, No. 8, pp. 979-985 (1976).
- Jager, et al., "Clinical Observations in the Treatment of Leprosy Reaction with Cyclic Imides", *Int. J. Lepr. Other Mycobact Dis.*, vol. 39, No. 2, p. 589 (1971).
- Jeltsch, M. et al., "Hypeplasia of Lymphatic Vessels in VEGF-C Transgenic Mice", *Science*, vol. 276, pp. 1423-1426 (1997).
- Jenkins, et al., "Thalidomide, Orogenital Ulcers, and Risk of Teratogenicity", *The Lancet*, vol. 1, No. 8417-18, p. 511 (1985).
- Jenkins, et al., "Thalidomide in Severe Orogenital Ulceration", *The Lancet*, vol. 2, No. 8417-18, pp. 1424-1426 (1984).
- Jennings, et al., "Effect of Actinomycin D on the Production of Acute Phase Protein in the Rabbit", *Experientia*, vol. 25, pp. 305-306 (1969).

US 7,435,745 B2

Page 5

- Jew, et al., "Thalidomide in Erythema Nodosum Leprosum", *DIAP*, vol. 24, No. 5, pp. 482-483 (1990).
- Johnke, et al., "Thalidomide Treatment of Prurigo Nodularis", *Ugeskr Laeger*, vol. 155, No. 38, pp. 3028-3030 (1993) (Abstract Only).
- Jönsson, N., "Chemical Structure and Teratogenic Properties IV. An Outline of a Chemical Hypothesis for the Teratogenic Action of Thalidomide", *Acta Pharm. Succica*, vol. 9, pp. 543-562 (1972).
- Jönsson, N., "Chemical Structure and Teratogenic Properties I. Synthesis and Teratogenic Activity in Rabbits of some Derivatives of Phthalimide, Isoindole-1-one, 1,2-Benzisothiazoline-3-one-1,1-Dioxide and 4(3II)-Quinazolinone", *Acta Pharm Succica*, vol. 9, pp. 431-436 (1972).
- Jönsson, N., "Chemical Structure and Teratogenic Properties III a Review of Available Data on Structure-Activity Relationships and Mechanism of Action of Thalidomide Analogues", *Acta Pharm. Succica*, vol. 9, pp. 521-542 (1972).
- Jorizzo, et al., "Thalidomide Effects in Behcet's Syndrome and Pustular Vasculitis", *Arch. Intern. Med.*, vol. 146, No. 5, pp. 878-881 (1986).
- Juret, et al., "Absence d'effet Carcino-Frérateur du Talidomide vis-à-vis de deux Tumerus Greffées", *Société de Biologie*, vol. 23, pp. 246-249 (1963).
- Kaitin, K.I., "Graft-versus-Host Disease", *N. Engl. J. Med.*, vol. 325, No. 5, pp. 357-358 (1991).
- Kaplan, G. et al., "TNF α regulation of HIV1: Biology and Therapy", *Res. in Immunology*, vol. 145, No. 8-9, pp. 685-690 (1994).
- Katsuta, et al., "Carcinogenesis in Tissue Culture. 3. Effects of the Second Treatments on DAB-Induced Proliferating Liver Cells of Normal Rats in Culture", *Jpn. J. Exp. Med.*, vol. 35, No. 4, pp. 231-248 (1965).
- Keenan, R.J. et al., "Immunosuppressive Properties of Thalidomide", *Transplantation*, vol. 52, No. 5, pp. 908-910 (1991).
- Kim, K. J. et al., "Inhibition of Vascular Endothelial Growth Factor-Induced Angiogenesis Suppresses Tumor Growth in vivo", *Ltrs. to Nat.*, vol. 362, pp. 841-844 (1993).
- Kitamoto, Y. et al., "Vascular Endothelial Growth Factor is an Essential Molecule for Mouse Kidney Development: Glomerulogenesis and Nephrogenesis", *J. Clin. Invest.*, vol. 99, No. 10, pp. 2351-2357 (1997).
- Klug, S. et al., "Embryotoxic Effects of Thalidomide Derivatives in the Non-Human Primate Callithrix Jacchus", *Arch. Toxicol.* vol. 68, pp. 203-205 (1994).
- Knighton, D. et al., "Avascular and Vascular Phases of Tumor Growth in the Chick Embryo", *Br. J. Canc.*, vol. 35, pp. 347-356 (1977).
- Knop, et al., "Thalidomide in the Treatment of Sixty Cases of Chronic Discoid Lupus Erythematosus", *Br. J. Dermatol.*, vol. 108, No. 4, pp. 461-466 (1983).
- Koch, H.P. et al., "4 Thalidomide and Congeners as Anti-inflammatory Agents", *Progress in Medicinal Chemistry*, vol. 22, pp. 166-242 (1985).
- Korn, et al., "The Second International Workshop on Scleroderma Research", *Matrix*, vol. 13, No. 5, pp. 427-429 (1993).
- Kroger, et al., "Synergistic Effects of Thalidomide and Poly (ADP-ribose) Polymerase Inhibition on Type II Collagen-Induced Arthritis in Mice", *Inflammation*, vol. 20, No. 2, pp. 203-215 (1996).
- Kundu, et al., "Prurigo Nodularis in an HIV Positive Man (2)", *Genitourinary Medicine*, vol. 71, No. 2, pp. 129-130 (1995).
- Kürkcüoğlu, N. et al., "Thalidomide in the treatment of Recurrent Necrotic Mucocutaneous Aphthae", *Brit. J. of Dermatol.*, vol. 112, No. 5, pp. 632 (1985).
- Lane, et al., "Treatment of Actinic Prurigo with Intermittent Short-Course Topical 0.05% Clobetasol 17-Propionate. A Preliminary Report", *Arch. Dermatol.*, vol. 126, No. 9, pp. 1211-1213 (1990).
- Languillon, J., "The Effects of Thalidomide on Leprosy Reaction", *Int. J. Lepr. Other Mycobact. Dis.*, vol. 39, No. 2, pp. 590-592 (1971).
- Larsson, H., "Treatment of Severe Colitis in Behcet's Syndrome with Thalidomide (CG-217)", *J. of Intern. Med.*, vol. 228, pp. 405-407 (1990).
- Ledo, E., "Photodermatosis. Part I: Photobiology, Photoimmunology, and Idiopathic photodermatoses", *Int. J. Dermatol.*, vol. 32, No. 6, pp. 387-396 (1993).
- Lehner, T., et al., "Thalidomide, Orogenital Ulcers, and the Risk of Teratogenesis", *The Lancet*, vol. 8423, pp. 288-289 (1985).
- Lenicque, "Action of Thalidomide on the Induction of Tentacles in Regenerating Hydra Littoralis", *Acta. Zool.*, pp. 127-139 (1967).
- Levy, et al., "Treatment of Erythema Nodosum Leprosum with Thalidomide", *The Lancet*, vol. 2, No. 824, pp. 324-325 (1973).
- Lien, W. M. et al., "The Blood Supply of Experimental Liver Metastases. II. A microcirculatory Study of the Normal and Tumor Vessels of the Liver with the Use of Perfused Silicone Rubber", *Surgery*, vol. 68, No. 2, pp. 334-340 (1970).
- Lo, et al., "Treatment of Discoid Lupus Erythematosus", *Int. J. Dermatol.*, vol. 28, No. 8, pp. 497-507 (1989).
- Londono, F., "Thalidomide in the Treatment of Actinic Prurigo", *Int. J. Dermatol.*, vol. 12, No. 5, pp. 326-328 (1973).
- Lovell, et al., "Thalidomide in Actinic Prurigo", *Br. J. Dermatol.*, vol. 108, No. 4, pp. 467-471 (1983).
- Lopez, J. et al., "Thalidomide as Therapy for Intestinal Chronic GVHD", *Bone Marrow Transplantation*, vol. 11, No. 3, pp. 251-252 (1993).
- Louzir, B. et al., "Erythroleucemie chez un patient ayant une maladie de Behcet et traite au long cours par thalidomide", *Annales de Medecine Interne*, vol. 143, pp. 479-480 (1992).
- Lueprasitsakul, W. et al., "Effect of Thalidomide on the Incidence of Iodine-Induced and Spontaneous Lymphocytic Thyroiditis and Spontaneous Diabetes Mellitus in the BB/Wor Rat", *Acta Endocrinologica*, vol. 123, pp. 79-83 (1990).
- Lüers, H., "Failure of Mutagenic Action of Thalidomide in Drosophila", *The Lancet*, vol. II, No. 7258, p. 1332 (1962).
- Magana-Garcia, M., "Antimalarials for Children", *J. Am. Acad. Dermatol.*, vol. 30, No. 3, p. 510 (1994).
- Maione, T. E. et al., "Inhibition of Angiogenesis by Recombinant Human Platelet Factor-4 and Related Peptides", *Science*, vol. 2, pp. 77-78 (1990).
- Makonkawkeyoon, S. et al., "Thalidomide Inhibits the Replication of Human Immunodeficiency virus type 1", *Proc. Natl. Acad. Sci.*, vol. 90, pp. 5974-5978 (1993).
- Marin-Padilla, M. et al. "Thalidomide Induced alterations in the Blastocyst and Placenta of the Armadillo, Dasypus Novemcinctus Mexicanus, Including a Choriocarcinoma", *The Am. J. of Pathol.*, vol. 43, No. 6, pp. 999-1016 (1963).
- Mascaro, et al., "Thalidomide in the Treatment of Recurrent, Necrotic, and Giant Mucocutaneous Aphthae and Aphthosis", *Arch. Derm.*, vol. 115, pp. 636-637 (1979).
- Matsubara, et al., "Inhibition of Human Endothelial Cell Proliferation by Gold Compounds", *J. Clin. Invest.*, vol. 79, pp. 1440-1446 (1987).
- Matsuyama, T. et al., "Cytokines and HIV Infection: is AIDS a Tumor Necrosis Factor Disease?", *AIDS*, vol. 5, No. 12, pp. 1405-1417 (1991).
- Mauad, "Melhoras Clínicas Obtidas em Doentes Cancerosos Avançados com Tratamento Pela Talidomida Associada a Hormônios", *Anais Paulistas Medicina e Cirurgia*, pp. 15-39 (1963).
- Maurice, et al., "The Effect of Thalidomide on Arachidonic Acid Metabolism in Human Polymorphonuclear Leukocytes and Platelets", *Br. J. Dermatol.*, vol. 115, No. 6, pp. 677-680 (1986).
- McCarthy, et al., "Thalidomide for the Therapy of Graft-versus-Host Disease Following Allogeneic Bone Marrow Transplantation", *Biomed. Pharmacother.*, vol. 43, No. 9, pp. 693-697 (1989).
- McKenna, et al., "Linear IgA Disease, Oral Ulceration and Crohn's Disease", *Br. J. Dermatol.*, vol. 127, pp. 67-68 (1992).
- Meza, D. et al., "Managing the Gastrointestinal Complications of AIDS", *Drug Therapy*, vol. 23, No. 11, pp. 74-83 (1993).
- Miller, et al., "Zusammentreffen Einer Thalidomid-Induzierten Fehlbildung mit Einem Malignen Lymphom Hohen Malignitätsgrades", *Monatsschr. Kinderheilkd.*, vol. 128, pp. 27-29 (1980).
- Miller, et al., "Treatment of Chronic Erythema Nodosum Leprosum with Cyclosporine A Produces Clinical and Immunohistologic Remission", *Int. J. Lepr. Other Mycobact. Dis.*, vol. 55, No. 3, pp. 441-449 (1987).
- Misery, L. et al., "Remission of Langerhans Cell Histiocytosis with Thalidomide Treatment", *Clinical and Experimental Dermatol.*, vol. 18, No. 5, p. 487 (1993).

US 7,435,745 B2

Page 6

- Miura, M. et al., "Potentiating Effect of Thalidomide on Methylcholanthrene Oncogenesis in Mice", *Experientia*, vol. 26, Fasc. 3, pp. 305-306 (1970).
- Miyachi, Y., "A Possible Mechanism of Action of Thalidomide on Rheumatoid Arthritis", *Arthritis and Rheuma.*, vol. 28, No. 7, p. 836 (1985).
- Miyachi, et al., "Effects of Thalidomide on the Generation of Oxygen Intermediates by Zymosan-Stimulated Normal Polymorphonuclear Leukocytes", *Arch. Dermatol. Res.*, vol. 274, Nos. 3-4, pp. 363-367 (1982).
- Mohri, et al., "Negative Effect of Thalidomide and Relative Substances on the Growth of HeLa Cells", *Chem. Pharm. Bull.*, vol. 16, pp. 2289-2292 (1968).
- Moncada, et al., "Thalidomide—Effect on T Cell Subsets as a Possible Mechanism of Action", *Int. J. Lepr. Other Mycobact. Dis.*, vol. 53, No. 2, pp. 201-205 (1985).
- Montrucchio, et al., "Tumor Necrosis Factor α -Induced Angiogenesis Depends on In Situ Platelet-Activating Factor Biosynthesis", *J. Exp. Med.*, vol. 180, pp. 377-382 (1994).
- Moulin, et al., "Treatment of Jessner-Kanof Disease with Thalidomide", *Ann. Dermatol. Venereol.*, vol. 10, No. 8, pp. 611-614 (1983).
- Mshana, et al., "Thymus-Dependent Lymphocytes in Leprosy. II. Effect of Chemotherapy on T-Lymphocyte Subpopulations", *J. Clin. Immunol.*, vol. 2, No. 2, pp. 69-74 (1982).
- Mückter, et al., "Thalidomide and Tumor", *Antimicrobial Agents and Chemotherapy*, pp. 531-538 (1965).
- Mummery, C.L. et al., "Screening for Cytotoxicity in Neuroblastoma Cells-I. Dependence of Growth Inhibition on the Presence of Serum", *Toxicology Letters*, vol. 18, pp. 201-209 (1983).
- Munro, et al., "Pyoderma Gangrenosum Associated with Behcet's Syndrome-Response to Thalidomide", *Clin. Exp. Dermatol.*, vol. 13, No. 6, pp. 408-410 (1988).
- Muthukkaruppan, Vr. et al., "Angiogenesis in the Mouse Cornea", *Science*, vol. 205, No. 28, pp. 1416-1418 (1979).
- Naafs, B. et al., "Thalidomide Therapy An Open Trial", *Int. J. of Dermatol.*, vol. 24, No. 2, pp. 131-134 (1985).
- Naafs, B., Bangkok Workshop on Leprosy Research. Treatment of Reactions and Nerve Damage. *Int. J. Lepr. Other Mycobact. Dis.*, vol. 64, No. 4, Suppl. S21-28 (1996).
- Nguyen, M. et al., "Elevated Levels of the Angiogenic Peptide Basic Fibroblast Growth Factor in Urine of Bladder Cancer Patients", *J. of the Natl. Canc. Inst.*, vol. 85, No. 3, pp. 241-242 (1993).
- Nguyen, M. et al., "Elevated Levels of the Angiogenic Peptide Basic Fibroblast Growth Factor in Urine of Patients with a Wide Spectrum of Cancers", *J. of the Natl. Canc. Inst.*, vol. 86, No. 5, pp. 356-361 (1994).
- Nicolas, J., et al., "Interferon Alfa Therapy in Severe Unresponsive Subacute Cutaneous Lupus Erythematosus", *New Engl. J. Med.*, vol. 321, No. 22, pp. 1550-1551 (1989).
- Nicolau, et al., "Thalidomide: Treatment of Severe Recurrent Aphthous Stomatitis in Patients with AIDS", *DICP*, vol. 24, No. 11, pp. 1054-1056 (1990).
- Nielsen, et al., "Thalidomide Enhances Superoxide Anion Release from Human Polymorphonuclear and Leukocytes", *Acta. Pathol. Microbiol. Immunol. Scand. [C]*, vol. 94, No. 6, pp. 233-237 (1986).
- Obeso, J. et al., "Methods in Laboratory Investigation A Hemangioendothelioma-Derived Cell Line: Its Use as a Model for the Study of Endothelial Cell Biology", *Laboratory Investigation*, vol. 63, No. 2, pp. 259-269 (1990).
- Oikawa, T. et al., "Eponemycin, A Novel Antibiotic is a Highly Powerful Angiogenesis Inhibitor", *Biochem. and Biophys. Res. Comm.*, vol. 181, No. 3, pp. 1070-1077 (1991).
- Olson, et al., "Thalidomide (N-phthaloylglutimide) in the Treatment of Advanced Cancer", *Clin. Pharmacol. Therap.*, vol. 6, No. 3, pp. 292-297 (1965).
- Orzalesi, M., "Il Danno Iatrogeno in Neonatologica", *Ped. Med. Chir.*, vol. 14, pp. 105-112 (1992).
- Ostraat, et al., "Thalidomide Prolongs Graft Survival in Rat Cardiac Transplants", *Transplant Proc.*, vol. 24, No. 6, pp. 2624-2625 (1992).
- Otsuka, et al., "A New Potent Angiogenesis Inhibitor, FR-118487", *J. of Microbiol. and Biotechno.*, vol. 1, No. 3, pp. 163-168 (1991).
- Paller, et al., "Proceedings of the Concurrent Sessions", *Pediatr. Dermatol.*, vol. 9, No. 4, pp. 397-406 (1992).
- Passaniti, A. et al., "Methods in Laboratory Investigation A Simple, Quantitative Method for Assessing Angiogenesis and Antiangiogenic Agents Using Reconstituted Basement Membrane, Heparin, and Fibroblast Growth Factor", *Laboratory Investigation*, vol. 67, No. 4, pp. 519-528 (1992).
- Patey, O. et al., "Thalidomide et colite ulcereuse dans la maladie de Behcet", *Gastroenterol. Clin. Biol.*, vol. 13, pp. 104-110 (1989).
- Pearson, et al., "Treatment of Moderately Severe Erythema Nodosum Leprosum with Thalidomide—A Double-Blind Controlled Trial", *Lepr. Rev.*, vol. 40, No. 2, pp. 111-116 (1969).
- Pfordte, "Über die Beeinflussung des Serumproperdinsystems Durch Verschiedene Arzneimittel", *Pharmazie*, vol. 26, pp. 301-302 (1971).
- Phillips, et al., "Tumor Necrosis Factor Alpha (rhTNF) Fails to Stimulate Angiogenesis in the Rabbit Cornea", *Anatomical Rec.*, vol. 245, pp. 53-56 (1996).
- Powell, R.J. et al., "Investigation and Treatment of Orogenital Ulceration; studies on a Possible Mode of Action of Thalidomide", *Brit. J. of Dermatol.*, vol. 113, Supp. 28, pp. 141-144 (1985).
- Prigent, F. et al., "Sarcoïdose cutanée Traitement par la Thalidomide", *La Presse Medicale*, vol. 12, No. 47, p. 3007 (1983).
- Radeff, et al., "Recurrent Aphthous Ulcer in Patient Infected with Human Immunodeficiency Virus: Successful Treatment with Thalidomide", *J. Am. Acad. Dermatol.*, vol. 23, No. 3, Pt. 1, pp. 523-525 (1990).
- Rainsford, K.D., "Disease-Modifying Antirheumatic and Immunoregulatory Agents", *Bailliere's Clinical Rheumatology*, vol. 4, No. 3, pp. 405-432 (1990).
- Rajan, et al., "A Clinical Study of Thalidomide Comparing Pre-Treatment and Post-Treatment Reactional Episodes and Corticosteroid Requirements", *Lepr. India*, vol. 55, No. 1, 111-116 (1983).
- Randall, T., "Investigational New Drug (US) 'Orphan' Trials now Use Thalidomide from two Sources", *JAMA*, vol. 263, No. 11, p. 1474 (1990).
- Revuz, J., "Actualite Du Thalidomide", *Ann. Dermatol. Venereol.*, vol. 117, pp. 313-321 (1990).
- Revuz, J. et al., "Crossover Study of Thalidomide vs. Placebo in Severe Recurrent Aphthous Stomatitis", *Arch. Dermatol.*, vol. 126, pp. 923-927 (1990).
- Rhoton, A. J., "Role for Thalidomide in Primary Biliary Cirrhosis Treatment?", *Gastroenterology*, vol. 105, No. 3, p. 956 (1993).
- Roe, F.J.C., "Pathology Thalidomide and Neoplasia", *Nature*, vol. 200, pp. 1016-1017 (1963).
- Roe, et al., "Tumour-Incidence in Progeny of Thalidomide-Treated Mice", *British J. Cancer*, pp. 331-333, (1965).
- Ruggenini, et al., "Taldomide e Tumori Sperimentali", *Cancro*, vol. 20, pp. 39-55 (1967).
- Rustin, et al., "Pyoderma Gangrenosum Associated with Behcet's Disease: Treatment with Thalidomide", *J. Am. Acad. Dermatol.*, vol. 23, No. 5, Pt. 1, pp. 941-944 (1990).
- Ryan, et al., "Thalidomide to Treat Esophageal Ulcer in AIDS (6)", *New Engl. J. Med.*, vol. 327, No. 3, pp. 208-209 (1992).
- Salven, P. et al., "Serum Vascular Endothelial Growth Factor Is Often Elevated in Disseminated Cancer", *Clinical Cancer Research*, vol. 3, pp. 647-651 (1997).
- Sampaio, et al., "Prolonged Treatment with Recombinant Interferon Gamma Induces Erythema Nodosum Lepromatous Leprosy Patients", *J. Exp. Med.*, vol. 175, No. 6, pp. 1729-1737 (1992).
- Sampaio, et al., "Thalidomide Selectively Inhibits Tumor Necrosis Factor Alpha Production by Stimulated Human Monocytes", *J. Exp. Med.*, vol. 173, No. 3, pp. 699-703 (1991).
- Santis, H.R., "Aphthous Stomatitis and its Management", *Curr. Opin. Dent.*, vol. 1, No. 6, pp. 763-768 (1991) (Abstract Only).
- Santos, et al., "In Vitro Tumor Necrosis Factor Production by Mononuclear Cells from Lepromatous Leprosy Patients and from Patients with Erythema Nodosumleprosum", *Clin. Immunol. Immunopathol.*, vol. 67, No. 3 I, pp. 199-203 (1993).
- Sato, K. et al., "Increased Concentration of Vascular Endothelial Growth Factor/Vascular Permeability Factor in Cyst Fluid of Enlarging and Recurrent Thyroid Nodules", *Journal of Clinical Endocrinology and Metabolism*, vol. 82, No. 6, pp. 1968-1972 (1997).

US 7,435,745 B2

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- Schweigerer, L. et al., "Angiogenesis and Angiogenesis Inhibitors in Paediatric Diseases", *European J. of Pediatric*, vol. 151, pp. 472-476 (1992).
- Shannon, et al., "Inhibition of de Novo IgM Antibody Synthesis by Thalidomide as a Relevant Mechanism of Action in Leprosy", *Scand. J. Immunol.*, vol. 13, No. 6, p. 553-562 (1981).
- Shannon, et al., "Thalidomide's Effectiveness in Erythema Nodosum Leprosum is Associated with a Decrease in CD4+ Cells in the Peripheral Blood", *Lepr. Rev.*, vol. 63, No. 1, pp. 5-11 (1992).
- Shealy, Y.F. et al., "D- and L-Thalidomide", *Chemistry and Industry*, pp. 1030-1031 (1965).
- Sheehan, N.J., "Thalidomide Neurotoxicity and Rheumatoid Arthritis", *Arthritis and Rheuma.*, vol. 29, No. 10, p. 1296 (1986).
- Sherman, M. et al., "Thalidomide: A Twenty-Five Year Perspective", *Food Drug Cosmetic Law J.*, vol. 41, pp. 458-466 (1986).
- Sheskin, J., "The Treatment of Leprosy Reaction in Lepromatous Leprosy. Fifteen Years' Experience with Thalidomide", *Int. J. Dermatol.*, vol. 19, No. 6, pp. 318-322 (1980).
- Sheskin, et al., "In Vivo Measurements of Iron, Copper and Zinc in the Skin of Prurigo Nodularis Patients Treated with Thalidomide", *Dermatologica*, vol. 162, No. 2, pp. 86-90 (1981).
- Shi, G. et al., "Kringle Domains and Plasmin Denaturation", *Biochem. and Biophys. Res. Comm.*, vol. 178, No. 1, pp. 360-368 (1991).
- Sidky, Y. et al., "Inhibition of Angiogenesis by Interferons: Effects on Tumor- and Lymphocyte-induced Vascular Responses", *Canc. Res.*, vol. 47, pp. 5155-5161 (1987).
- Silverman, W.A., "Medical Inflation", *Persp. Biol. Med.*, pp. 617-637 (1980).
- Srivastava, A. et al., "The Prognostic Significance of Tumor Vascularity in Intermediate-Thickness Skin Melanoma", *Am. J. Pathol.*, vol. 133, pp. 419-423 (1988) (abstract from Medline: Accession No. 06745734).
- Style, A., "Early Diagnosis and Treatment of Leprosy in the United States", *American Family Physician*, vol. 52, No. 1, 172-178 (1995).
- Sugiura, et al., "Effect of Thalidomide on Transplantable Mouse, Rat, and Hamster Tumors", *GANN*, vol. 55, pp. 57-60 (1964).
- Swift, T.R., "Thalidomide in Erythema Nodosum Leprosum", *Lancet*, vol. 2, No. 835, p. 966 (1973).
- Szydlowska, et al., "On the Application of Thalidomide as a Block of Functional Groups of Proteins in Histochemical Investigations", *Folia Histo. Cytochem.*, vol. 16, No. 3, pp. 233-240 (1978).
- Tamura, et al., "Combination Thalidomide and Cyclosporine for Cardiac Allograft Rejection. Comparison with Combination Methylprednisolone and Cyclosporine", *Transplantation*, vol. 49, No. 1, p. 20-25 (1990).
- Tanaka, Y. et al., "Vascular Endothelial Growth Factor in Diabetic Retinopathy", *The Lancet*, vol. 349, p. 1520 (1997).
- Taylor, S. et al., "Protamine is an Inhibitor of Angiogenesis", *Nature*, vol. 297, pp. 307-312 (1982).
- Teppo, L. et al., "Thalidomide-Type Malformations and Subsequent Osteosarcoma", *The Lancet*, vol. II, No. 8034, p. 405 (1977).
- "Thalidomide 20 Years On", *The Lancet*, vol. II, No. 8245, pp. 510-511 (1981).
- "Thalidomide", *The Merck Index*, 11th ed., p. 1458 (1989).
- Theophilus, S., "Treatment with Thalidomide in Steroid Dependency and Neuritis", *Lepr. India*, vol. 52, No. 3, pp. 423-428 (1980).
- Thomas, et al., "Effect of Thalidomide on Liver Regeneration in Rat", *Indian J. Exp. Biol.*, vol. 10, pp. 314-315 (1972).
- Thomas, et al., "Lack of Thalidomide Induced Aplasia in Regenerating Tail of Lizard, Hemidactylus Flaviviridis", *Indian J. Exp. Biol.*, vol. 10, pp. 316-317 (1972).
- Thomas, L. et al., "Successful Treatment of Adult's Langerhans Cell Histiocytosis with Thalidomide", *Archives of Dermatol.*, vol. 129, pp. 1261-1264 (1993).
- Torry, R. J. et al., "Angiogenesis in the Uterus: Potential Regulation and Relation to Tumor Angiogenesis", *Am. J. of Reproductive Immunology*, vol. 27, pp. 171-179 (1992).
- Traldi, et al., "L'impiego Dell'imide Dell'Acido N'ftalilglutammico (Thalidomide) Nella Terapia Sintomatica del Vomito di Molti Pazienti Affetti da Neoplasie Maligne o Causato Dalla Somministrazione di Cloridato di Mecloretamina", *Cancro*, vol. 18, pp. 336-341 (1965).
- Trautman, J. R., "Treatment of Hansen's Disease", *Curtis*, vol. 18, No. 1, 62-65 (1976).
- Van den Broek, H., "Treatment of Prurigo Nodularis with Thalidomide", *Arch. Dermatol.*, vol. 116, No. 5, pp. 571-572 (1980).
- Vasilescu, et al., "Cercetari Privind Actiunea Talidomidei Asupra Celulelor Cultivate in Vitro", *Cerc. Fiziol.*, vol. 13, No. 4, pp. 293-300 (1968).
- Verhaul, et al., "Combination Oral Antiangiogenic Therapy with Thalidomide and Sulindac Inhibits Tumor Growth in Rabbits", *Brit. J. Cancer*, vol. 79, No. 1, pp. 114-118 (1999).
- Vicente, T. et al., "In Vitro Activity of Thalidomide Against *Mycobacterium avium* Complex", *Archives of Internal Medicine*, vol. 153, p. 334 (1993).
- Villa, et al., "Antimytotic Effect of Thalidomide and its Metabolites on the Chick Embryo Blood Cells", *Haematol. Latina*, vol. 6, pp. 217-221 (1963).
- Villa, et al., "Cytological Effects of Thalidomide", *The Lancet*, pp. 725 (1963).
- Vladutiu, A., "Another Chance for Thalidomide?", *The Lancet*, vol. I, No. 7444, pp. 981-982 (1966).
- Vogelsang, G. B. et al., "Thalidomide for the Treatment of Chronic Graft-Versus-Host Disease", *New England J. of Med.*, vol. 326, pp. 1055-1059 (1992).
- Vogelsang, et al., "Therapy of Chronic Graft-v-Host Disease in a Rat Model", *Blood*, vol. 74, No. 1, No. 1, pp. 507-511 (1989).
- Vogelsang, et al., "Thalidomide Induction of Bone Marrow Transplantation Tolerance", *Transplant Proc.*, vol. 19, No. 1, Pt. 3, pp. 2658-2661 (1987).
- Vogelsang, et al., "Treatment and Prevention of Acute Graft-versus-Host Disease with Thalidomide in a Rat Model", *Transplantation*, vol. 41, No. 5, pp. 644-647 (1986).
- Vogt, B. et al., "Inhibition of Angiogenesis in Kaposi's Sarcoma by Captopril", *The Lancet*, vol. 349, p. 1148 (1997).
- Waters, M.F. et al., "Treatment of Ulcerative Colitis with Thalidomide", *Brit. Med. J.*, vol. 1, No. 6166, pp. 792 (1979).
- Waters, M., "Use of Thalidomide in Leprosy", *BMJ*, vol. 303, No. 6800, p. 470 (1991).
- Waters, M.F., "An Internally-Controlled Double Blind Trial of Thalidomide in Severe Erythema Nodosum Leprosum", *Lepr. Rev.*, vol. 42, No. 1, pp. 26-42 (1971).
- Weidner, J. et al., "Tumor Angiogenesis and Metastasis-Correlation in Invasive Breast Carcinoma", *The New England J. of Med.*, vol. 324, No. 1, pp. 1-8 (1991).
- Weidner, N. et al., "Tumor Angiogenesis Correlates with Metastasis in Invasive Prostate Cancer", *Am. J. of Pathol.*, vol. 143, No. 2, pp. 401-409 (1993).
- Weidner, N. et al., "Tumor Angiogenesis: A New Significant and Independent Prognostic Indicator in Early-Stage Breast Carcinoma", *J. of Nat. Canc. Inst.*, vol. 84, No. 24, 1875-1887 (1992).
- White, C. W. et al., "Treatment of Pulmonary Hemangiomas with Recombinant Interferon Alfa-2a", *The New England J. of Med.*, vol. 32, No. 18, pp. 1197-1200 (1989).
- Williams, K. M., "Enantiomers in Arthritic Disorders", *Pharmacol. & Therapeutics*, vol. 46, No. 2, pp. 273-295 (1990).
- Williams, et al., "Thalidomide Hypersensitivity in AIDS", *Lancet*, vol. 337, pp. 436-437 (1991).
- Winkelmann, et al., "Thalidomide Treatment of Prurigo Nodularis", *Acta. Derm. Venereol.*, vol. 64, No. 5, pp. 412-417 (1984).
- Wood, et al., "The Potential use of Thalidomide in the Therapy of Graft-versus-Host Disease—A Review of Clinical and Laboratory Information", *Leuk. Res.*, vol. 14, No. 5, pp. 395-399 (1990).
- Woodyatt, P.B., "Thalidomide", *The Lancet*, p. 750. (1962).
- Wulff, et al., "Development of Polyneuropathy During Thalidomide Therapy", *Br. J. Dermatol.*, vol. 112, No. 4, pp. 475-480 (1985).
- Yazici, et al., "Practical Treatment Recommendations for Pharmacotherapy of Behcet's Syndrome", *Drugs*, vol. 42, No. 5, pp. 796-804 (1991).
- Yoshimura, T. et al., "Cloning, Sequencing, and Expression of Human Macrophage Stimulating Protein (MSP, MST1) Confirms MSP as a member of the Family of Kringle Proteins and Locates the MSP Gene on Chromosome 3", *The J. of Biol. Chem.*, vol. 268, No. 21, pp. 15461-15468 (1993).

US 7,435,745 B2

Page 8

- Youle, et al., "Treatment of Resistant Aphthous Ulceration with Thalidomide in Patients Positive for HIV Antibody", *BMJ*, vol. 298, No. 6671, p. 432 (1989).
- Youle, et al., "Thalidomide in Hyperalgetic Pharyngeal Ulceration of AIDS", *Lancet*, vol. 335, No. 8705, p. 1591 (1990).
- Yue, t. et al., "2-Methoxyestradiol, an Endogenous Estrogen Metabolite, Induces Apoptosis in Endothelial Cells and Inhibits Angiogenesis: Possible Role for Stress-Activated Protein Kinase Signaling Pathway and Fas Expression", *Molecular Pharmacology*, vol. 51, pp. 951-962 (1997).
- Ziche, M. et al., "Nitric Oxide Synthase Lies Downstream from Vascular Endothelial Growth Factor-induced but Not Basic Fibroblast Growth Factor-Induced Angiogenesis", *J. Clin. Invest.*, vol. 99, No. 11, pp. 2625-2634 (1997).
- Zwart, D., "Treatment of Grade II Astrocytoma with Thalidomide", *Arzneim.-Forsch.*, vol. 16, No. 12, pp. 1688-1689 (1966).
- Braun, A.G. et al., "Teratogen Metabolism," Energy Research Abstracts, *U.S. Department of Energy, Technical Information Center Office of Scientific & Technical Information*, (1984) 9(8):5508:15072.
- Braun, A.G. et al., "Teratogen Metabolism: Activation of Thalidomide and Thalidomide Analogues to Products that Inhibit the Attachment of Cells to Concanavalin A Coated Plastic Surfaces," Energy Research Abstracts, *U.S. Department of Energy, Technical Information Center Office of Scientific & Technical Information*, (1984) 9(8):5508:15071.
- Braun, A.G. et al., "Teratogen Metabolism : Activation of Thalidomide and Thalidomide Analogues to Products that Inhibit the Attachment of Cells to Concanavalin a Coated Plastic Surfaces," *Biochemical Pharmacology* (1984) 33(9):1471-1477.
- Braun, A.G. et al., "Teratogen Metabolism : Activation of Thalidomide and Thalidomide Analogues to Products that Inhibit the Attachment of Cells to Concanavalin a Coated Plastic Surfaces," Revised Version, *U.S. Department of Commerce, National Technical Information Services*, Harvard Medical School: Boston, MA (1982) DE84006118.
- Gordon, G. "Mutagenic and Cytotoxic Studies with Thalidomide," (unpublished Ph.D. dissertation, The John Hopkins U. 1981) (microformed on UMI Dissertation Abstracts database 8120023).
- Gordon, G.B. et al., "Thalidomide Teratogenesis: Evidence for a Toxic Arene Oxide Metabolite," *Federation of American Societies for Experimental Biology Federation Proceedings* (1981) 40(3): 2508.
- Gordon, G.B. et al., "Thalidomide Teratogenesis: Evidence for a Toxic Arene Oxide Metabolite," *Proc. Natl. Acad. Sci.* (1981) 78(4): 2545-2548.
- Lisek, C.A. "Structural Elucidation of a Hydroxylated Metabolite of Thalidomide: Evidence Supporting an Arene Oxide Intermediate," (unpublished Ph.D. dissertation, The John Hopkins U. 1985) (microformed on UMI Dissertation Abstracts database 8609337).

* cited by examiner

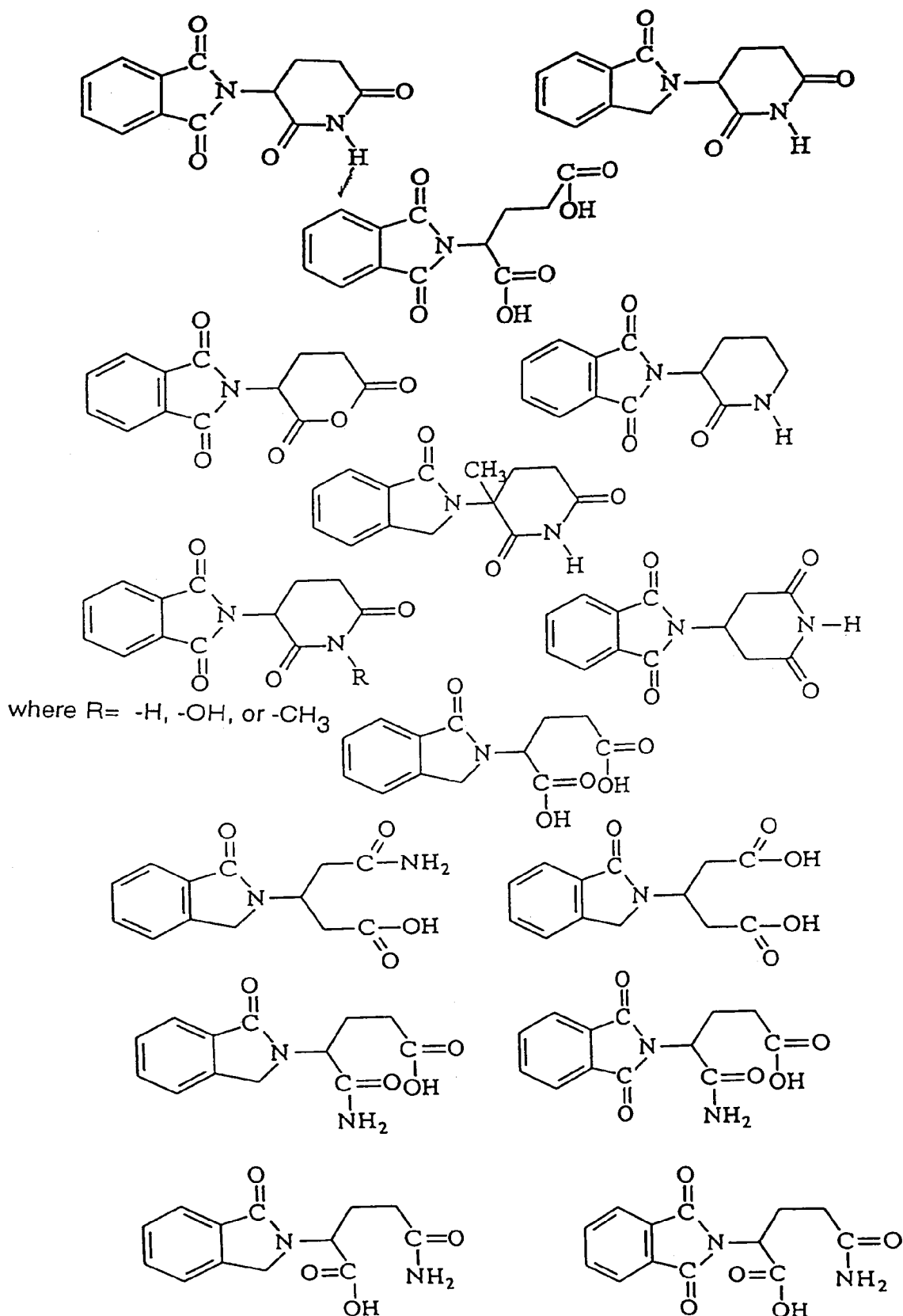


FIGURE 1

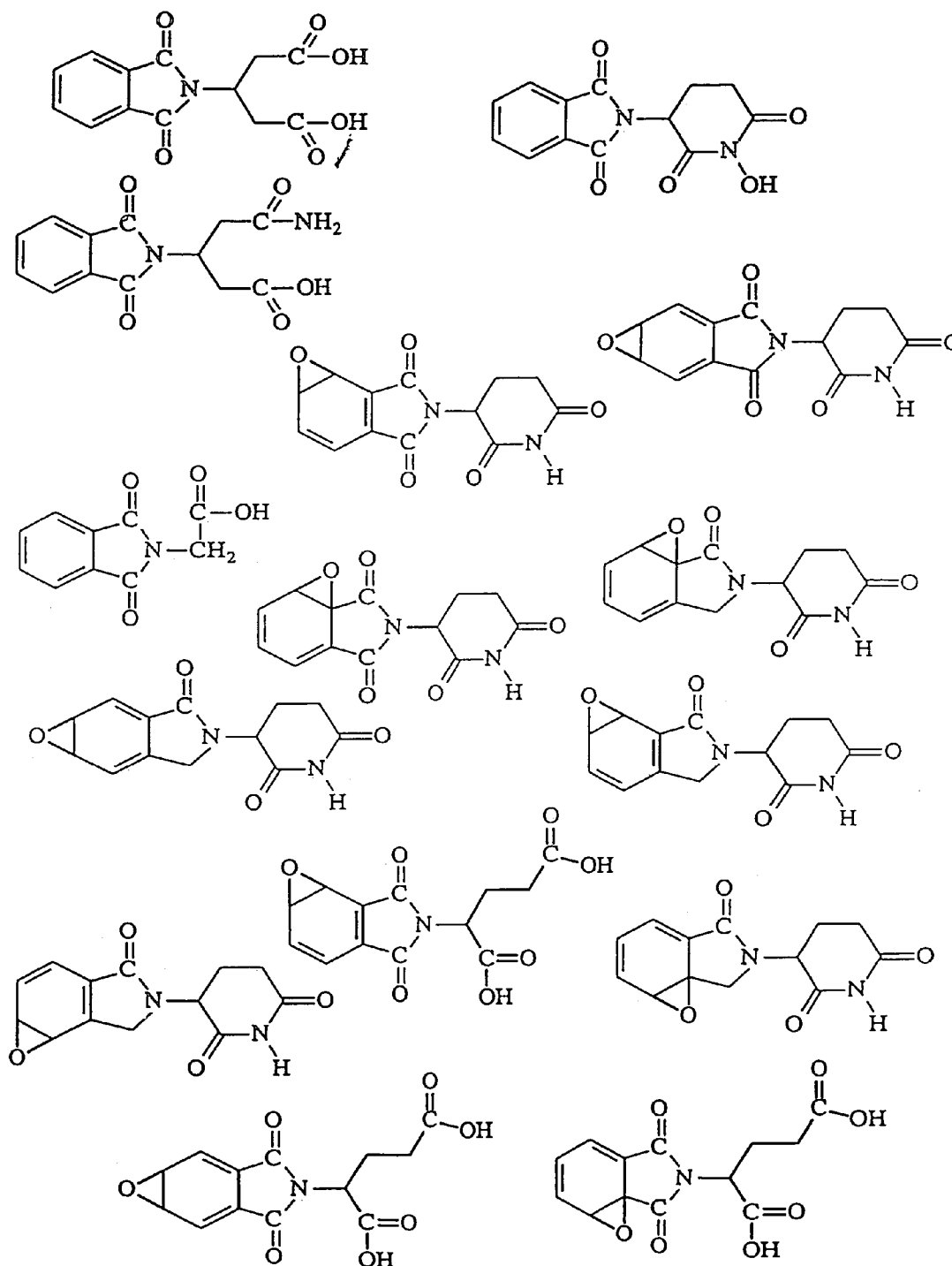


Figure 2

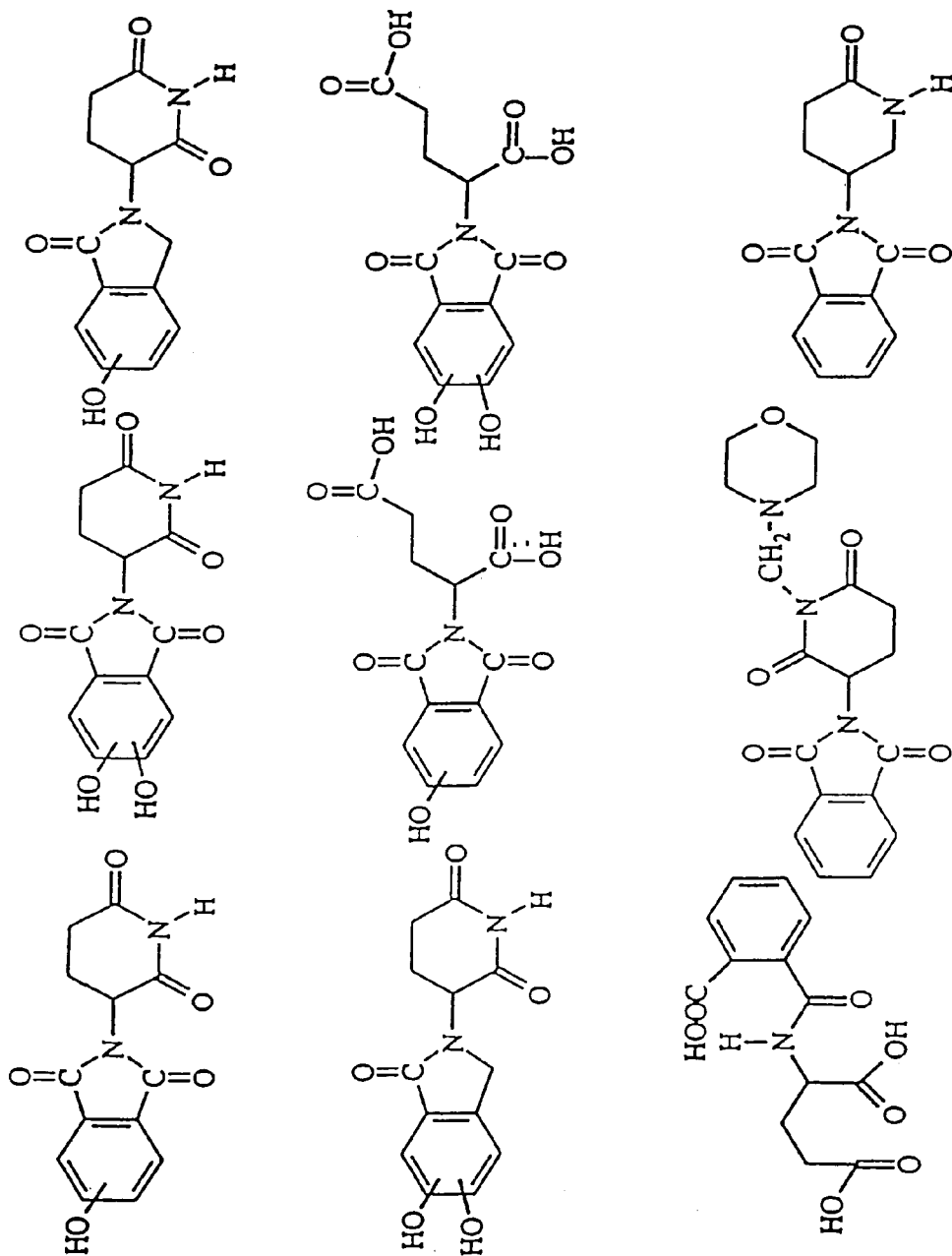


Figure 3

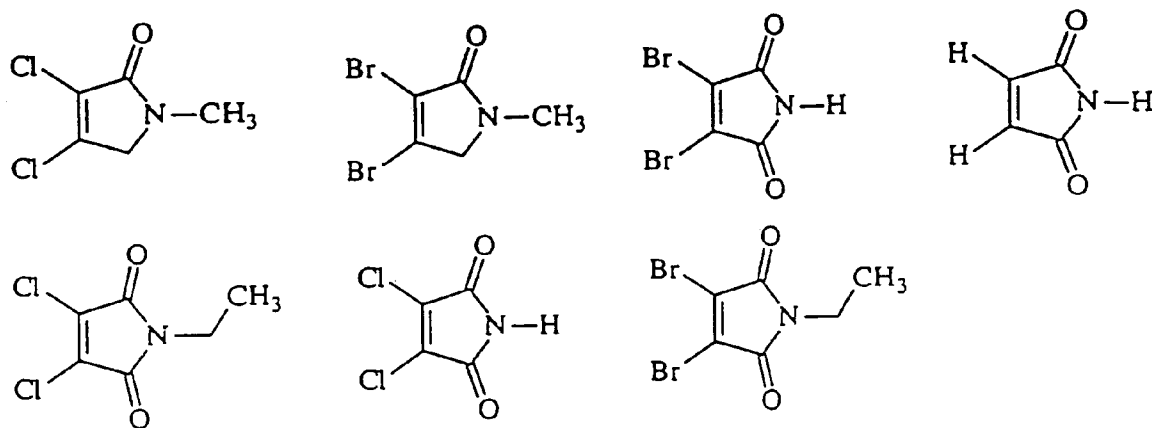


Figure 4

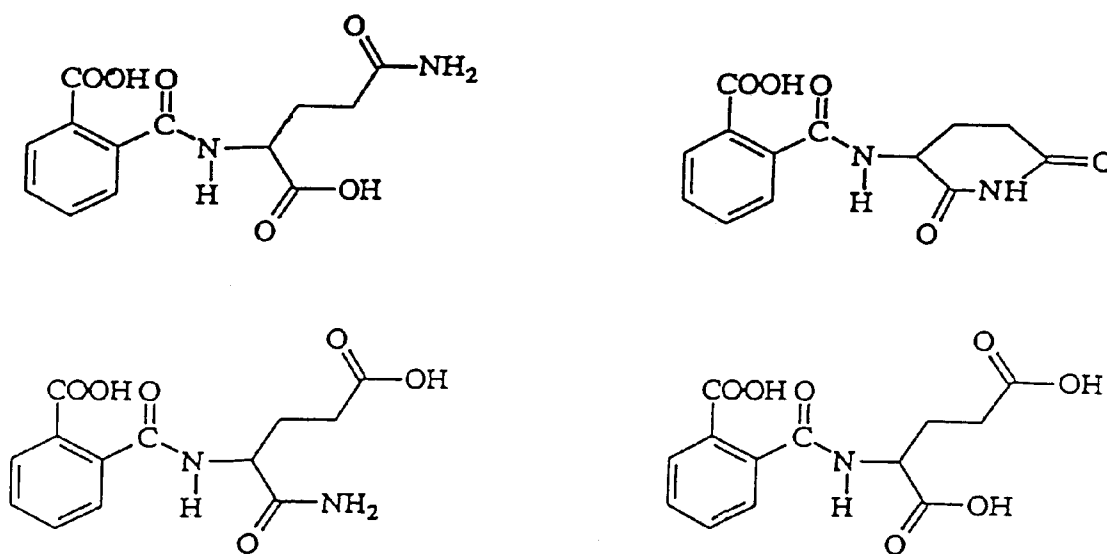


Figure 5

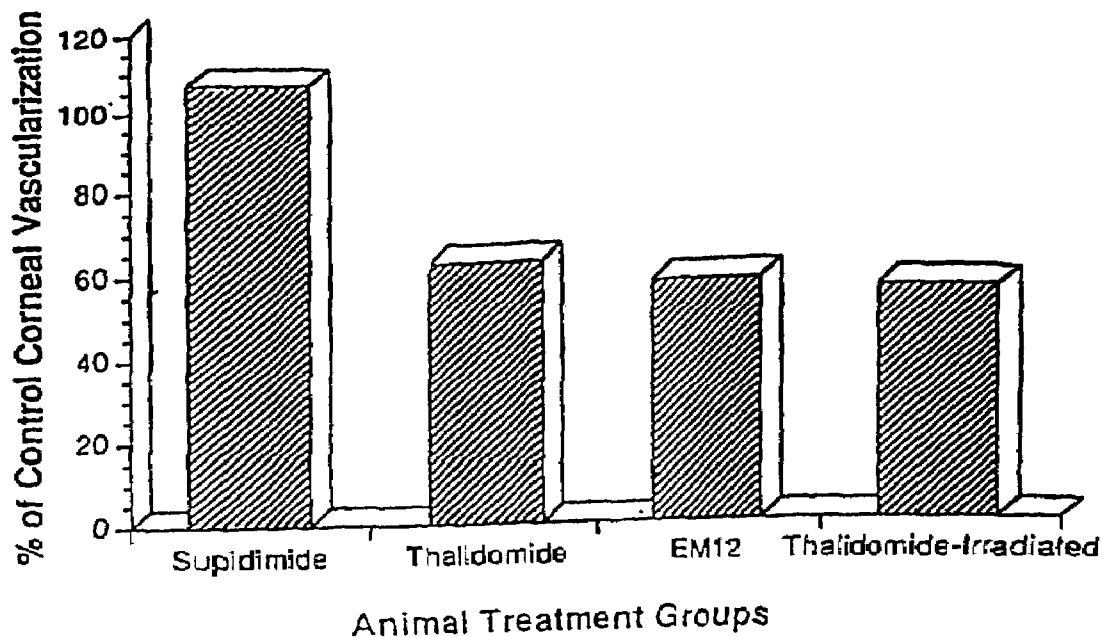


Figure 6

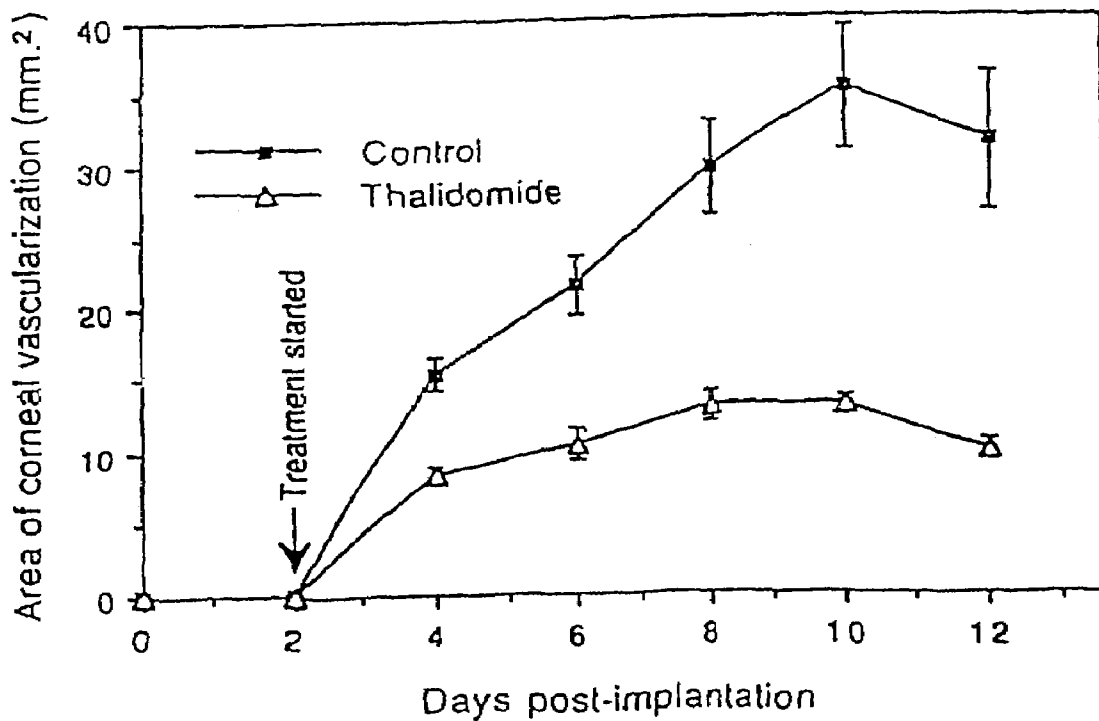


Figure 7

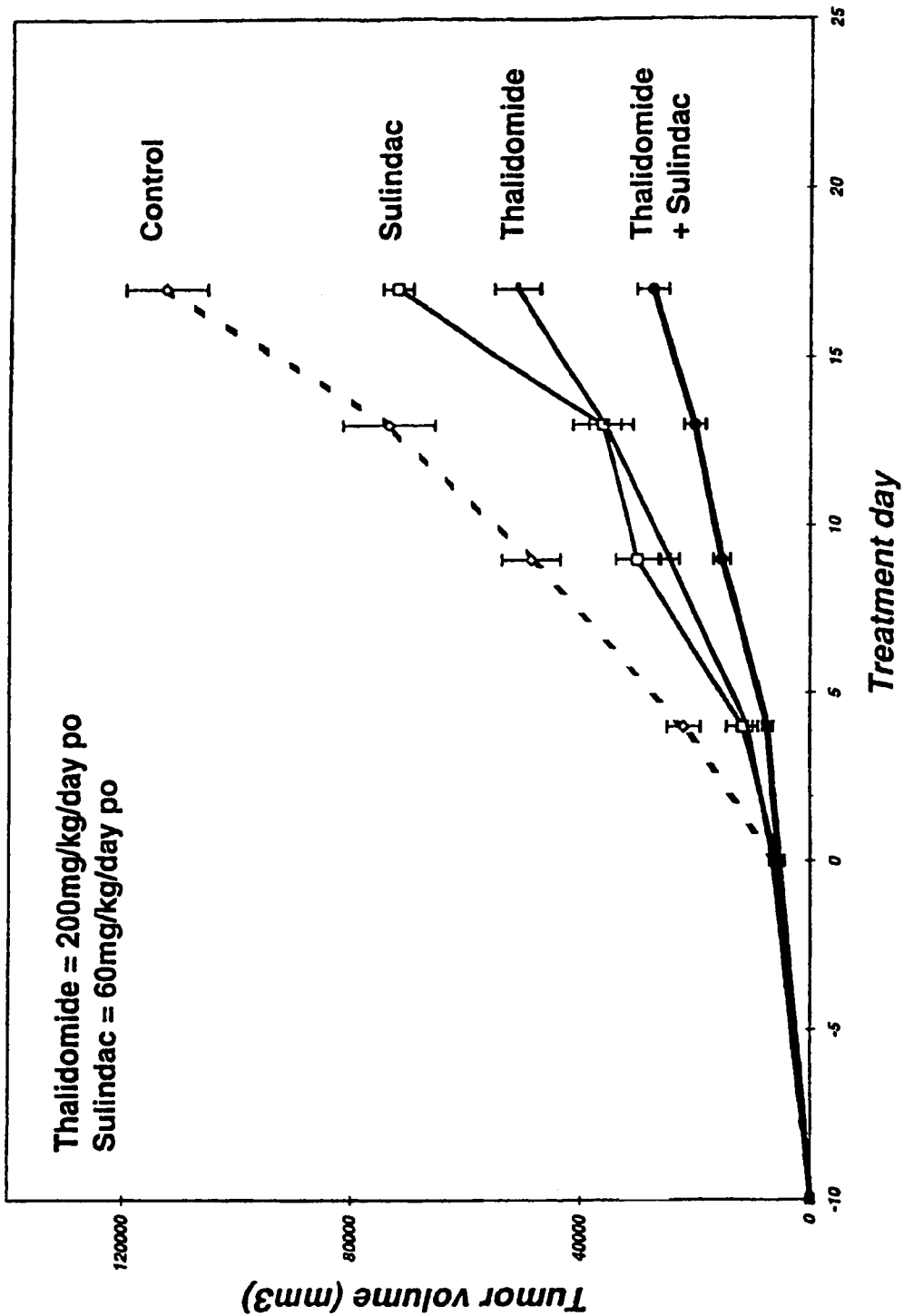


FIGURE 8

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METHODS AND COMPOSITIONS FOR INHIBITION OF ANGIOGENESIS

RELATED APPLICATIONS

This application is a continuation of U.S. application Ser. No. 09/287,377, filed Apr. 7, 1999, now abandoned, which is a continuation of U.S. application Ser. No. 08/963,058, filed Nov. 3, 1997, now abandoned, which claims priority to U.S. provisional application Ser. No. 60/028,708, filed Nov. 5, 1996, all of which are incorporated herein in their entireties by reference.

TECHNICAL FIELD

The present invention relates to methods and compositions for preventing unwanted angiogenesis in a human or animal. More particularly, the present invention relates to a method for preventing unwanted angiogenesis, particularly in angiogenesis dependent or associated diseases, by administration of compounds such as thalidomide and related compounds.

BACKGROUND OF THE INVENTION

As used herein, the term "angiogenesis" means the generation of new blood vessels into a tissue or organ. Under normal physiological conditions, humans or animals only undergo angiogenesis in very specific restricted situations. For example, angiogenesis is normally observed in wound healing, fetal and embryonal development and formation of the corpus luteum, endometrium and placenta. The control of angiogenesis is a highly regulated system of angiogenic stimulators and inhibitors.

The control of angiogenesis has been found to be altered in certain disease states and, in many cases, the pathological damage associated with the disease is related to uncontrolled angiogenesis.

Both controlled and uncontrolled angiogenesis are thought to proceed in a similar manner. Endothelial cells and pericytes, surrounded by a basement membrane, form capillary blood vessels. Angiogenesis begins with the erosion of the basement membrane by enzymes released by endothelial cells and leukocytes. The endothelial cells, which line the lumen of blood vessels, then protrude through the basement membrane. Angiogenic stimulants induce the endothelial cells to migrate through the eroded basement membrane. The migrating cells form a "sprout" off the parent blood vessel, where the endothelial cells undergo mitosis and proliferate. The endothelial sprouts merge with each other to form capillary loops, creating the new blood vessel. In the disease state, prevention of angiogenesis could avert the damage caused by the invasion of the new microvascular system.

Persistent, unregulated angiogenesis occurs in a multiplicity of disease states, tumor metastasis and abnormal growth by endothelial cells and supports the pathological damage seen in these conditions. The diverse pathological states created due to unregulated angiogenesis have been grouped together as angiogenic dependent or angiogenic associated diseases. Therapies directed at control of the angiogenic processes could lead to the abrogation or mitigation of these diseases.

One example of a disease mediated by angiogenesis is ocular neovascular disease. This disease is characterized by invasion of new blood vessels into the structures of the eye such as the retina or cornea. It is the most common cause of blindness and is involved in approximately twenty eye diseases. In age-related macular degeneration, the associated

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visual problems are caused by an ingrowth of chorioidal capillaries through defects in Bruch's membrane with proliferation of fibrovascular tissue beneath the retinal pigment epithelium. Angiogenic damage is also associated with diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, neovascular glaucoma and retrolental fibroplasia. Other diseases associated with corneal neovascularization include, but are not limited to, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sjogren's syndrome, acne rosacea, phlyctenulosis, syphilis, Mycobacteria infections, lipid degeneration, chemical burns, bacterial ulcers, fungal ulcers, Herpes simplex infections, Herpes zoster infections, protozoan infections, Kaposi's sarcoma, Mooren's ulcer, Terrien's marginal degeneration, marginal keratolysis, rheumatoid arthritis, systemic lupus, polyarteritis, trauma, Wegener's sarcoidosis, Scleritis, Stevens-Johnson disease, radial keratotomy, pemphigoid and corneal graft rejection.

Diseases associated with retinal/choroidal neovascularization include, but are not limited to, diabetic retinopathy, macular degeneration, sickle cell anemia, sarcoid, syphilis, pseudoxanthoma elasticum, Paget's disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis/vitritis, mycobacterial infections, Lyme's disease, systemic lupus erythematosus, retinopathy of prematurity, Eales' disease, Behcet's disease, infections causing a retinitis or chorioiditis, presumed ocular histoplasmosis, Best's disease, myopia, optic pits, Stargardt's disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma and post-laser complications. Other diseases include, but are not limited to, diseases associated with rubeosis (neovascularization of the angle) and diseases caused by the abnormal proliferation of fibrovascular or fibrous tissue including all forms of proliferative vitreoretinopathy.

Another disease in which angiogenesis is believed to be involved is rheumatoid arthritis. The blood vessels in the synovial lining of the joints undergo angiogenesis. In addition to forming new vascular networks, the endothelial cells release factors and reactive oxygen species that lead to pannus growth and cartilage destruction. The factors involved in angiogenesis may actively contribute to, and help maintain, the chronically inflamed state of rheumatoid arthritis.

Factors associated with angiogenesis may also have a role in osteoarthritis. The activation of the chondrocytes by angiogenic-related factors contributes to the destruction of the joint. At a later stage, the angiogenic factors would promote new bone formation. Therapeutic intervention that prevents the bone destruction could halt the progress of the disease and provide relief for persons suffering with arthritis.

Chronic inflammation may also involve pathological angiogenesis. Such disease states as ulcerative colitis and Crohn's disease show histological changes with the ingrowth of new blood vessels into the inflamed tissues. Bartonellosis, a bacterial infection found in South America, can result in a chronic stage that is characterized by proliferation of vascular endothelial cells. Another pathological role associated with angiogenesis is found in atherosclerosis. The plaques formed within the lumen of blood vessels have been shown to have angiogenic stimulatory activity.

One of the most frequent angiogenic diseases of childhood is the hemangioma. In most cases, the tumors are benign and regress without intervention. In more severe cases, the tumors progress to large cavernous and infiltrative forms and create clinical complications. Systemic forms of hemangiomas, the

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hemangiomas, have a high mortality rate. Therapy-resistant hemangiomas exist that cannot be treated with therapeutics currently in use.

Angiogenesis is also responsible for damage found in hereditary diseases such as Osler-Weber-Rendu disease, or hereditary hemorrhagic telangiectasia. This is an inherited disease characterized by multiple small angiomas, tumors of blood or lymph vessels. The angiomas are found in the skin and mucous membranes, often accompanied by epistaxis (nosebleeds) or gastrointestinal bleeding and sometimes with pulmonary or hepatic arteriovenous fistula.

Angiogenesis is prominent in solid tumor formation and metastasis. Angiogenic factors have been found associated with several solid tumors such as rhabdomyosarcomas, retinoblastoma, Ewing's sarcoma, neuroblastoma, and osteosarcoma. A tumor cannot expand without a blood supply to provide nutrients and remove cellular wastes. Tumors in which angiogenesis is important include solid tumors, and benign tumors such as acoustic neuroma, neurofibroma, trachoma and pyogenic granulomas. Prevention of angiogenesis could halt the growth of these tumors and the resultant damage to the animal due to the presence of the tumor.

It should be noted that angiogenesis has been associated with blood-borne tumors such as leukemias, any of various acute or chronic neoplastic diseases of the bone marrow in which unrestrained proliferation of white blood cells occurs, usually accompanied by anemia, impaired blood clotting, and enlargement of the lymph nodes, liver, and spleen. It is believed that angiogenesis plays a role in the abnormalities in the bone marrow that give rise to leukemia-like tumors.

Angiogenesis is important in two stages of tumor metastasis. The first stage where angiogenesis stimulation is important is in the vascularization of the tumor which allows tumor cells to enter the blood stream and to circulate throughout the body. After the tumor cells have left the primary site, and have settled into the secondary, metastasis site, angiogenesis must occur before the new tumor can grow and expand. Therefore, prevention of angiogenesis could lead to the prevention of metastasis of tumors and possibly contain the neoplastic growth at the primary site.

Knowledge of the role of angiogenesis in the maintenance and metastasis of tumors has led to a prognostic indicator for breast cancer. The amount of neovascularization found in the primary tumor was determined by counting the microvessel density in the area of the most intense neovascularization in invasive breast carcinoma. A high level of microvessel density was found to correlate with tumor recurrence. Control of angiogenesis by therapeutic means could possibly lead to cessation of the recurrence of the tumors.

Angiogenesis is also involved in normal physiological processes such as reproduction and wound healing. Angiogenesis is an important step in ovulation and also in implantation of the blastula after fertilization. Prevention of angiogenesis could be used to induce amenorrhea, to block ovulation or to prevent implantation by the blastula.

In wound healing, excessive repair or fibroplasia can be a detrimental side effect of surgical procedures and may be caused or exacerbated by angiogenesis. Adhesions are a frequent complication of surgery and lead to problems such as small bowel obstruction.

Several kinds of compounds have been used to prevent angiogenesis. Taylor et al. have used protamine to inhibit angiogenesis, see Taylor et al., *Nature* 297:307 (1982). The toxicity of protamine limits its practical use as a therapeutic. Folkman et al. have disclosed the use of heparin and steroids to control angiogenesis. See Folkman et al., *Science* 221:719 (1983) and U.S. Pat. Nos. 5,001,116 and 4,994,443. Steroids,

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such as tetrahydrocortisol, which lack glucocorticoid activity, have been found to be angiogenic inhibitors.

Other factors found endogenously in animals, such as a 4 kDa glycoprotein from bovine vitreous humor and a cartilage derived factor, have been used to inhibit angiogenesis. Cellular factors such as interferon inhibit angiogenesis. For example, interferon α or human interferon β has been shown to inhibit tumor-induced angiogenesis in mouse dermis stimulated by human neoplastic cells. Interferon β is also a potent inhibitor of angiogenesis induced by allogeneic spleen cells. See Sidky et al., *Cancer Research* 47:5155-5161 (1987). Human recombinant α interferon (α /A) was reported to be successfully used in the treatment of pulmonary hemangiomas, an angiogenesis-induced disease. See White et al., *New England J. Med.* 320:1197-1200 (1989).

Other agents which have been used to inhibit angiogenesis include ascorbic acid esters and related compounds. See Japanese Kokai Tokkyo Koho No. 58-131978. Sulfated polysaccharide DS 4152 also shows angiogenic inhibition. See Japanese Kokai Tokkyo Koho No. 63-119500. A fungal product, fumagillin, is a potent angiostatic agent in vitro. The compound is toxic in vivo, but a synthetic derivative, AGM 12470, has been used in vivo to treat collagen II arthritis. Fumagillin and O-substituted fumagillin derivatives are disclosed in EPO Publication Nos. 0325199A2 and 0357061A1.

PCT Application No. WO 92/14455 to Kaplan et al. is directed to a method for controlling abnormal concentration of TNF- α by administering thalidomide or thalidomide derivatives to a patient with toxic concentrations of TNF- α .

The above compounds are either topical or injectable therapeutics. Therefore, there are drawbacks to their use as a general angiogenic inhibitor and lack adequate potency. For example, in prevention of excessive wound healing, surgery on internal body organs involves incisions in various structures contained within the body cavities. These wounds are not accessible to local applications of angiogenic inhibitors. Local delivery systems also involve frequent dressings which are impracticable for internal wounds, and increase the risk of infection or damage to delicate granulation tissue for surface wounds.

Thus, a method and composition are needed that are capable of inhibiting angiogenesis and which are easily administered. A simple and efficacious method of treatment would be through the oral route. If an angiogenic inhibitor could be given by an oral route, the many kinds of diseases discussed above, and other angiogenic dependent pathologies, could be treated easily. The optimal dosage could be distributed in a form that the patient could self-administer.

SUMMARY OF THE INVENTION

In accordance with the present invention, compositions and methods are provided that are effective in inhibiting unwanted angiogenesis. These compositions are easily administered by different routes, including orally, and can be given in dosages that are safe and provide angiogenic inhibition at internal sites. The present invention provides a method of treating mammalian diseases mediated by undesired and uncontrolled angiogenesis by administering a composition comprising an anti-angiogenic compound in a dosage sufficient to inhibit angiogenesis.

The present invention also includes angiogenic inhibiting compounds that contain an epoxide group. These angiogenic inhibiting compounds can be administered to a human or animal alone or with epoxide hydrolase inhibiting compounds.

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The present invention also includes compositions comprising an anti-angiogenesis compound and an antiinflammatory compound. The antiinflammatory compound can be either a steroidal or nonsteroidal antiinflammatory compound. Non-steroidal antiinflammatory compounds, called NSAIDs, are preferred.

The present invention is especially useful for treating certain ocular neovascular diseases such as macular degeneration. The compounds which are contemplated as part of the present invention preferably can be given orally to the patient and thereby halt the progression of the disease. Other disease that can be treated using the present invention are diabetic retinopathy, neovascular glaucoma and retrolental fibroplasia.

Accordingly, it is an object of the present invention to provide a compound and method to inhibit unwanted angiogenesis in a human or animal.

It is yet another object of the present invention to provide a composition for inhibiting angiogenesis by oral administration of the composition.

It is another object of the present invention to provide a treatment for diseases mediated by angiogenesis.

It is yet another object of the present invention to provide a treatment for macular degeneration.

It is yet another object of the present invention to provide a treatment for all forms of proliferative vitreoretinopathy including those forms not associated with diabetes.

It is yet another object of the present invention to provide a treatment for solid tumors.

It is yet another object of the present invention to provide a method and composition for the treatment of blood-born tumors such as leukemia.

It is another object of the present invention to provide a method and composition for the treatment of hemangioma.

It is another object of the present invention to provide a method and composition for the treatment of retrolental fibroplasia.

It is another object of the present invention to provide a method and composition for the treatment of psoriasis.

It is another object of the present invention to provide a method and composition for the treatment of Kaposi's sarcoma.

It is another object of the present invention to provide a method and composition for the treatment of Crohn's diseases.

It is another object of the present invention to provide a method and composition for the treatment of diabetic retinopathy.

Another object of the present invention is to provide a method and composition comprising thalidomide and antiinflammatory drugs for the treatment of angiogenesis dependent diseases.

It is another object of the present invention is to provide a method and composition comprising thalidomide and steroidal antiinflammatory drugs for the treatment of angiogenesis dependent diseases.

It is yet another object of the present invention is to provide a method and composition comprising thalidomide and nonsteroidal, antiinflammatory drugs for the treatment of angiogenesis dependent diseases.

It is another object of the present invention is to provide a method and composition comprising angiogenesis inhibiting compounds and antiinflammatory drugs for the treatment of angiogenesis dependent diseases.

Yet another object of the present invention is to provide a method and composition comprising angiogenesis inhibiting

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compounds and steroidal antiinflammatory drugs for the treatment of angiogenesis dependent diseases.

Another object of the present invention is to provide a method and composition comprising angiogenesis inhibiting compounds and nonsteroidal antiinflammatory drugs for the treatment of angiogenesis dependent diseases.

It is an object of the present invention to provide a method and composition antiinflammatory drugs for the treatment of angiogenesis dependent diseases.

It is another object of the present invention to provide a method and composition comprising steroidal antiinflammatory drugs for the treatment of angiogenesis dependent diseases.

It is yet another object of the present invention to provide a method and composition comprising nonsteroidal antiinflammatory drugs for the treatment of angiogenesis dependent diseases.

Another object of the present invention is to provide a method and composition comprising thalidomide and antiinflammatory drugs for the treatment of cancer.

Yet another object of the present invention is to provide a method and composition comprising thalidomide and steroidal antiinflammatory drugs for the treatment of cancer.

Another object of the present invention is to provide a method and composition comprising thalidomide and nonsteroidal antiinflammatory drugs for the treatment of cancer.

It is an object of the present invention to provide a method and composition comprising antiinflammatory drugs for the treatment of cancer.

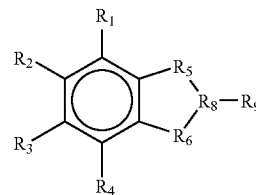
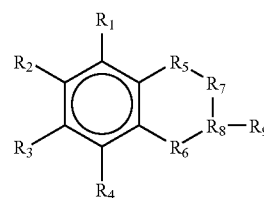
It is another object of the present invention to provide a method and composition comprising steroidal antiinflammatory drugs for the treatment of cancer.

It is yet another object of the present invention to provide a method and composition comprising nonsteroidal antiinflammatory drugs for the treatment of cancer.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiments and the appended claims.

BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1 through 3 are a listing of representative compounds in the genus represented by the following general formulas:



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-continued

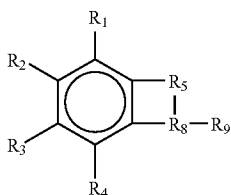


FIG. 4 is a listing of representative compounds in the genus represented by the following general formula:

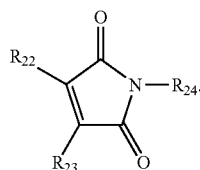


FIG. 5 is a listing of representative compounds in the genus represented by the following general formula:

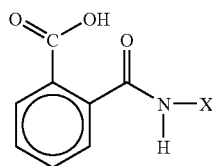


FIG. 6 shows the effect of thalidomide and EM12 on angiogenesis in a rabbit cornea model of angiogenesis.

FIG. 7 shows the effect of thalidomide on the area of corneal vascularization in a rabbit cornea model of angiogenesis.

FIG. 8 shows the effect of thalidomide, sulindac, or a combination of the two on the inhibition of tumor growth of V2-carcinoma in New Zealand White female rabbits.

DETAILED DESCRIPTION

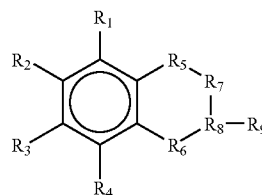
The present invention includes compositions and methods for the treatment of diseases that are mediated by angiogenesis. One embodiment of the present invention is the use of thalidomide or the metabolites of thalidomide as disclosed herein to inhibit unwanted angiogenesis. The present invention also includes compounds which cause dysmelia in the developing fetus and have anti-angiogenic activity. The present invention comprises a method of treating undesired angiogenesis in a human or animal comprising the steps of administering to the human or animal with the undesired angiogenesis a composition comprising an effective amount of a teratogenic compound that is anti-angiogenic.

Compounds that can be used in accordance with the present invention include compounds included in the following general formulae. Examples of compounds that have anti-angiogenic properties having one of the following three formulae (A), (B), or (C):

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C)

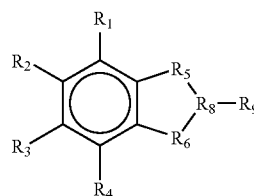
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A)

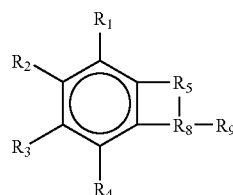
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B)

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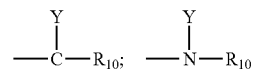
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C)

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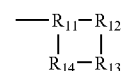
In the above formulae, A), B), and C), R_1 , R_2 , R_3 and R_4 can be selected from: —H; —OH; =O, straight and branched chain alkanes, alkenes, alkynes; cyclic alkanes, alkenes, and alkynes; combinations of cyclic and acyclic alkanes, alkenes, and alkynes; alcohol, aldehyde, ketone, carboxylic acids, esters, or other moieties in combination with acyclic, cyclic, or combination acyclic/cyclic moieties; aza; amino; —XO_n, or —O—XO_n, where X=N and n=2; X=S and n=2 or 3; or X=P and n=1-3; and halogens, R_5 , R_6 , R_7 , and R_8 are each independently selected from:

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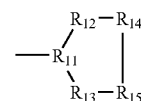
or —O— where Y is optional and is the same as defined above for R_5 ; and R_{10} is the same as defined above for R_1 , or when Y is absent, R_{10} is =O; and R_9 is a moiety having formula D), E), F), G) or H):

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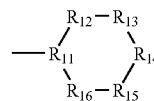
D)

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E)

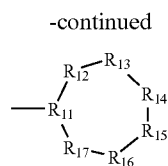
65



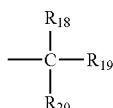
F)

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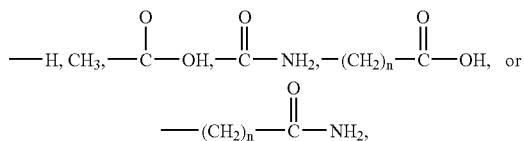
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where each of R_{11} - R_{17} is independently the same as defined above for R_5 ;



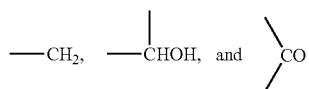
where R_{18} , R_{19} , and R_{20} are, independently selected from



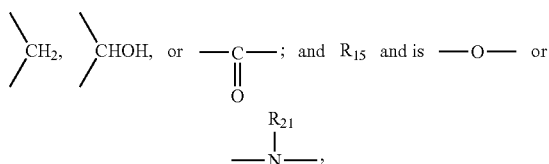
and $n=1$ to 4.

Accordingly, another aspect of the present invention features inhibiting angiogenesis in a mammal by administering a therapeutic composition comprising one of the above-described compounds in a dosage sufficient to inhibit angiogenesis.

In preferred embodiments, the compound has formula B), where R_5 and R_6 are selected from the group consisting of:



and R_9 has formula F) or H); and R_{14} and R_{16} are selected from the group consisting of:

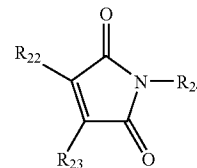


where R_{21} is ---H , ---CH_3 , or ---OH . Specific preferred compounds according to this aspect of the present invention include thalidomide, its precursors, metabolites and analogs. Particular analogs include EM-12, N-phthaloyl-DL-glutamic acid (PGA) or N-phthaloyl-DL-glutamine anhydride. Examples of compounds that are members of this genus are listed in FIGS. 1 through 3. It is to be understood that the compounds included as part of the present invention are not to be limited to those compounds shown in FIGS. 1 through 3

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and include all other compounds that are members of the genus described by the general formulas herein.

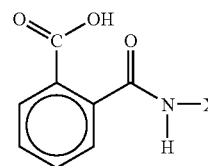
Compounds of the following formula that have anti-angiogenic properties:



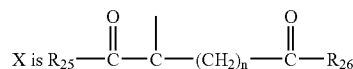
where R_{22} and R_{23} are (independently), ---H , ---F , ---Cl , ---Br , ---I , ---CH_3 , or $\text{---CH}_2\text{---CH}_3$; and R_{24} is ---H , ---CH_3 , or $\text{---CH}_2\text{---CH}_3$.

The present invention also features inhibiting angiogenesis in a mammal by administering a compound according to the above formulae in a dosage sufficient to inhibit angiogenesis. Examples of specific compounds that are members of this genus are listed in FIG. 4.

Angiogenesis inhibition hydrolysis products of thalidomide having the following general formula can be used in practicing the present invention:

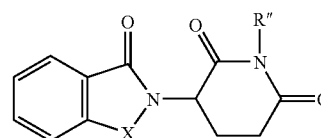
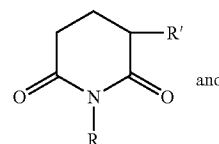


where X is R_6 as defined above, or



and R_{25} and R_{26} are, independently, ---OH , ---H , or NH_2 , and $n=1$ through 4. Examples of such compounds are shown in FIG. 5.

Angiogenesis inhibition compounds having the following general formula can be used in practicing the present invention:

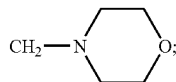


wherein R is selected from the group consisting of hydrogen, alkyl radicals of 1 to 6 carbon atoms, the phenyl radical, and

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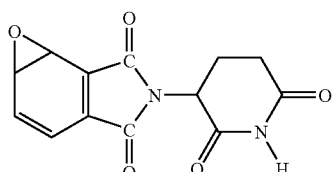
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the benzyl radical; wherein R' is selected from the group consisting of the phthalimido radical and the succinimido radical; wherein X is CH₂ or C=O; and wherein R'' is H, —CH₂CH₃, —C₆H₅, —CH₂C₆H₅, —CH₂CH=CH₂, or

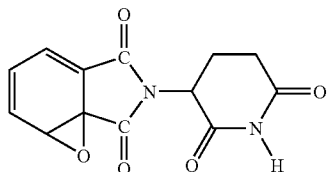


and hydrolysis products of the compounds wherein R'' is H and the piperidino ring or both the piperidino and the imido ring are hydrolyzed.

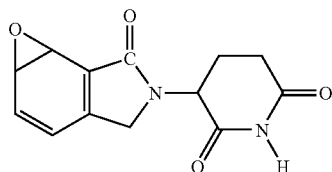
Another set of compounds that are considered part of the present invention are the epoxides of thalidomide, EM-12 and EM-138. Representative epoxide compounds are shown as follows:



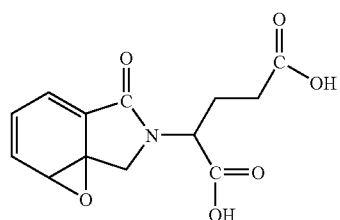
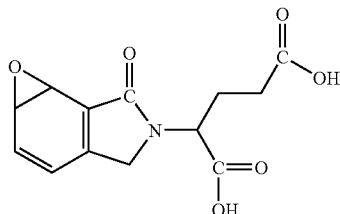
Epoxides of thalidomide



Epoxides of EM 12



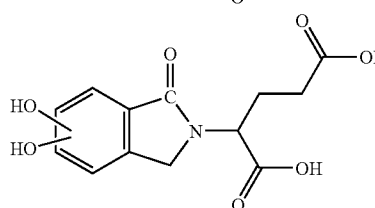
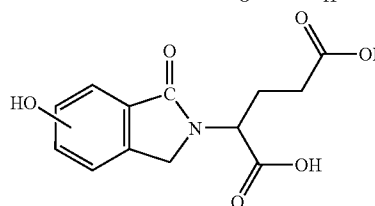
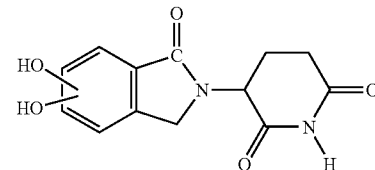
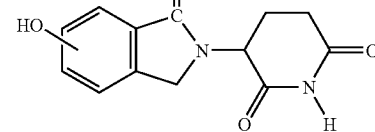
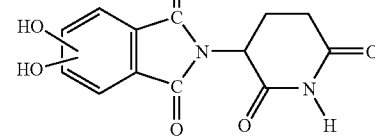
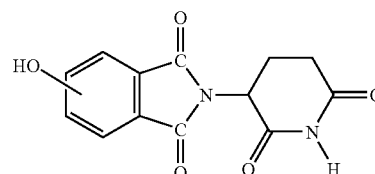
Epoxides of EM 138



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It should be understood that the epoxide can be attached at the 6,1 site on the benzene ring, the 1,2 site, the 2,3 site 3,4 or the 4,5 site. All of these compounds are contemplated as part of the present invention.

The epoxides of the thalidomide, EM-12, and EM-138 can be hydrolyzed to the following compounds:



It is to be understood that the hydroxyl group can be on carbons 1, 2, 3, 4, 5 and 6 of the benzene ring. Also contemplated as part of the present invention are dihydroxyl compounds wherein the two hydroxyl groups are located bis to each other on carbons 1, 2, 3, 5 and 6 of the above compounds. The epoxides, the hydrolysis products of the epoxides, and the hydrolysis products of the thalidomide are all contemplated to be part of the present invention.

It is known that epoxides are hydrolyzed by a group of enzymes known as epoxide hydrolases. There is a class of compounds which are epoxide hydrolase inhibitors. Examples of these compounds are valpromide (2-propylpentanamide) and valproic acid (2-propylpentanoic acid). Because epoxides are important angiogenesis inhibitors, it is contemplated as part of the present invention, compositions

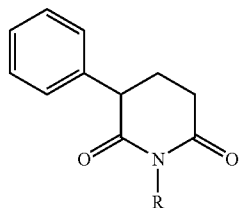
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comprising any of the angiogenesis inhibitors compounds recited herein in combination with epoxide hydrolase inhibitors. The epoxide hydrolase inhibitors can be administered to a human or animal together or sequentially. The epoxide group appears to be an important substituent common to several angiogenesis inhibitors. The use of epoxide hydrolase inhibitors to potentiate the activity of any angiogenesis inhibitor containing an epoxide is contemplated as part of the present invention. For example, the epoxide hydrolase inhibitors can be administered with the following epoxide-containing anti-angiogenesis compounds: AGM 1470, Eponimycin, microbial metabolites of *Scolecobasidium arenarium* designated f/2015, fr/111142 and fr/18487. See Oikawa, *Biochem Biophys. Res. Comm.*, Vol. 81:1070 (1971) and Otsuka, *J. Microbial. Biotech.*, Vol 1:163 (1991).

It is contemplated as an embodiment of the present invention the use of the epoxide containing angiogenesis inhibitors with or without epoxide hydrolase inhibitors as a treatment for diseases mediated by elevated or toxic levels of TNF- α . TNF- α has been recognized as manifesting a dose dependent toxicity. If present at low levels for a long period of time, TNF- α can result in cachexia. Cachexia is a general weight loss and wasting occurring in the course of some chronic diseases such as cancer, opportunistic infections of AIDS, inflammatory diseases, parasitic diseases, tuberculosis, and high dose IL-2 therapy. The epoxide containing angiogenesis inhibitors, with or without epoxide hydrolase inhibitors, are also effective in treating diseases such as septic shock, leprosy and graph vs. host disease.

Other embodiments are within the present invention. For example, other dysmelia-causing compounds can be used according to the present invention, e.g. 4-methylphthalic acid, pyridoxine, vasopressin, acetazolamide, or a compound having the following formula (where R=H, —OH, or —CH₃):



Other compounds which are teratogens, such as valproic acid (2-propylpentanoic acid), the retinoids, such as *cis*-retinoic acid, and rifampin may also be used in accordance with the invention.

In summary, the preferred compounds are thalidomide, as well as analogs, hydrolysis products, metabolites and precursors of thalidomide that are teratogenic, and, more specifically, that cause dysmelia. However, it is to be understood that it is not necessary for a compound to have both teratogenic activity and angiogenesis inhibiting activity to be considered part of the present invention. Dysmelia-causing compounds can be identified by the general procedures of Helm, *Arzneimittelforschung*, 31(i/6):941-949 (1981), in which rabbit pups are examined after exposure to the compound in utero. The compounds can generally be purchased, e.g., from Andrulic Pharmaceuticals, Beltsville, Md., or synthesized according to known procedures. It is to be understood that the compounds of the present invention can exist as enantiomers

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and that the racemic mixture of enantiomers or the isolated enantiomers are all considered as within the scope of the present invention.

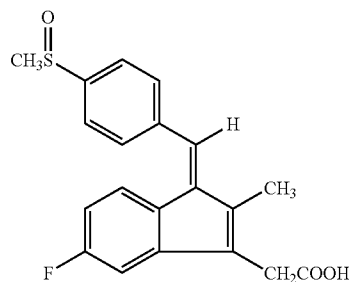
Many of the compounds that are contemplated as part of the present invention can be enriched in optically active enantiomers of the compounds specified above. Specifically, Blaschke has reported that the S enantiomers may be disproportionately responsible for the dysmelia-producing effect of these compounds. See, generally Blaschke, *Arzneimittelforschung* 29:1640-1642 (1979). The above described articles generally describe procedures to obtain optically active preparations of the compounds of interest. See, e.g. Shealy et al., *Chem. Indus.* 1030 (1965); and Casini et al., *Farmaco Ed. Sci.* 19:563 (1964).

In another embodiment, the invention also includes the inhibition of angiogenesis and the treatment of angiogenesis dependent diseases by administering antiinflammatory compounds, either alone or in combination with other angiogenesis inhibiting compounds, such as those described above.

These antiinflammatory compounds may be either steroids or nonsteroidal antiinflammatory drugs (NSAIDs). Examples of steroids which may be used in the invention include, but are not limited to, cortisone, cortisol, corticosterone, hydrocortisone, hydrocortisol, prednisone, prednisolone, dexamethasone, beclomethasone, betamethasone, mometasone, mometasone furoate, budesonide, triamcinolone acetamide, and fluticasone. Preferred steroids are prednisone, hydrocortisone, cortisol, dexamethasone, betamethasone, and beclomethasone. Especially preferred steroids are hydrocortisone, dexamethasone, and betamethasone.

Examples of NSAIDs which may be used in the invention include, but are not limited to, aspirin, acetaminophen, ibuprofen, esculetin, phenidone, quercetin, ketoprofen, nordihydroguaiaretic acid (NDGA), sulindac, sulindac sulfone, sulindac sulfide, indomethacin, NS-398 (a cyclooxygenase-2 inhibitor), cyclooxygenase-1 inhibitors, methylheptyl imidazole, furegrelate sodium, SKF525AHCL, thromboxane inhibitors, toradol, ecase, salsalate, diflunisal, mefenamic acid, naproxen, naproxen sodium, floctafenine, meclofenamate, phenylbutazone, oxyphenbutazone, diclofenac, etodolac, fenoprofen, flufenamic acid, flurbiprofen, pirofen, tolmetin, apazone, fenbufen, nabumetone, oxaprozin, piroxicam, salicylate, and tenoxicam. Preferred NSAIDs are sulindac, sulindac sulfone, sulindac sulfide, indomethacin, NS-398, methylheptyl imidazole, furegrelate sodium, and SKF525AHCL. Especially preferred NSAIDs are indomethacin and sulindac.

Sulindac, which includes (Z)-5-Fluoro-2-methyl-1-[[4-(methyl-sulfinyl)phenyl]methylene]-1H-indene-3-acetic acid, or *cis*-5-fluoro-2-methyl-1-[p-(methylsulfinyl)benzylidene]indene-3-acetic acid, has the following structure:



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Such compounds can be used to treat angiogenesis dependent diseases. Such compounds can be used alone or in combination with other angiogenesis inhibiting compounds to treat angiogenesis dependent diseases, such as cancer.

The compounds described above can be provided as pharmaceutically acceptable formulations using formulation methods known to those of ordinary skill in the art. These formulations can be administered by standard routes. In general, the combinations may be administered by the topical, transdermal, oral, rectal or parenteral (e.g., intravenous, subcutaneous or intramuscular) route. In addition, the combinations may be incorporated into biodegradable polymers allowing for sustained release of the compound, the polymers being implanted in the vicinity of where drug delivery is desired, for example, at the site of a tumor. The biodegradable polymers and their use are described, for example, in detail in Brem et al., *J. Neurosurg.* 74:441-446 (1991).

The dosage of the compound will depend on the condition being treated, the particular compound, and other clinical factors such as weight and condition of the human or animal and the route of administration of the compound. It is to be understood that the present invention has application for both human and veterinary use. For oral administration to humans, a dosage of between approximately 0.1 to 300 mg/kg/day, preferably between approximately 0.5 and 50 mg/kg/day, and most preferably between approximately 1 to 10 mg/kg/day, is generally sufficient.

The formulations include those suitable for oral, rectal, ophthalmic, (including intravitreal or intracaineral) nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intratracheal, and epidural) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by conventional pharmaceutical techniques. Such techniques include the step of bringing into association the active ingredient and the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredient 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, 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 liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil emulsion and as a bolus, etc.

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 ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface active or dispersing agent. Molded tablets may be made by molding, in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may be optionally coated or scored and may be formulated so as to provide a slow or controlled release of the active ingredient therein.

Formulations suitable for topical administration in the mouth include lozenges comprising the ingredients in a flavored basis, 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 ingredient to be administered in a suitable liquid carrier.

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Formulations suitable for topical administration to the skin may be presented as ointments, creams, gels and pastes comprising the ingredient to be administered in a pharmaceutical acceptable carrier. A preferred topical delivery system is a transdermal patch containing the ingredient to be administered.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

Formulations suitable for nasal administration, wherein the carrier is a solid, include a coarse powder having a particle size, for example, in the range of 20 to 500 microns which is administered in the manner in which snuff is administered, i.e., by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations, wherein the carrier is a liquid, for administration, as for example, a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient.

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 parenteral administration include aqueous and non-aqueous 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. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in freeze-dried (lyophilized) conditions requiring only the addition of the sterile liquid carrier, for example, water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose, as herein above recited, or an appropriate fraction thereof, of the administered ingredient.

It should be understood that in addition to the ingredients, particularly mentioned above, the formulations of the present 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 flavoring agents.

Diseases associated with corneal neovascularization that can be treated according to the present invention include but are not limited to, diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, neovascular glaucoma and retrolental fibroplasia, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sjogren's syndrome, acne rosacea, phlyctenulosis, syphilis, Mycobacteria infections, lipid degeneration, chemical burns, bacterial ulcers, fungal ulcers, Herpes simplex infections, Herpes zoster infections, protozoan infections, Kaposi's sarcoma, Mooren's ulcer, Terrien's marginal degeneration, marginal keratolysis, trauma, rheumatoid arthritis, systemic lupus, polyarteritis, Wegener's sarcoidosis, scleritis, Stevens-Johnson disease, radial keratotomy, pemphigoid and corneal graft rejection.

Diseases associated with retinal/choroidal neovascularization that can be treated according to the present invention include, but are not limited to, diabetic retinopathy, macular degeneration, sickle cell anemia, sarcoid, syphilis, pseudox-

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anthoma elasticum, Paget's disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis/vitritis, mycobacterial infections, Lyme's disease, systemic lupus erythematosus, retinopathy of prematurity, Eales' disease, Behcet's disease, infections causing a retinitis or choroiditis, presumed ocular histoplasmosis, Best's disease, myopia, optic pits, Stargardt's disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma and post-laser complications. Other diseases include, but are not limited to, diseases associated with rubeosis (neovascularization of the angle) and diseases caused by the abnormal proliferation of fibrovascular or fibrous tissue including all forms of proliferative vitreoretinopathy, whether or not associated with diabetes.

Diseases associated with chronic inflammation can be treated by the compositions and methods of the present invention. Diseases with symptoms of chronic inflammation include inflammatory bowel diseases such as Crohn's disease and ulcerative colitis, psoriasis, sarcoidosis and rheumatoid arthritis. Angiogenesis is a key element that these chronic inflammatory diseases have in common. The chronic inflammation depends on continuous formation of capillary sprouts to maintain an influx of inflammatory cells. The influx and presence of the inflammatory cells produce granulomas and, thus, maintain the chronic inflammatory state. Inhibition of angiogenesis by the compositions and methods of the present invention would prevent the formation of the granulomas and alleviate the disease.

The compositions and methods of the present invention can be used to treat patients with inflammatory bowel diseases such as Crohn's disease and ulcerative colitis. Both Crohn's disease and ulcerative colitis are characterized by chronic inflammation and angiogenesis at various sites in the gastrointestinal tract. Crohn's disease is characterized by chronic granulomatous inflammation throughout the gastrointestinal tract consisting of new capillary sprouts surrounded by a cylinder of inflammatory cells. Prevention of angiogenesis by the compositions and methods of the present invention inhibits the formation of the sprouts and prevents the formation of granulomas.

Crohn's disease occurs as a chronic transmural inflammatory disease that most commonly affects the distal ileum and colon but may also occur in any part of the gastrointestinal tract from the mouth to the anus and perianal area. Patients with Crohn's disease generally have chronic diarrhea associated with abdominal pain, fever, anorexia, weight loss and abdominal swelling. Ulcerative colitis is also a chronic, non-specific, inflammatory and ulcerative disease arising in the colonic mucosa and is characterized by the presence of bloody diarrhea.

The inflammatory bowel diseases also show extraintestinal manifestations such as skin lesions. Such lesions are characterized by inflammation and angiogenesis and can occur at many sites other than the gastrointestinal tract. The compositions and methods of the present invention are also capable of treating these lesions by preventing the angiogenesis, thus, reducing the influx of inflammatory cells and the lesion formation.

Sarcoidosis is another chronic inflammatory disease that is characterized as a multisystem granulomatous disorder. The granulomas of this disease may form anywhere in the body, and, thus, the symptoms depend on the site of the granulomas and whether the disease active. The granulomas are created by the angiogenic capillary sprouts providing a constant supply of inflammatory cells.

The compositions and methods of the present invention can also treat the chronic inflammatory conditions associated

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with psoriasis. Psoriasis, a skin disease, is another chronic and recurrent disease that is characterized by papules and plaques of various sizes. Prevention of the formation of the new blood vessels necessary to maintain the characteristic lesions leads to relief from the symptoms.

Another disease which can be treated according to the present invention is rheumatoid arthritis. Rheumatoid arthritis is a chronic inflammatory disease characterized by non-specific inflammation of the peripheral joints. It is believed that the blood vessels in the synovial lining of the joints undergo angiogenesis. In addition to forming new vascular networks, the endothelial cells release factors and reactive oxygen species that lead to pannus growth and cartilage destruction. The factors involved in angiogenesis may actively contribute to, and help maintain, the chronically inflamed state of rheumatoid arthritis.

Other diseases that can be treated according to the present invention are hemangiomas, Osler-Weber-Rendu disease, or hereditary hemorrhagic telangiectasia, solid or blood borne tumors and acquired immune deficiency syndrome.

The compositions and methods of the present invention include the use of angiogenesis inhibiting compounds and antiinflammatory compounds, such as steroids or nonsteroidal antiinflammatory drugs (NSAIDs). The compositions and methods of the present invention include the combination of angiogenesis inhibiting compounds, such as thalidomide or thalidomide-like analogs or AMG-1470, EM-12 or EM-138 with antiinflammatory compounds, such as steroids or nonsteroidal antiinflammatory drugs (NSAIDs). The compositions and methods of the present invention also include the use antiinflammatory compounds alone. These compositions can be used to treat angiogenesis dependent diseases.

For example, it has been found that indomethacin (5 mg/kg) inhibits bFGF induced angiogenesis by 59% and VEGF induced angiogenesis by 61%. Similarly, sulindac (25 mg/kg) has been found to inhibit bFGF induced angiogenesis by 50% and VEGF induced angiogenesis by 55%. Sulindac is metabolized in vivo to two metabolites: sulindac sulfide and sulindac sulfone.

Sulindac sulfide actively inhibits prostaglandin synthesis, while sulindac sulfone does not; however, both were found to be inhibitors of angiogenesis. Sulindac sulfide was found to inhibit bFGF induced neovascularization by 34%, and sulindac sulfone exhibited 31% inhibition.

Other NSAIDs have also been found to inhibit angiogenesis associated neovascularization. For example, carbomethyleptyl imidazole and furegrelate sodium, both thromboxane inhibitors, inhibit bFGF induced neovascularization 32% and 22%, respectively. Another thromboxane inhibitor, SKF525AHCL, which promotes prostaglandin production, was found to inhibit bFGF induced neovascularization by 25%. The specific cyclooxygenase-2 inhibitor NS-398 also inhibited bFGF induced neovascularization by 25%.

When sulindac is combined with thalidomide, there is an additive effect in the inhibition of angiogenesis. When sulindac is combined with other angiogenesis inhibiting compounds, such as AMG 1470, EM-12 or EM-138, there is an additive effect in the inhibition of angiogenesis. Angiogenesis in the eye, in the assay described in Example 2, is produced by the presence of bFGF (basic fibroblastic growth factor) and VEGF (vascular endothelial cell growth factor). Inhibition of such angiogenesis was shown with the NSAIDs, steroids, thalidomide, or the combination of thalidomide and an NSAID, sulindac.

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AGENT	DOSE	bFGF ¹	VEGF ²
Aspirin	100 mg/kg	8	—
Acetaminophen	100 mg/kg	0	0
Ibuprofen	20 mg/kg	7	11
Hydrocortisone	20 mg/kg	50	40
Sulindac	25 mg/kg	52	54
Thalidomide	200 mg/kg	42	44
Thalidomide + Sulindac	200 mg/kg (thal) + 25 mg/kg (sulindac)	65	74
AGM 1470	30 mg/kg qod	48	
AGM 1470 + Sulindac	30 mg/kg qod (AGM) + 25 mg/kg (sulindac)	70	

¹% Inhibition of bFGF induced angiogenesis²% Inhibition of VEGF induced angiogenesis

The inhibition of VEGF by 74% demonstrates the additive effect when thalidomide and sulindac are combined. The data for hydrocortisone, sulindac, thalidomide and thalidomide +sulindac differ significantly from the controls ($p < 0.0001$). Studies with the composition comprising thalidomide and sulindac on V2 carcinoma in rabbits has demonstrated a T/C (treated to control ratio) of 0.32 after 18 days of oral treatment with thalidomide 200 mg/kg combined with 25 mg/kg sulindac.

An additive effect is also seen in the combination of non-steroidal antiinflammatory drugs and other angiogenesis inhibiting compounds such as the combination of sulindac and AMG-1470. AMG-1470 is a known angiogenesis inhibiting compound as shown in Brem et al. Minimal drug resistance occurs after prolonged antiangiogenic therapy with AMG-1470, *Surgical Forum* 45 (0), 1994, pp 674-677, herein incorporated by reference. As shown in the above chart, sulindac plus AGM-1470 shows a greater inhibitory effect upon angiogenesis than does either compound alone.

This invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

EXAMPLE I

The chick embryo chorioallantoic membrane assay described by Crum et al., *Science* 230:1375 et seq. (1985), is used to identify compounds that do not require further metabolic conversion. See also, U.S. Pat. No. 5,001,116, hereby incorporated by reference, which describes the CAM assay at col. 7 of the patent. Briefly, fertilized chick embryos were removed from their shell on day 3 or 4, and a methylcellulose disc containing the compound was implanted on the chorioallantoic membrane. The embryos were examined 48 hours later and, if a clear avascular zone appeared around the methylcellulose disc, the diameter of that zone was measured.

EXAMPLE II

Rabbit Cornea Angiogenesis Assay

Pellets for implantation into rabbit corneas were made by mixing 110 μ l of saline containing 12 μ g of recombinant bFGF (Takeda Pharmaceuticals-Japan) with 40 mg of sucralfate (Bukh Meditec-Denmark); this suspension was added to 80 μ g of 12% hydron (Interferon Sciences) in ethanol. 10 μ l

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aliquots of this mixture was then pipetted onto Teflon pegs and allowed to dry producing approximately 17 pellets. A pellet was implanted into corneal micropockets of each eye of an anesthetized female New Zealand white rabbit, 2 mm from the limbus followed by topical application of erythromycin ointment onto the surface of the cornea. The animals were fed daily from 2 days post-implantation by gastric lavage with either drug suspended in 0.5% carboxymethyl cellulose or 0.5% carboxymethyl cellulose alone. Thalidomide was purchased from Andrus Pharmaceutical (Maryland) and the EM-12 and Supidimide were kindly provided by Grunenthal GMBH (Germany). The animals were examined with a slit lamp every other day in a masked manner by the same corneal specialist. The area of corneal neovascularization was determined by measuring with a reticule the vessel length (L) from the limbus and the number of clock hours (C) of limbus involved. A formula was used to determine the area of a circular band segment: $C/12 * 3.1416 [r^2 - (r-L)^2]$ where $r = 6$ mm the measured radius of the rabbit cornea. Various mathematical models were utilized to determine the amount of vascularized cornea, and this formula was found to provide the most accurate approximation of the area of the band of neovascularization that grows towards the pellet.

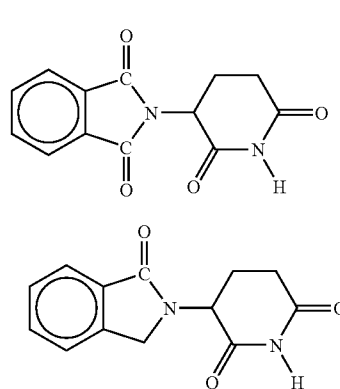
It is important to note that the rabbit cornea assay is preferable because it will generally recognize compounds that are inactive per se but are metabolized to yield active compounds. Thalidomide related compounds, as shown below in Example III, are known to be teratogens and are candidates for use in the present invention.

EXAMPLE III

Inhibition of bFGF Induced Corneal Neovascularization by Thalidomide and Related Analog Expressed as Percent of Median Control on Day 8

Pellets containing bFGF and sucralfate were implanted into micropockets of both corneas of rabbits according to Example II. Vessel ingrowth into clear cornea from the limbus was first noted on day 2 and treatments (200 mg/kg orally) were begun on this day. The area of corneal neovascularization was measured from day 4 through day 12. Day 8 measurements were used for comparison between groups. No regression of vessels and near maximal neovascularization was seen at this time point. Statistical analysis was performed with ANOVA with ranked data to account for interexperimental variation and to guard against a non-normal distribution of data (i.e. outliers) by utilizing a nonparametric method.

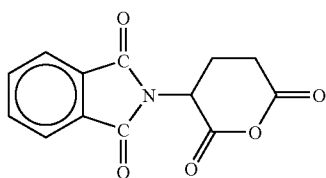
The compounds tested were as follows:



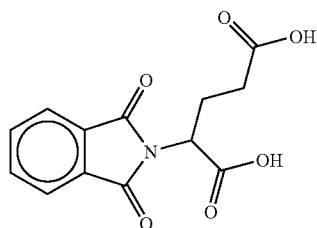
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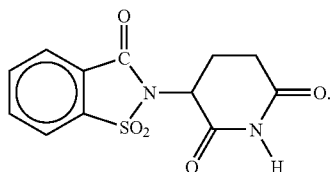
-continued
phthaloyl glutamic anhydride (PGA)



phthaloyl glutamic acid (PG Acid)



supidimide



Treatment with a dose of (200 mg/kg) of thalidomide resulted in an inhibition of the area of vascularized cornea that ranged from 30-51% in three experiments with a median inhibition of 36% (FIG. 6) (n=30 eyes, p=0.0001, 2 way ANOVA with ranked data). The inhibition of angiogenesis by thalidomide was seen after only two doses (FIG. 7). The rabbits did not demonstrate obvious sedation and there were no signs of toxicity or weight loss. The teratogenic analog EM-12, which shares the other properties of thalidomide was also inhibitory, with a median inhibition of 42% (n=10 eyes, p=0.002, 1-way ANOVA with ranked data). Supidimide, a nonteratogenic analog of thalidomide that retains the sedative properties of thalidomide, exhibited no activity. (area 107% of control, n=10 eyes, not statistically different from control). Other analogs, PGA and PG acid displayed weaker inhibitory effects than thalidomide (data not shown). The density of vessel ingrowth in thalidomide-treated animals was also markedly reduced.

EXAMPLE IV

EM-12 in Rabbit Cornea Assay

EM-12 was tested in the rabbit cornea assay described in Example II at 100 mg/kg/day and showed 21% inhibition, and at 200 mg/kg/day the assay showed 43% inhibition.

EXAMPLE V

Phthaloyl Glutamic Acid in CAM

Phthaloyl glutamic acid was tested in the above described CAM assay and exhibit an avascular zone with a mild scar.

EXAMPLE VI

Phthaloyl Glutamic Acid in Rabbit Cornea Assay

Phthaloyl glutamic acid described above at 200 mg/kg and exhibited 29% inhibition of angiogenesis.

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EXAMPLE VII

Phthaloyl Glutamic Anhydride in CAM Assay

Phthaloyl glutamic anhydride was test in the CAM assay described above and exhibited an avascular zone.

EXAMPLE VIII

Treatment of Crohn's Disease

A 32 year old female patient with Crohn's disease was treated using the methods of the present invention. The patient exhibited the characteristic symptoms of Crohn's disease, i.e., severe gastrointestinal involvement (including diarrhea and cramping) and a large skin lesion on the lower leg. Thalidomide was orally administered to her at a dosage of 100 mg twice a day. After treatment was continued for one week, the gastrointestinal symptoms, including the diarrhea and cramping, were lessened and the skin lesion resolved.

EXAMPLE IX

Corneal Micropocket Assay

Six to eight week old C57B16 male mice were obtained from Jackson Laboratories, MA. The mice were anesthetized, and 0.4x0.4 mm pockets were made in the stroma of the mouse cornea adjacent to the limbus. Pellets containing 80 ng of either bFGF or VEGF were implanted in the pellets. The pellets containing bFGF were implanted 1.0-1.2 mm from the limbal vessels, while the pellets containing VEGF were implanted 0.5-0.7 mm from the limbal vessels. Erythromycin was then topically applied.

The mice were then treated with varying doses of anti-inflammatory drugs as shown in the table below. The vascular response to the pellets was measured by maximal vessel length and number of clock hours of neovascularization 5 days after implantation of the bFGF pellets and 6 days after implantation of the VEGF pellets. The area of corneal neovascularization was calculated using the following formula which best approximated the area of neovascularization: Area (mm²)=[$\pi \times \text{clock hours} \times \text{length (mm)} \times 0.2 \text{ (mm)}$]. The results are shown in the following table.

AGENT	DOSE	bFGF ¹	VEGF ²	n	p-value
Acetaminophen	100 mg/kg	0	—	8	ns
Aspirin	10-160 mg/kg	0-11	—	8	ns
NDGA	25 mg/kg	30	—	8	ns
Esculetin	200 mg/kg	15	—	8	.02
Phenidone	100 mg/kg	17	—	8	<.01
Quercetin	300 mg/kg	18	—	8	<.01
Ibuprofen	25 mg/kg	6	8	23/8	ns/ns
Ketoprofen	80 mg/kg	30	41	8/8	<.01
Indomethacin	5 mg/kg	59	61	15/21	<.01/<.01
Sulindac	25 mg/kg	50	55	15/15	<.01/<.01

¹% Inhibition of bFGF induced angiogenesis

²% Inhibition of VEGF induced angiogenesis

Inhibitory effect is expressed in percentage representing the area of corneal neovascularization by either bFGF or VEGF compared to controls (n=8/experiment) of the experiments in which that particular drug was tested. N=the number of eyes that were tested and drugs were given once daily either sc, ip or oral as described.

EXAMPLE X

Six to eight week old C57B16 male mice were obtained from Jackson Laboratories, MA. The mice were anesthetized, and 0.4x0.4 mm pockets were made in the stroma of the mouse cornea adjacent to the limbus. Pellets containing 80 ng of either bFGF or VEGF were implanted. The pellets containing bFGF were implanted 1.0-1.2 mm from the limbal vessels, while the pellets containing VEGF were implanted 0.5-0.7 mm from the limbal vessels. Erythromycin was then topically applied.

The mice were then treated with thalidomide, indomethacin, sulindac, or combinations of thalidomide with either indomethacin or sulindac. The vascular response to the pellets was measured by maximal vessel length and number of clock hours of neovascularization 5 days after implantation of the bFGF pellets and 6 days after implantation of the VEGF pellets. The area of corneal neovascularization was calculated using the following formula which best approximates the area of neovascularization: Area (mm²)=[π×clock hours×length (mm)×0.2 (mm)]. The results are shown in the following table.

AGENT	DOSE	bFGF ¹	VEGF ²	n
Thalidomide	200 mg/kg	41	40	31/39
Indomethacin	5 mg/kg	59	61	15/21
Sulindac	25 mg/kg	50	55	15/15
Thalidomide + Indomethacin	200 mg/kg + 5 mg/kg	67	61	15/21
Thalidomide + Sulindac	200 mg/kg + 25 mg/kg	63 ³	74 ³	15/16

¹% Inhibition of bFGF induced angiogenesis

²% Inhibition of VEGF induced angiogenesis

³Inhibitory effect is significantly different from either agent alone (p < .01, tested by ANOVA)

Inhibitory effect is expressed in percentage representing the area of corneal neovascularization by either bFGF or VEGF compared to controls (n=8/experiment) of the experiments in which that particular drug was tested. N=the number of eyes that were tested and drugs were given once daily either sc, ip or oral as described.

EXAMPLE XI

Six to eight week old C57B16 male mice, obtained from Jackson Laboratories, MA, were anesthetized, and 0.4x0.4 mm pockets were made in the stroma of the mouse cornea adjacent to the limbus. Pellets containing 80 ng of bFGF were implanted 1.0-1.2 mm from the limbal vessels. Erythromycin was then topically applied.

The mice were then treated with sulindac, or one of the sulindac derivatives, sulindac sulfone or sulindac sulfide. The vascular response to the pellets was measured by maximal vessel length and number of clock hours of neovascularization 5 days after implantation of the bFGF pellets. The area of corneal neovascularization was calculated using the following formula which best approximates the area of neovascularization: Area (mm²)=[π×clock hours×length (mm)×0.2 (mm)]. The results are shown in the following table.

AGENT	DOSE	% inhibition	n
Sulindac	25 mg/kg	50	15
Sulindac sulfide	25 mg/kg	31	8
	50 mg/kg	34	8
Sulindac sulfone	25 mg/kg	31	16

Inhibitory effect is expressed in percentage representing the area of corneal neovascularization by bFGF compared to controls (n=8/experiment. N=the number of eyes that were tested and drugs were given once daily either sc, ip or oral as described.

EXAMPLE XII

Tumor Assay

New Zealand White female rabbits, weighing approximately 1.5 kg, obtained from Charles River, Mass., were used for propagating the V2-carcinoma. This tumor originates from a Shope virus-induced papilloma. Small 0.5x0.5 cm pieces were implanted intramuscularly in the right thigh. Treatment with 200 mg/kg/day thalidomide (n=14), 60 mg/kg/day sulindac (n=5), or a combination of thalidomide and sulindac (n=10) was started at day 10 after tumor implantation, when the mean volume of the tumor was 6 cm³. The control animals (n=13) were treated with methylcellulose. The rabbits were sacrificed 17 days after the start of treatment when mean volume of the control tumors was 100 cm³. The results of this experiment (FIG. 8) show that the combination of thalidomide and sulindac is more effective in reducing the size of V2-carcinoma tumors than either thalidomide or sulindac alone. The combination of thalidomide and sulindac inhibited tumor growth by 75% and was significantly different (p<0.05) from either agent alone or the control group.

Oral treatment with sulindac or thalidomide inhibited tumor growth by, respectively, 35% (n=5, p<0.01), and 55% (n=14, p<0.01). The data were collected in 3 separate experiments and each bar represents the standard error of the mean.

It should be understood, of course, that the foregoing relates only to preferred embodiments of the present invention and that numerous modifications or alterations may be made therein without departing from the spirit and the scope of the invention as set forth in the appended claims.

I claim:

1. A method of treating blood-borne tumors comprising administering to a patient in need of such treatment an effective amount of thalidomide in combination with dexamethasone.
2. The method of claim 1, wherein the amount of thalidomide administered is from 0.1 to 300 mg/kg/day.
3. The method of claim 2, wherein the amount of thalidomide administered is from 0.5 to 50 mg/kg/day.
4. The method of claim 3, wherein the amount of thalidomide administered is from 1 to 10 mg/kg/day.
5. The method of claim 1, wherein the thalidomide and dexamethasone are administered parenterally.
6. The method of claim 1, wherein the thalidomide and dexamethasone are administered orally.

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