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(54) Title: METHYLPHENIDATE DERIVATIVES AND USES OF THEM

(57) Abstract: The present invention provides methods of using compounds of formula (I) and salts and prodrugs thereof, wherein n, R¹ and R² are defined herein. The invention also provides certain novel compounds of formula I and pharmaceutical compositions comprising them.

![Graph](image-url)
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METHYLPHENIDATE DERIVATIVES AND USES OF THEM

FIELD OF THE INVENTION

[0001] The invention relates to uses of methylphenidate derivatives. The uses include inhibiting angiogenesis and treating angiogenic diseases and conditions.

BACKGROUND

[0002] Methylphenidate is the treatment of choice for children and adults diagnosed with attention deficit/hyperactivity disorder (ADHD), including its inattentive subtype (formerly known as attention deficit disorder or ADD). Certain derivatives of methylphenidate have also been proposed for the treatment of ADD (see U.S. Patent No. 6,025,502) and for the treatment of other neurological disorders and conditions (see U.S. Patents Nos. 5,859,249, 6,025,502 and 6,486,177 and PCT application WO 99/36403).


Further, some types of tumors have been reported to be decreased, while other types of tumors have been reported to be increased. See Dunnick and Hailey, Toxicology, 103:77-84 (1995), National Toxicology Program, Natl. Toxicol. Program Tech. Rep. Ser., 439:1-299 (1995) and Dunnick et al., Cancer Lett., 102:77-83 (1996).
SUMMARY OF THE INVENTION

[0005] The invention provides methods of using a compound of formula I

\[
(R^1)_n\begin{array}{c}
\text{O} \\
\text{HN}
\end{array}
\]

wherein n is an integer from 1 to 5, and each R\(^1\) is independently aryl, heteroaryl, alkyl, cycloalkyl, alkoxy, aryloxy, acyl, carboxyl, hydroxyl, halogen, amino, nitro, sulfon or sulphydryl. Each alkyl can optionally be substituted with hydroxyl, amino or sulphydryl. R\(^2\) is hydrogen or lower alkyl.

[0006] In a first embodiment, the invention provides a method of inhibiting angiogenesis in an animal. The method comprises administering an effective amount of a compound of formula I, or a pharmaceutically-acceptable salt or a prodrug thereof, to the animal.

[0007] In a second embodiment, the invention provides a method of treating an angiogenic disease or condition in an animal. The method comprises administering a therapeutically effective amount of a compound of formula I, or a pharmaceutically-acceptable salt or a prodrug thereof, to the animal.

[0008] In a third embodiment, the invention provides a method of treating a proliferative disorder in an animal. The method comprises administering a therapeutically effective amount of a compound of formula I, or a pharmaceutically-acceptable salt or a prodrug thereof, to the animal.

[0009] The invention also provides a compound of formula IA:

\[
(R^1)_n\begin{array}{c}
\text{O} \\
\text{HN}
\end{array}
\]

IA
where
   n is an integer from 1 to 5;
   each R^1 is independently a moiety of the formula –C(O)–R^6, –OR^7 or –C(O)–O–R^3;
   R^2 is hydrogen or lower alkyl;
   R^3 is hydrogen, alkyl, cycloalkyl or aryl;
   R^7 is aryl; and
   R^8 is cycloalkyl or aryl.

[0010] The invention further provides a pharmaceutical composition comprising a pharmaceutically-acceptable carrier and a compound of formula IA:

![Chemical Structure](image)

or a salt or prodrug thereof,

   where
   n is an integer from 1 to 5;
   each R^1 is independently a moiety of the formula –C(O)–R^6, –OR^7 or –C(O)–O–R^3;
   R^2 is hydrogen or lower alkyl;
   R^3 is hydrogen, alkyl, cycloalkyl or aryl;
   R^7 is aryl; and
   R^8 is cycloalkyl or aryl.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Figures 1A-C are graphs of OD at 530 nm for various additives to peripheral blood lymphocyte (PBL) cultures stimulated with 2 μg/ml, 5 μg/ml and 20 μg/ml phytohemagglutinin (PHA), respectively.

[0012] Figure 2 is a graph of OD at 530 nm for various additives to PBL cultures stimulated with 2 μg/ml PHA.

[0013] Figure 3 is a graph of concentration of IL-13 for various additives to PBL cultures stimulated with 2 μg/ml PHA.
Figure 4 is a graph of concentration of IFNγ for various additives to PBL cultures stimulated with 2 μg/ml PHA.

Figures 5A-B are graphs of OD at 530 nm for various additives to PBL cultures stimulated with 2 μg/ml and 5 μg/ml PHA, respectively.

Figure 6 is a graph of concentration of IL-13 for various additives to PBL cultures stimulated with 5 μg/ml PHA.

Figure 7 is a graph of concentration of TNFα for various additives to PBL cultures stimulated with 2 μg/ml PHA.

Figure 8 is a graph of concentration of IL-8 for various additives to PBL cultures stimulated with 2 μg/ml PHA.

Figures 9A-B are graphs of OD for various additives to THP-1 monocyte cultures stimulated with lipopolysaccharide (LPS). In Figure 9B, the top dark gray bar in each instance is c-Jun and the bottom light gray bar is NfκB.

Detailed Description of the Invention

In one aspect, compounds of formula I are useful in the practice of the present invention.

![Chemical Structure](image)

In Formula I, n is an integer from 1 to 5. Preferably n is 1 or 2.

Each R₁, which may be the same or different, is aryl, heteroaryl, alkyl, cycloalkyl, alkoxy, arylxoy, acyl, carboxyl, hydroxyl, halogen, amino, nitro, sulfo or sulfhydryl. Each alkyl can optionally be substituted with hydroxyl, amino or sulfhydryl. R₁ is preferably aryl, alkyl, cycloalkyl, alkoxy, aryloxy or acyl. More preferably R₁ is aryl, alkyl or cycloalkyl, even more preferably aryl, most preferably phenyl.

In formula I, R₂ is hydrogen or lower alkyl. Preferably, R₂ is –CH₃.

In one specific embodiment, the compound of formula II is particularly useful in the present invention:
[0023] "Acyl" means a moiety of the formula –C(O)–R³, wherein R³ is H, alkyl, cycloalkyl or aryl.

[0024] "Amino" means a moiety of the formula –NR⁴R⁵, wherein each of R⁴ and R⁵ is independently H or lower alkyl, preferably lower alkyl.

[0025] "Alkoxy" means a moiety of the formula –OR⁶, wherein R⁶ is alkyl. An example of an alkoxy group is methoxy (–O-CH₃).

[0026] "Alkyl" means a monovalent saturated straight-chain or branched hydrocarbon containing 1-8 carbon atoms. Each alkyl may, optionally, be substituted with one or more amino, hydroxyl or sulphhydryl groups.

[0027] "Aryl" means a monovalent mono-, bi- or tricyclic aromatic hydrocarbon moiety of 6 to 14 ring carbon atoms. Preferred is phenyl.

[0028] "Aryloxy" means a moiety of the formula –OR⁷, wherein R⁷ is aryl. An example of an alkoxy group is phenoxy.

[0029] "Carboxyl" means a moiety of the formula –C(O)–OR³, wherein R³ is H, alkyl, cycloalkyl or aryl.

[0030] "Cycloalkyl" means a saturated, monovalent mono- or bicyclic hydrocarbon moiety of three to ten ring carbon atoms. Preferably the cycloalkyl contains 4-8 ring carbon atoms. The most preferred cycloalkyl is cyclohexyl.

[0031] "Halogen" means chlorine, fluorine, bromine or iodine. Preferred is chlorine or bromine.

[0032] "Heteroaryl" means a monovalent monocyclic or bicyclic aromatic moiety of 5 to 12 ring atoms containing one, two, or three ring heteroatoms each of which is independently selected from N, O, and S, the remaining ring atoms being C.

[0033] "Hydroxyl" means –OH.
“Lower alkyl” means a saturated straight-chain or branched hydrocarbon containing 1-4 carbon atoms.

“Nitro” means –NO₂.

“Sulphydryl” means –SH.

“Sulfo” means –SO₃H.

“Prodrug” means any compound which releases an active parent drug according to formula I in vivo when such prodrug is administered to a mammalian subject. Prodrugs of a compound of formula I are prepared by modifying one or more functional group(s) present in the compound of formula I in such a way that the modification(s) may be cleaved in vivo to release the parent compound. Prodrugs include compounds of formula I wherein a hydroxy, amino, or sulphydryl group in a compound of formula I is bonded to any group that may be cleaved in vivo to generate the free hydroxyl, amino, or sulphydryl group, respectively. Examples of prodrugs include, but are not limited to, esters (e.g., acetate, formate, and benzoate derivatives), carbamates (e.g., N,N-dimethylaminocarbonyl) of hydroxy functional groups in compounds of formula I, and the like.

“Inhibit” or “inhibiting” is used herein to mean to reduce (wholly or partially) or to prevent.

“Treating” or “treatment” of a disease or condition includes: (1) preventing the disease or condition, i.e., causing the clinical symptoms of the disease or condition not to develop in a mammal that may be exposed to or predisposed to the disease or condition, but does not yet experience or display symptoms of the disease or condition; (2) inhibiting the disease or condition, i.e., arresting or reducing the development of the disease or condition or its clinical symptoms; or (3) relieving the disease or condition, i.e., causing regression of the disease or condition or its clinical symptoms, including curing the disease or condition.

An “effective amount” means the amount of a compound that, when administered to an animal for treating a disease or condition or for causing an effect is sufficient to do so. The “effective amount” can and will most likely vary depending on the compound, the disease or condition and its severity, or the effect sought to be caused, and the age, weight, etc., of the animal to be treated.

Methods of synthesizing the compounds of formula I useful in the present invention are known in the art. See, e.g., U.S. Patents Nos. 5,859,249 and 6,025,502, PCT application WO 99/36403, Pan et al., Eur. J. Pharmacol., 264, 177-182 (1994), Gatley et al.,

[0043] If the compound of the present invention contains one or more chiral centers, the compound can be synthesized enantioselectively or a mixture of enantiomers and/or diastereomers can be prepared and separated. The resolution of the compounds of the present invention, their starting materials and/or the intermediates may be carried out by known procedures, e.g., as described in the four volume compendium *Optical Resolution Procedures for Chemical Compounds*: Optical Resolution Information Center, Manhattan College, Riverdale, N.Y., and in *Enantiomers, Racemates and Resolutions*, Jean Jacques, Andre Collet and Samuel H. Wilen; John Wiley & Sons, Inc., New York, 1981, which are incorporated herein in their entirety. Basically, the resolution of the compounds is based on the differences in the physical properties of diastereomers by attachment, either chemically or enzymatically, of an enantiomerically pure moiety, resulting in forms that are separable by fractional crystallization, distillation or chromatography.

[0044] The pharmaceutically-acceptable salts of the compounds of formula I may also be used in the practice of the invention. Pharmaceutically-acceptable salts include conventional non-toxic salts, such as salts derived from inorganic acids (such as hydrochloric, hydrobromic, sulfuric, phosphoric, nitric, and the like), organic acids (such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, glutamic, aspartic, benzoic, salicylic, oxalic, ascorbic acid, and the like) or bases (such as the hydroxide, carbonate or bicarbonate of a pharmaceutically-acceptable metal cation or organic cations derived from N,N-dibenzylethylenediamine, D-glucosamine, or ethylenediamine). The salts are prepared in a conventional manner, e.g., by reacting the free base form of the compound with an acid.

[0045] It is to be understood that the scope of this invention encompasses not only the use of the compounds of formula I themselves, but also the salts and prodrugs thereof. In addition, the present invention contemplates the use of the isomers of the compounds of formula I, and of the salts and prodrugs thereof, including pure isomers and various mixtures of isomers.
Compounds of formula I, pharmaceutically-acceptable salts thereof or prodrugs thereof, can be used to inhibit angiogenesis. Angiogenesis is the process of new blood vessel formation in the body. Angiogenesis is also used herein to mean the same as, or to include, neovascularization, vascularization, arterialization and vasculogenesis.

Compounds of formula I, pharmaceutically-acceptable salts thereof or prodrugs thereof, can also be used to treat angiogenic diseases and conditions. An angiogenic disease or condition is a disease or condition involving, caused by, exacerbated by, or dependent on, angiogenesis. Specific angiogenic diseases and conditions treatable according to the invention include neoplastic diseases, hypertrophy (e.g., cardiac hypertrophy induced by thyroid hormone), connective tissue disorders (e.g., rheumatoid arthritis and atherosclerosis), psoriasis, ocular angiogenic diseases, cardiovascular diseases, cerebral vascular diseases, endometriosis, polyposis, obesity, diabetes-associated diseases and hemophilic joints. The compounds of formula I, pharmaceutically-acceptable salts thereof or prodrugs thereof, can also be used to inhibit the vascularization required for embryo implantation, thereby providing a method of birth control.

The compounds of formula I, pharmaceutically-acceptable salts thereof or prodrugs thereof, will be particularly useful for the treatment of ocular angiogenic diseases. Ocular angiogenic diseases include diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasias, and rubecosis. The compounds of formula I, pharmaceutically-acceptable salts thereof or prodrugs thereof, will be especially useful for the treatment of diabetic retinopathy and macular degeneration.

The compounds of formula I, pharmaceutically-acceptable salts thereof or prodrugs thereof, will also be particularly useful for the treatment of neoplastic diseases. Neoplastic diseases treatable with the compounds of formula I, pharmaceutically-acceptable salts thereof or prodrugs thereof, include malignant tumors (e.g., tumors of the bladder, brain, breast, cervix, colon, rectum, kidney, liver, lung, ovary, pancreas, prostate, stomach and uterus), tumor metastasis, and benign tumors (e.g., hemangiomas, acoustic neuromas, neurofibromas, trachomas and pyogenic granulomas). The compounds of formula I, pharmaceutically-acceptable salts thereof or prodrugs thereof, will be especially useful for the treatment of tumors of the brain, breast, colon, liver and pancreas, most especially tumors of the brain (e.g., glioblastomas).
In addition to being able to inhibit angiogenesis, the compounds of formula I, pharmaceutically-acceptable salts thereof or prodrugs thereof, have been found to be able to inhibit the proliferation of cells, reduce the growth of cancer cells, inhibit the production of cytokines, inhibit Ras and RAP-1, and inhibit the production of NFκB and AP-1. Thus, the compounds of formula I, pharmaceutically-acceptable salts thereof or prodrugs thereof, will also be particularly useful for the treatment of a variety of proliferative disorders, including angiogenic diseases and conditions, especially neoplastic diseases (see above), and other cancers and other proliferative disorders.

Cancers treatable with the compounds of formula I, pharmaceutically-acceptable salts thereof or prodrugs thereof, include carcinomas, sarcomas, lymphomas, leukemias, solid tumors and hematologic malignancies. Specific cancers treatable with the compounds of formula I, pharmaceutically-acceptable salts thereof or prodrugs thereof, include brain cancers, head and neck cancers, breast cancers, ovarian cancers, prostate cancers, gastric cancers, colon cancers, pancreatic cancers, bladder cancers, thyroid cancers, hepatic cancers, lung cancers, bone cancers and skin cancers. The compounds of formula I, pharmaceutically-acceptable salts thereof or prodrugs thereof, will be especially useful for the treatment of brain cancers, breast cancers, colon cancers, liver cancers, pancreatic cancers, skin cancers, lymphomas and leukemias.

Other proliferative disorders include mesangial cell proliferation disorders, fibrotic disorders and hyperproliferative skin disorders. Mesangial cell proliferative disorders refer to disorders brought about by abnormal proliferation of mesangial cells. Mesangial cell proliferative disorders include renal diseases, such as glomerulonephritis, diabetic nephropathy, malignant nephrosclerosis, thrombotic microangiopathy syndromes and glomerulopathies. Fibrotic disorders refer to the abnormal formation of extracellular matrices. Examples of fibrotic disorders include hepatic cirrhosis, pulmonary fibrosis and atherosclerosis. Hyperproliferative skin disorders include psoriasis, skin cancer and epidermal hyperproliferation.

To treat an animal in need of treatment, a compound of formula I, pharmaceutically-acceptable salt thereof or prodrug thereof, is administered to the animal. Preferably, the animal is a mammal, such as a rabbit, goat, dog, cat, horse or human. Most preferably, the animal is a human.
[0054] Effective dosage forms, modes of administration and dosage amounts for the compounds of the invention may be determined empirically, and making such determinations is within the skill of the art. It is understood by those skilled in the art that the dosage amount will vary with the particular compound employed, the disease or condition to be treated, the severity of the disease or condition, the route(s) of administration, the rate of excretion of the compound, the duration of the treatment, the identify of any other active ingredient(s) being administered to the animal, the age, size and species of the animal, and like factors known in the medical and veterinary arts. In general, a suitable daily dose of a compound of the present invention will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect. However, the daily dosage will be determined by an attending physician or veterinarian within the scope of sound medical judgment. If desired, the effective daily dose may be administered as two, three, four, five, six or more sub-doses, administered separately at appropriate intervals throughout the day. Administration of the compound should be continued until an acceptable response is achieved.

[0055] The compounds useful in the present invention (i.e., the compounds of formula I and the pharmaceutically-acceptable salts and prodrugs thereof) may be administered to an animal patient for therapy by any suitable route of administration, including orally, nasally, rectally, vaginally, parenterally (e.g., intravenously, intraspinally, intraperitoneally, subcutaneously, or intramuscularly), intracisternally, transdermally, intracranially, intracerebrally, and topically (including buccally and sublingually). The preferred routes of administration are orally and topically.

[0056] While it is possible for a compound useful in the present invention to be administered alone, it is preferable to administer the compound as a pharmaceutical formulation (composition). The pharmaceutical compositions useful in the invention comprise one or more compounds of formula I, or pharmaceutically-acceptable salts or prodrugs thereof, as active ingredient(s) in admixture with one or more pharmaceutically-acceptable carriers and, optionally, with one or more other compounds, active ingredient(s) or other materials. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the animal. Pharmaceutically-acceptable carriers are well known in the art. Regardless of the route of administration selected, the compounds of the present invention are formulated into pharmaceutically-
acceptable dosage forms by conventional methods known to those of skill in the art. See, e.g., Remington's Pharmaceutical Sciences.

Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, powders, granules or as a solution or a suspension in an aqueous or non-aqueous liquid, or an oil-in-water or water-in-oil liquid emulsions, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia), and the like, each containing a predetermined amount of a compound or compounds useful in the present invention as an active ingredient. A compound or compounds useful in the present invention may also be administered as bolus, electuary or paste.

In solid dosage forms for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient(s) is (are) mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), 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, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in microencapsulated form.

Liquid dosage forms for oral administration of the compounds of the invention include pharmaceutically-acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient(s), the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active compound(s), may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Pharmaceutical formulations for intraocular injection of a compound or compounds of the invention into the eyeball include solutions, emulsions, suspensions, particles, capsules, microspheres, liposomes, matrices, etc. See, e.g., U.S. Patent No.
6,060,463, U.S. Patent Application Publication No. 2005/0101582, and PCT application WO 2004/043480, the complete disclosures of which are incorporated herein by reference. For instance, a pharmaceutical formulation for intraocular injection may comprise one or more compounds of the invention in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or non-aqueous solutions, suspensions or emulsions, which may contain antioxidants, buffers, suspending agents, thickening agents or viscosity-enhancing agents (such as a hyaluronic acid polymer). Examples of suitable aqueous and nonaqueous carriers include water, saline (preferably 0.9%), dextrose in water (preferably 5%), buffers, dimethylsulfoxide, alcohols and polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like). These compositions may also contain adjuvants such as wetting agents and emulsifying agents and dispersing agents. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as polymers and gelatin. Injectable depot forms can be made by incorporating the drug into microcapsules or microspheres made of biodegradable polymers such as polylactide-polyglycolide. Examples of other biodegradable polymers include poly(orthoesters), poly(glycolic) acid, poly(lactic) acid, polycaprolactone and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes (composed of the usual ingredients, such as dipalmitoyl phosphatidylcholine) or microemulsions which are compatible with eye tissue. Depending on the ratio of drug to polymer or lipid, the nature of the particular polymer or lipid components, the type of liposome employed, and whether the microcapsules or microspheres are coated or uncoated, the rate of drug release from microcapsules, microspheres and liposomes can be controlled.

The compounds of the invention can also be administered surgically as an ocular implant. For instance, a reservoir container having a diffusible wall of polyvinyl alcohol or polyvinyl acetate and containing a compound or compounds of the invention can be implanted in or on the sclera. As another example, a compound or compounds of the invention can be incorporated into a polymeric matrix made of a polymer, such as polycaprolactone, poly(glycolic) acid, poly(lactic) acid, poly(anhydride), or a lipid, such as sebacic acid, and may be implanted on the sclera or in the eye. This is usually accomplished with the animal receiving a topical or local anesthetic and using a small incision made behind the cornea. The matrix is then inserted through the incision and sutured to the sclera.
A preferred embodiment of the invention is local topical administration of the compounds of the invention to the eye, and a particularly preferred embodiment of the invention is a topical pharmaceutical composition suitable for application to the eye. Topical pharmaceutical compositions suitable for application to the eye include solutions, suspensions, dispersions, drops, gels, hydrogels and ointments. See, e.g., U.S. Patent No. 5,407,926 and PCT applications WO 2004/058289, WO 01/30337 and WO 01/68053, the complete disclosures of all of which are incorporated herein by reference.

Topical formulations suitable for application to the eye for treatment of an angiogenic disease or condition comprise one or more compounds of the invention in an aqueous or nonaqueous base. The topical formulations can also include absorption enhancers, permeation enhancers, thickening agents, viscosity enhancers, agents for adjusting and/or maintaining the pH, agents to adjust the osmotic pressure, preservatives, surfactants, buffers, salts (preferably sodium chloride), suspending agents, dispersing agents, solubilizing agents, stabilizers and/or tonicity agents. Topical formulations suitable for application to the eye for treatment of an angiogenic disease or condition will preferably comprise an absorption or permeation enhancer to promote absorption or permeation of the compound or compounds of the invention into the eye and/or a thickening agent or viscosity enhancer that is capable of increasing the residence time of a compound or compounds of the invention in the eye. See PCT applications WO 2004/058289, WO 01/30337 and WO 01/68053. Exemplary absorption/permeation enhancers include methysulfonylmethane, alone or in combination with dimethylsulfoxide, carboxylic acids and surfactants. Exemplary thickening agents and viscosity enhancers include dextrans, polyethylene glycols, polyvinylpyrrolidone, polysaccharide gels, Gelrite®, cellulotic polymers (such as hydroxypropyl methylcellulose), carboxyl-containing polymers (such as polymers or copolymers of acrylic acid), polyvinyl alcohol and hyaluronic acid or a salt thereof.

Liquid dosage forms (e.g., solutions, suspensions, dispersions and drops) can be prepared, for example, by dissolving, dispersing, suspending, etc. a compound or compounds of the invention in a vehicle, such as, for example, water, saline, aqueous dextrose, glycerol, ethanol and the like, to form a solution, dispersion or suspension. If desired, the pharmaceutical formulation may also contain minor amounts of non-toxic auxillary substances, such as wetting or emulsifying agents, pH buffering agents and the like,
for example sodium acetate, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, etc.

[0069] Aqueous solutions and suspensions can include, in addition to a compound or compounds of the invention, preservatives, surfactants, buffers, salts (preferably sodium chloride), tonicity agents and water. If suspensions are used, the particle sizes should be less than 10 μm to minimize eye irritation. If solutions or suspensions are used, the amount delivered to the eye should not exceed 50 μl to avoid excessive spillage from the eye.

[0070] Colloidal suspensions are generally formed from microparticles (i.e., microspheres, nanospheres, microcapsules or nanocapsules, where microspheres and nanospheres are generally monolithic particles of a polymer matrix in which the formulation is trapped, adsorbed, or otherwise contained, while with microcapsules and nanocapsules the formulation is actually encapsulated). The upper limit for the size of these microparticles is about 5μ to about 10μ.

[0071] Ophthalmic ointments include a compound or compounds of the invention in an appropriate base, such as mineral oil, liquid lanolin, white petrolatum, a combination of two or all three of the foregoing, or polyethylene-mineral oil gel. A preservative may optionally be included.

[0072] Ophthalmic gels include a compound or compounds of the invention suspended in a hydrophilic base, such as Carbol 940 or a combination of ethanol, water and propylene glycol (e.g., in a ratio of 40:40:20). A gelling agent, such as hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose or ammoniated glycyrrhizinate, is used. A preservative and/or a tonicity agent may optionally be included.

[0073] Hydrogels are formed by incorporation of a swellable, gel-forming polymer, such as those listed above as thickening agents or viscosity enhancers, except that a formulation referred to in the art as a “hydrogel” typically has a higher viscosity than a formulation referred to as a “thickened” solution or suspension. In contrast to such preformed hydrogels, a formulation may also be prepared so to form a hydrogel in situ following application to the eye. Such gels are liquid at room temperature but gel at higher temperatures (and thus are termed “thermoreversible” hydrogels), such as when placed in contact with body fluids. Biocompatible polymers that impart this property include acrylic acid polymers and copolymers, N-isopropylacrylamide derivatives and ABA block
copolymers of ethylene oxide and propylene oxide (conventionally referred to as “poloxamers” and available under the Pluronic® tradename from BASF-Wayndotte).

Preferred dispersions are liposomal, in which case the formulation is enclosed within liposomes (microscopic vesicles composed of alternating aqueous compartments and lipid bilayers).

Eye drops can be formulated with an aqueous or nonaqueous base also comprising one or more dispersing agents, solubilizing agents or suspending agents. Drops can be delivered by means of a simple eye dropper-capped bottle or by means of a plastic bottle adapted to deliver liquid contents dropwise by means of a specially shaped closure.

The compounds of the invention can also be applied topically by means of drug-impregnated solid carrier that is inserted into the eye. Drug release is generally effected by dissolution or bioerosion of the polymer, osmosis, or combinations thereof. Several matrix-type delivery systems can be used. Such systems include hydrophilic soft contact lenses impregnated or soaked with the desired compound of the invention, as well as biodegradable or soluble devices that need not be removed after placement in the eye. These soluble ocular inserts can be composed of any degradable substance that can be tolerated by the eye and that is compatible with the compound of the invention that is to be administered. Such substances include, but are not limited to, poly(vinyl alcohol), polymers and copolymers of polyacrylamide, ethylacrylate and vinylpyrrolidone, as well as cross-linked polypeptides or polysaccharides, such as chitin.

Dosage forms for the other types of topical administration (i.e., not to the eye) or for transdermal administration of compounds of the invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches, drops and inhalants. The active ingredient may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any buffers, or propellants which may be required. The ointments, pastes, creams and gels may contain, in addition to the active ingredient, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof. Powders and sprays can contain, in addition to the active ingredient, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder or mixtures of these substances. Sprays can additionally contain customary propellants such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and
propane. Transdermal patches have the added advantage of providing controlled delivery of compounds of the invention to the body. Such dosage forms can be made by dissolving, dispersing or otherwise incorporating one or more compounds of the invention in a proper medium, such as an elastomeric matrix material. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate-controlling membrane or dispersing the compound in a polymer matrix or gel.

[0078] Formulations of the pharmaceutical compositions for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more compounds of the invention with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active compound. Formulations of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

[0079] Pharmaceutical formulations include those suitable for administration by inhalation or insufflation or for nasal or intraocular administration. For administration to the upper (nasal) or lower respiratory tract by inhalation, the compounds of the invention are conveniently delivered from an insufflator, nebulizer or a pressurized pack or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide, or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

[0080] Alternatively, for administration by inhalation or insufflation, the composition may take the form of a dry powder, for example, a powder mix of one or more compounds of the invention and a suitable powder base, such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules or cartridges, or, e.g., gelatin or blister packs from which the powder may be administered with the aid of an inhalator, insufflator or a metered-dose inhaler.

[0081] For intranasal administration, compounds useful in the invention may be administered by means of nose drops or a liquid spray, such as by means of a plastic bottle
atomizer or metered-dose inhaler. Liquid sprays are conveniently delivered from pressurized packs. Typical of atomizers are the Mistometer (Wintrop) and Medihaler (Riker).

[0082] Drops, such as eye drops or nose drops, may be formulated with an aqueous or nonaqueous base also comprising one or more dispersing agents, solubilizing agents or suspending agents. Drops can be delivered by means of a simple eye dropper-capped bottle or by means of a plastic bottle adapted to deliver liquid contents dropwise by means of a specially shaped closure.

[0083] Pharmaceutical compositions suitable for parenteral administrations comprise one or more compounds useful in the invention in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or non-aqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

[0084] Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[0085] These compositions may also contain adjuvants such as wetting agents, emulsifying agents and dispersing agents. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like in the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

[0086] In some cases, in order to prolong the effect of the active ingredient(s), it is desirable to slow the absorption of the active ingredient(s) from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the active ingredient(s) then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-
administered active ingredient(s) is accomplished by dissolving or suspending the active ingredient(s) in an oil vehicle.

[0087] Injectable depot forms are made by forming microencapsule matrices of the active ingredient(s) in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of active ingredient(s) to polymer, and the nature of the particular polymer employed, the rate of release of the active ingredient(s) can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the active ingredient(s) in liposomes or microemulsions which are compatible with body tissue. The injectable materials can be sterilized for example, by filtration through a bacterial-retaining filter.

[0088] The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampules and vials, and may be stored in a lyophilized condition requiring only the addition of the sterile liquid carrier, for example water for injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the type described above.

[0089] Additional objects, advantages, and novel features of this invention will become apparent to those skilled in the art upon examination of the following examples thereof, which are not intended to be limiting.

EXAMPLES

EXAMPLE 1:

[0090] Whole blood was drawn from GR283, a human volunteer with known allergies, into a glass vacutainer tube containing no anticoagulant. This blood was allowed to clot, and the serum was removed by centrifugation and then heat inactivated by placing it in a water bath at 56°C for 30 minutes. Whole blood from GR283 was also drawn into a glass vacutainer tube containing heparin and used for peripheral blood lymphocytes (PBL) isolation as follows. Whole blood was layered over room temperature Histopaque 1077 solution and centrifuged at 2000 rpm for 15 minutes at room temperature. Cells at the plasma-Histopaque interface were then removed and washed with culture medium (IMDM medium with 10% heat-inactivated GR283 serum plus 1% penicillin/streptomycin) at 37°C.

[0091] The compound of formula II (see above) and methylphenidate (both obtained from Dr. Jeffrey D. Winkler, University of Pennsylvania, Philadelphia, Pennsylvania) in
culture medium were added to wells of a 96-well plate to give final concentrations of 5 μg/ml, 15 μg/ml and 50 μg/ml of the compound of formula II and of methylphenidate. Sterile 18 MΩ water, the solvent for the compound of formula II, and dexamethasone (obtained from Sigma) (final concentration of 10 μg/ml in water) were used as controls. Then, GR283’s PBL in culture medium were added to the wells to give a final concentration of 150,000 cells per well, and the plates were incubated at 37°C, 5% CO₂ for 24 hours. After this incubation, phytohemagglutinin (PHA) in culture medium was added to give final concentrations of 2 μg/ml, 5 μg/ml or 20 μg/ml, final total volume of 200 μl/well, and the cells were incubated for an additional 72 hours at 37°C, 5% CO₂. All cultures were performed in triplicate.

[0092] At the end of this incubation, cell clumping was examined by photographing representative wells with a digital camera mounted to an inverted microscope. The compound of formula II reduced the amount of cell clumping induced by 5 μg/ml PHA in a dose-dependent manner. The compound of formula II attenuated cell clumping, presumably, as a result of decreased expression of cellular adhesion molecules on the surfaces of the cells.

[0093] Cell proliferation was assayed by adding 20 μl of Promega cell titer solution to each well and incubating the plate for an additional 4 hours. Promega cell titer solution is a solution containing a tetrazolium dye that is reduced by living cells to a formazan dye, and this reduction is proportional to the number of living cells present in the well. After the 4-hour incubation, the optical density (OD) at 530 nm of each well was measured. The OD at 530 nm for blank wells containing no cells was subtracted from the OD of the experimental wells. The results of the proliferation assays are presented in Figures 1A-C. As can be seen from Figures 1A-C, the compound of formula II (Cpd. II) and dexamethasone (Dex) significantly inhibited the proliferation of PBL stimulated with PHA in a dose-dependent manner. Methylphenidate (MP) showed a significant effect at its highest dose and the lowest PHA dose. Otherwise, methylphenidate did not significantly reduce the proliferation of the PHA-stimulated PBL.

EXAMPLE 2:

[0094] Whole blood was drawn from GR467, a human volunteer with known allergies, and processed as described in Example 1 to give heat-inactivated serum and PBL. The compound of formula II and methylphenidate in culture medium (made using heat-inactivated GR467 serum) were added to wells of a 96-well plate to give final concentrations
of 5 μg/ml, 15 μg/ml and 25 μg/ml of the compound of formula II and 15 μg/ml methylphenidate. Water and dexamethasone (final concentration of 10 μM) were used as controls. Then, GR467’s PBL in culture medium were added to the wells to give a final concentration of 150,000 cells per well, and the plates were incubated at 37°C, 5% CO₂ for 24 hours. After this incubation, PHA was added to give a final concentration of 2 μg/ml, final total volume of 200 μl/well, and the cells were incubated for an additional 72 hours at 37°C, 5% CO₂. All cultures were performed in triplicate.

[0095] At the end of this incubation, cell proliferation was determined as described in Example 1. The results are presented in Figure 2. As can be seen from Figure 2, the compound of formula II (Cpd II) and dexamethasone (Dex) significantly inhibited the proliferation of PBL, both unstimulated and stimulated with PHA, whereas methylphenidate did not.

[0096] The release of cytokines by the PBL was also measured by culturing the PBL in 1 ml tubes, at 1.3 x 10⁶ cells per ml, with 15 μg/ml of the compound of formula II, 15 μg/ml methylphenidate or 10 μM dexamethasone at 37°C, 5% CO₂ for 24 hours. After this incubation, PHA was added to give a final concentration of 2 μg/ml, and the cells were incubated for an additional 96 hours at 37°C, 5% CO₂. All cultures were performed in triplicate. Cells were then removed by centrifugation at 1000 rpm for 10 minutes, and the culture medium collected.

[0097] IL-13 is made by activated T_h2 cells, and IL-13’s primary targets are B-cells and monocytes. IL-13 stimulates humoral immune responses, and it has been implicated in the pathogenesis of asthma. IL-13 is secreted by lymphoma cell lines and may be an autocrine growth factor. IL-13 is also expressed in pancreatic cancer. However, IL-13 has also been reported to inhibit the growth of other types of tumors, such as gliomas and renal cell carcinomas.

[0098] IFNγ is a proinflammatory cytokine made by activated T-cells and other cells. IFNγ can activate neutrophils, endothelial cells and macrophages, as well as cause an increase in MHC molecule expression. IFNγ drives the cell-mediated immune response. IFNγ plays an important role in the immune-mediated rejection of established tumors. IFNγ has antiproliferative effects on some tumors, can have apoptotic effects on others, can induce the production of angiostatic chemokines and enhances the immunogenicity of tumor cells.
Release of IL-13 and interferon gamma (IFNγ) into the culture medium was measured by ELISA. To perform the ELISA, matched pairs of antibodies against human IL-13 and IFNγ were purchased from Pierce Biotechnology and Biosource, respectively. ELISA strip well plates were coated with 10 μg/ml of antibody (in phosphate-buffered saline (PBS)) to IL-13 and 4 μg/ml of antibody to IFNγ (in PBS) overnight at room temperature. The plates were then blocked using a 4% BSA solution in PBS for one hour, followed by the addition of 50 μl of experimental culture medium per well in duplicate. The plates were incubated at room temperature for one hour and then washed using 50 mM Tris pH 8.0 with 0.1% Tween 20. Then, solutions of 400 ng/ml biotinylated second antibody to IL-13 and 500 ng/ml biotinylated second antibody to IFNγ were made in blocking buffer, and 100 μl were added per well. The plates were incubated for 1 hour and washed again. A 1:8000 dilution of Streptavidin HRP (Pierce Biotechnology) conjugate was made in blocking buffer, and 100 μl were added to the wells and incubation continued for 30 minutes. A final wash step was performed, after which 100μl Pierce Biotechnology TMB substrate were added to each well. Color was developed for 30 minutes and stopped by adding 100 μl 0.18 N H2SO4. OD was determined using microplate reader with a 450 nM filter.

The results for IL-13 are shown in Figure 3. As can be seen, the compound of formula II (Cpd. II) and dexamethasone (Dex) significantly inhibited IL-13 release induced by PHA. Methylphenidate (MP) did not inhibit the release of IL-13. Indeed, methylphenidate increased the release of IL-13 by the PHA-stimulated cells.

The results for IFNγ are shown in Figure 4. As can be seen, the compound of formula II (Cpd. II) and dexamethasone (Dex) significantly inhibited IFNγ release in both unstimulated cells and in cells stimulated with PHA. Methylphenidate (MP) had some effect on the release of IFNγ by unstimulated cells, but did not significantly suppress the release of IFNγ from cells stimulated with PHA. Indeed, methylphenidate increased the release of IFNγ by the PHA-stimulated cells.

EXAMPLE 3:

Whole blood was drawn from GR191, a normal human volunteer, and processed as described in Example 1 to give heat-inactivated serum and PBL. The compound of formula II and methylphenidate in culture medium (made using heat-inactivated GR191 serum) were added to wells of a 96-well plate to give final concentrations of 5 μg/ml, 15
μg/ml, 25 μg/ml and 50 μg/ml of the compound of formula II and 50 μg/ml methylphenidate. Water, mouse nerve growth factor (Upstate Biotechnology, Inc) (NGF) (final concentration of 250 ng/ml) and dexamethasone (final concentration of 10 μM) were used as controls. Then, GR191’s PBL in culture medium were added to the wells to give a final concentration of 150,000 cells per well, and the plates were incubated at 37°C, 5% CO₂ for 24 hours. After this incubation, PHA was added to give final concentrations of 2 μg/ml and 5 μg/ml, final total volume of 200 μl/well, and the cells were incubated for an additional 72 hours at 37°C, 5% CO₂. All cultures were performed in triplicate.

[0103] At the end of this incubation, cell proliferation was determined as described in Example 1. The results are presented in Figures 5A-B. As can be seen from Figures 5A-B, the compound of formula II (Cpd. II) and dexamethasone (Dex) significantly inhibited the proliferation of PBL, both unstimulated and stimulated with PHA, whereas methylphenidate (MP) did not.

[0104] The release of cytokines by the PBL was also measured by culturing the PBL in 1 ml tubes, at 1 x 10⁶ cells per ml, with 15 μg/ml and 50 μg/ml of the compound of formula II or 10 μM dexamethasone at 37°C, 5% CO₂ for 24 hours. After this incubation, PHA was added to give a final concentration of 5 μg/ml, and the cells were incubated for an additional 72 hours at 37°C, 5% CO₂. All cultures were performed in triplicate. Cells were then removed by centrifugation at 1000 rpm for 10 minutes.

[0105] The supernatants were collected, and the concentrations of IL-13 and tumor necrosis factor alpha (TNFα) in the supernatants were measured by ELISA. The IL-13 ELISA was performed as described in Example 2. The results are presented in Figure 6. As can be seen in Figure 6, the compound of formula II (Cpd. II) and dexamethasone (Dex) significantly inhibited the release of IL-13 from the PHA-stimulated PBL.

[0106] TNFα is a proinflammatory cytokine made by activated T-cells and other cells. TNFα causes endothelial cells to express adhesion molecules and may play a role in the recruitment of immune cells to the sites of inflammation. TNFα has been detected in multiple solid and hematologic malignancies. A number of different intracellular signals are induced by TNFα, including signals for both cells survival through NFκB and AP-1and cell death through caspase activation. NFκB is a key regulator of cell survival and promoter of carcinogenesis in multiple tumor types.
The TNFα ELISA was performed as described in Example 2 using matched pair antibodies from Pierce Endogen (2 μg/ml for the coating antibody and 250 ng/ml for the second antibody). The results are presented in Figure 7. As can be seen in Figure 7, the compound of formula II (Cpd. II) and dexamethasone (Dex) significantly inhibited the release of TNFα from PHA-stimulated PBL.

The cells were further analyzed by flow cytometry. Annexin was used to determine populations of dead or dying cells. Anti-CD69 antibody was used to establish the level of cellular activation. Antibody to T-cell receptor αβ (TCR) was also used. Recombinant Annexin 5 (PE and FITC conjugates) and the antibodies were all purchase from Caltag (Burlington, CA) and used following the manufacturer's recommendations. The following results were observed.

**Cell Death:**

Annexin staining of TCR-positive cells increased from 7.3% (background) to 45% and 23% with 50 μg/ml and 15 μg/ml of the compound of formula II, respectively, signifying an increase in cell death in the T-cell population. Stimulation with PHA at 5 μg/ml increased the annexin staining of TCR-positive cells to 67%. This indicates that PHA can also induce cell death in the T-cell population. Cell death decreased slightly as a result of treatment with PHA plus 15 μg/ml of the compound of formula II (62% of the TCR-positive cells stained for annexin with PHA and IMM 0001 versus 67% with PHA alone). PHA plus 50 μg/ml of the compound of formula II caused 87% cell death in the TCR-positive subset of cells as seen by annexin staining. These results show that the higher 50 μg/ml concentration of the compound of formula II caused significant death of T-cells, whereas the lower 15 μg/ml concentration did not. Dexamethasone rescued the PHA-induced increase in annexin staining of TCR-positive cells (decreased from 84% to 48%), demonstrating that the control compound is working properly.

**Activation Of T-Cells:**

CD69 + TCR staining (activated T cells) was not detected in any of the controls (nil, compound of formula II alone and dexamethasone alone). PHA increased CD69 + TCR staining to 84%. Only PHA caused T-cell activation as detectable by increased CD 69 staining. CD69 + TCR staining of PHA-stimulated cells dropped from 84% to 54% with 50
µg/ml of the compound of formula II and to 64% with 15 µg/ml of the compound of formula II. Dexamethasone was less effective than the compound of formula II at reducing the CD69 + TCR staining of PHA-stimulated cells. Thus, the compound of formula II is more effective at decreasing T-cell activation than dexamethasone, a potent anti-inflammatory.

EXAMPLE 4:

[0111] Whole blood was drawn from GR-192, a normal human volunteer, and processed as described in Example 1 to give heat-inactivated serum and PBL. Then, GR-192’s PBL were cultured in 1 ml tubes, at 1.3 x 10^6 cells per ml, with 15 µg/ml of the compound of formula II (in culture medium made using 10% heat-inactivated GR-192 serum) or 10 µM dexamethasone, at 37°C, 5% CO₂ for 24 hours. After this incubation, PHA was added to give a final concentration of 2 µg/ml, and the cells were incubated for an additional 96 hours at 37°C, 5% CO₂. All cultures were performed in triplicate. Cells were then removed by centrifugation at 1000 rpm for 10 minutes, and the culture medium collected.

[0112] Release of IL-8 into the culture medium was measured by ELISA. IL-8 is a pro-inflammatory cytokine and a potent chemoattractant and activator of neutrophils. It has also been reported to be a chemoattractant and activator of T-lymphocytes and eosinophils. IL-8 is produced by immune cells (including lymphocytes, neutrophils, monocytes and macrophages), fibroblasts and epithelial cells. IL-8 has potent angiogenic activity.

[0113] To perform the ELISA, matched pairs of antibodies against human IL-8 were purchased from Pierce Biotechnology and Biosource, respectively. ELISA strip well plates were coated with 2 µg/ml of antibody to IL-8 (in phosphate-buffered saline (PBS)) overnight at room temperature. The plates were then blocked using a 4% BSA solution in PBS for one hour, followed by the addition of 50 µl of experimental culture medium per well in duplicate. The plates were incubated at room temperature for one hour and then washed using 50 mM Tris pH 8.0 with 0.1% Tween 20. Then, solutions of 100 ng/ml biotinylated second antibody to IL-8 were made in blocking buffer, and 100 µl were added per well. The plates were incubated for 1 hour and washed again. A 1:8000 dilution of Strepavidin HRP (Pierce Biotechnology) conjugate was made in blocking buffer, and 100 µl were added to the wells and incubation continued for 30 minutes. A final wash step was performed, after which 100 µl Pierce Biotechnology TMB substrate were added to each well. Color was developed for 30
minutes and stopped by adding 100 μl 0.18 N H₂SO₄. OD was determined using microplate reader with a 450 nm filter.

[0114] The results are shown in Figure 8. As can be seen, the compound of formula II (Cpd. II) and dexamethasone (Dex) significantly inhibited IL-8 release induced by PHA.

[0115] A CD4-positive human T-lymphocyte cell line (TRiPS), which was isolated from an influenza-immunized donor and is specific for hemagglutinin peptide 307-319, was stimulated for passage using approximately 4x10⁵ cells on day 18-20 after a previous stimulation. Cells were washed once in cold Iscove’s Modified Dulbecco Minimal Essential Medium (IMDM, Sigma) plus 10% fetal bovine serum (FBS; American Type Culture Collection (ATCC)) and resuspended in 1.0 ml cold IMDM medium containing a 1:500 dilution of anti-CD3 monoclonal antibody OKT3 (prepared from mouse ascites fluid). Cells were incubated with antibody for 30 minutes on ice, then washed with cold medium without FBS and combined with approximately 2x10⁶ irradiated normal human donor peripheral blood leukocytes (PBL), as feeder cells, in medium plus 50 U/ml human IL-2 (Xenometrix). Cultures were expanded by the addition of fresh IMDM medium with FBS plus IL-2 on day 3. Day of culture is measured from the day of stimulation with OKT3. Cells can be used for experiments starting on day 7 (at maximum proliferation), typically on day 14 (most sensitive to re-stimulation) and up until day 21 (resting cells approaching senescence).

[0116] Activation experiments were performed by withdrawing an aliquot of cells and washing twice with warmed (37°C) IMDM. For each specific assay, 2x10⁵ viable cells were pre-incubated in a total volume of 0.9 ml warmed IMDM medium containing 15 μg/ml of the compound of formula II or 10 μM dexamethasone for 15 minutes at 37°C. An aliquot of 2x10⁵ CD3/CD28 Dynabeads (Dynal), as activating stimulus, in 0.1 ml warmed IMDM was then added, and the cultures incubated 24 hours at 37°C. Supernatants of the cell cultures were harvested after pelleting the cells by centrifugation.

[0117] Cytokine content was assayed by specific IL-8 ELISA as described above. It was found that the compound of formula II had no effect on IL-8 production by the TRiPS cell line.

EXAMPLE 5:

[0118] THP-1 is a monocyte cell line obtained from American Type Culture Collection (ATCC) (catalog no. TIB-202). THP-1 cells were placed in medium (RPMI
containing 10% FCS and 8 ng/ml monothioglycerol (obtained from Sigma)) at a concentration of 250,000 cells per ml and incubated with 15 μg/ml of compound of formula II or 10 μM dexamethasone for one hour at 37 °C and 5% CO₂. After 1 hour, lipopolysaccharide (LPS) (obtained from Sigma) was added to the cultures to give a final concentration of 200 ng/ml, and the cells were then incubated for an additional 4 hours or for an additional 24 hours. After the incubation, the cells were centrifuged, and the supernatants were collected. The concentrations of IL-8 and TNFα in the supernatants were determined by ELISA.

[0119] The concentrations of IL-8 in the supernatants were determined by ELISA performed as described in Example 4. The results are presented in Table 1 below. As can be seen in Table 1, the compound of formula II (Cpd. II) and dexamethasone (Dex) significantly inhibited the release of IL-8 from the LPS-stimulated monocytes.

[0120] The TNFα ELISA was performed as described in Example 2. The results are presented in Table 2 below. As can be seen in Table 2, the compound of formula II (Cpd. II) and dexamethasone (Dex) significantly inhibited the release of TNFα from the LPS-stimulated monocytes.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time Of Incubation</th>
<th>Mean IL-8 Concentration (pg/ml)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no additives)</td>
<td>4 hours</td>
<td>75.96 ± 12.73</td>
<td>N/A</td>
</tr>
<tr>
<td>LPS</td>
<td>4 hours</td>
<td>2844.60 ± 180.55</td>
<td>N/A</td>
</tr>
<tr>
<td>LPS + Cpd II</td>
<td>4 hours</td>
<td>2185.00 ± 78.30</td>
<td>23%</td>
</tr>
<tr>
<td>LPS + Dex</td>
<td>4 hours</td>
<td>2102.18 ± 52.20</td>
<td>26%</td>
</tr>
<tr>
<td>Control (no additives)</td>
<td>24 hours</td>
<td>46.09 ± 22.42</td>
<td>N/A</td>
</tr>
<tr>
<td>LPS</td>
<td>24 hours</td>
<td>6653.20 ± 193.18</td>
<td>N/A</td>
</tr>
<tr>
<td>LPS + Cpd II</td>
<td>24 hours</td>
<td>4490.20 ± 264.46</td>
<td>33%</td>
</tr>
<tr>
<td>LPS + Dex</td>
<td>24 hours</td>
<td>2300.00 ± 283.41</td>
<td>66%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time Of Incubation</th>
<th>Mean TNFα Concentration (pg/ml)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no additives)</td>
<td>24 hours</td>
<td>1.415 ± 1.464</td>
<td>N/A</td>
</tr>
<tr>
<td>LPS</td>
<td>24 hours</td>
<td>138.655 ± 0.601</td>
<td>N/A</td>
</tr>
<tr>
<td>LPS + Cpd II</td>
<td>24 hours</td>
<td>65.370 ± 0.891</td>
<td>53%</td>
</tr>
<tr>
<td>LPS + Dex</td>
<td>24 hours</td>
<td>94.759 ± 8.755</td>
<td>32%</td>
</tr>
</tbody>
</table>
EXAMPLE 6:

The Jurkat T-lymphocyte leukemia cell line was obtained from American Type Culture Collection (ATCC), Rockville, MD (catalog no. TIB-152). Jurkat cells, at $1 \times 10^5$ cells/ml, were cultured at 37 °C and 5% CO$_2$ in IMDM medium (ATCC) with 10% FCS for 72 hours with 7.5 µg/ml or 15 µg/ml of the compound of formula II (Cpd II). Following the incubation, the cells were washed with Hepes buffered saline, split into three equal volumes, and then incubated with 5 µM ethidium bromide dimer-1 (ETH-D1) (obtained from Molecular Probes) and 5 µM calcein AM solution (obtained from Promega) for one hour at 37 °C and 5% CO$_2$ in 96-well culture plates to assay for cell viability. The fluorescence in each well was measured using a plate reader at excitation/emission 485/530 nm and 530/645 nm. Relative percentage of dead to live cells was calculated by dividing ETH-D1 fluorescence by calcein AM fluorescence. The results are shown in Table 3 below.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Relative Percentage Dead/Live</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no additives)</td>
<td>20.85% ± 1.42%</td>
</tr>
<tr>
<td>7.5 µg/ml Cpd II</td>
<td>16.74% ± 2.15%</td>
</tr>
<tr>
<td>15 µg/ml Cpd II</td>
<td>40.79% ± 1.81%</td>
</tr>
</tbody>
</table>

EXAMPLE 7:

Passage 4 (i.e., four cell population doublings) human umbilical vein endothelial cells (HUVECs), human source lot number 9713 (obtained from ATCC) in 1 ml of endothelial growth medium-2 (EGM-2) (obtained from Cambrex) were mixed with 30 µg of the compound of formula II (Cpd II) in endothelial basal medium-2 (EBM-2) (Cambrex) or 30 µg methylphenidate (MP) in EBM-2. Water (vehicle for the two test compounds) was used as a control, and the PI3 kinase inhibitor, LY 294002 (Sigma), at 50 µM, was included as a positive control. Then, the cells were seeded at 10,000 cells/well into the wells of a plate contained in a tube formation assay kit obtained from BD Biosciences, Rockville, MD. The wells of the plate contained an extracellular matrix protein gel. Fetal calf serum (FCS) (ATCC) was added to a final concentration of 5% to initiate tube formation. Then, the plates were incubated for 18 hours at 37 °C and 5% CO$_2$. Following the incubation, the plates were photographed with a digital camera attached to an inverted microscope (Olympus IMT-2 set at a phase contrast (PC) of 10).
When endothelial cells are cultured on extracellular matrix protein gels in the presence of angiogenic signals, they arrange themselves into structures loosely resembling capillary blood vessels. To establish the basal tube formation for this assay, cells were treated with the same amount of water as present in the solutions of Cpd II and MP. This treatment produced a lattice of endothelial cell structures with multiple branch points. Treatment with Cpd II and LY 294002 reduced the amount of branching and cellular interaction in the wells, leaving the cells in isolated clusters. MP had no observable effect on the ability of the endothelial cells to organize into structures resembling capillary blood vessels. These data indicate that Cpd II, but not MP, interferes with this step of angiogenesis.

EXAMPLE 8:

Passage 4 HUVECs, lot number 9713, in either EGM-2 plus 50 ng/ml vascular endothelial growth factor (VEGF) (obtained from Sigma) or in EGM-2 complete medium (containing 2% FCS, hydrocortisone, human fibroblast growth factor B, VEGF, recombinant insulin-like growth factor-1, ascorbate, human epithelial growth factor, gentamycin and heparin) (obtained from Cambrex) were put into the wells of a 96-well tissue culture plate at 5,000 cells well. The following additives were added to the cells: water (vehicle control); 5 μg/ml of the compound of formula II (Cpd II); 15 μg/ml Cpd II; or 30 μg/ml of Cpd II. After 48 hours of culture at 37 °C and 5% CO₂, cell proliferation was evaluated by the Promega cell titer assay as described in Example 1, except that the plates were incubated for only 2 hours after addition of the Promega cell titer reagent.

The results are shown in Table 4 below. As can be seen from Table 4, Cpd II reduced the number of cells detected in the wells in a dose-dependent manner. The reductions seen with 15 μg/ml Cpd II and 30 μg/ml Cpd II were statistically significant. Since wells with no growth factors were not included, it is not possible to determine if the reductions in cell numbers seen with Cpd II are due to inhibition of proliferation or a cytotoxic effect.
TABLE 4

<table>
<thead>
<tr>
<th>Sample</th>
<th>Medium</th>
<th>Mean OD at 530 nm</th>
<th>p value (versus vehicle control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no additives)</td>
<td>EGM-2 + VEGF</td>
<td>0.141 ± 0.004</td>
<td>N/A</td>
</tr>
<tr>
<td>Vehicle control (water added)</td>
<td>EGM-2 + VEGF</td>
<td>0.224 ± 0.011</td>
<td>N/A</td>
</tr>
<tr>
<td>5 µg/ml Cpd II</td>
<td>EGM-2 + VEGF</td>
<td>0.189 ± 0.014</td>
<td>0.0324</td>
</tr>
<tr>
<td>15 µg/ml Cpd II</td>
<td>EGM-2 + VEGF</td>
<td>0.132 ± 0.022</td>
<td>0.0069</td>
</tr>
<tr>
<td>30 µg/ml Cpd II</td>
<td>EGM-2 + VEGF</td>
<td>0.046 ± 0.012</td>
<td>0.0003</td>
</tr>
<tr>
<td>Control (no additives)</td>
<td>EGM-2 + growth factors</td>
<td>0.243 ± 0.002</td>
<td>N/A</td>
</tr>
<tr>
<td>Vehicle control (water added)</td>
<td>EGM-2 + growth factors</td>
<td>0.299 ± 0.011</td>
<td>N/A</td>
</tr>
<tr>
<td>5 µg/ml Cpd II</td>
<td>EGM-2 + growth factors</td>
<td>0.271 ± 0.022</td>
<td>0.1131</td>
</tr>
<tr>
<td>15 µg/ml Cpd II</td>
<td>EGM-2 + growth factors</td>
<td>0.239 ± 0.019</td>
<td>0.0283</td>
</tr>
<tr>
<td>30 µg/ml Cpd II</td>
<td>EGM-2 + growth factors</td>
<td>0.066 ± 0.003</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

EXAMPLE 9:

[H0126] HepG2 is a human hepatic cancer cell line, which was obtained from ATCC. HepG2 cells were grown to confluence in 25 cm² flasks in IMDM medium containing 10% FCS. Then, the cells were trypsinized as follows. The medium in each flask was aspirated and replaced with 5 ml of 0.025% trypsin/EDTA (Cambrex). The cells were monitored on a microscope until they no longer adhered to the flasks. Then, 5 ml of trypsin neutralizing solution (TNS) (Cambrex) were added to each flask to stop the reaction. The cell suspension was centrifuged at 1000 rpm for 10 minutes, and the supernatants were aspirated. The cells were reconstituted in fresh medium and counted. Then, 4 ml of the cell suspension in medium containing at 1.22 x 10⁶ cells/ml were mixed with an additional 1 ml of medium. Next, 0.5 ml/well of the resulting cell suspension was added to wells in a 24-well culture plate (about 500,000 cells/well). The cells were treated as indicated in Table 5 below and incubated for 24 hours at 37 °C, with or without 5% CO₂. The supernatants were removed from the wells and centrifuged to remove debris. Next, the supernatants were analyzed for erythropoietin (EPO) production. EPO was measured by ELISA using a kit obtained from R & D Systems, Minneapolis, MN (catalog no. DE900) following the manufacturer’s instructions.

[H0127] The results are shown in Table 5 below. As can be seen from Table 5, Cpd II significantly inhibited the release of EPO from the HepG2 cells. A decrease in EPO would have an inhibitory effect on angiogenesis. A viability assay was not performed, but the morphology of the cells appeared normal based on microscopic analysis.
**TABLE 5**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Units/ml EPO</th>
<th>p value versus hypoxia alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no treatment)</td>
<td>74.90 ± 2.65</td>
<td>N/A</td>
</tr>
<tr>
<td>Hypoxia (5% CO₂)</td>
<td>108.39 ± 2.81</td>
<td>N/A</td>
</tr>
<tr>
<td>Hypoxia + 15 µg/ml Cpd II</td>
<td>71.60 ± 2.01</td>
<td>0.005</td>
</tr>
<tr>
<td>Hypoxia + 25 µM LY 294002</td>
<td>52.99 ± 1.04</td>
<td>0.016</td>
</tr>
</tbody>
</table>

**EXAMPLE 10:**

[0128] Passage 4 HUVECs, lot number 9713, were put into the wells of a 48-well tissue culture plate at 20,000 cells/well in 500 µl of EGM-2 complete medium (but without serum or ascorbate) supplemented with ITSS (insulin, transferrin and sodium selenite) (obtained from Sigma). Also, passage 4 HUVECs, human source lot number 7016 (obtained from ATCC), were put into the wells of a 48-well tissue culture plate at 20,000 cells/well in 500 µl of EGM-2 complete medium (but without serum or ascorbate) supplemented with ITSS. The following additives were added to the cells: water (vehicle control) and 15 µg/ml of the compound of formula II (Cpd II). After incubation for 1 hour at 37 °C and 5% CO₂, LPS was added to give a final concentration of 200 ng/ml, and the cells were incubated overnight at 37 °C and 5% CO₂. After this incubation, the supernatants were collected, and the amount of IL-8 in the supernatants determined by ELISA as described in Example 4.

[0129] The results are shown in Table 6 below. As can be seen in Table 6, Cpd II complete eliminated IL-8 release by the 7016 HUVECs and decreased IL-8 release by 90% in the 9713 HUVECs.

**TABLE 6**

<table>
<thead>
<tr>
<th>Cells</th>
<th>Treatment</th>
<th>IL-8 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7016 HUVECs</td>
<td>Control (LPS only)</td>
<td>53.3</td>
</tr>
<tr>
<td>7016 HUVECs</td>
<td>LPS + 15 µg/ml Cpd II</td>
<td>Below detection</td>
</tr>
<tr>
<td>9713 HUVECs</td>
<td>Control (LPS only)</td>
<td>485.0</td>
</tr>
<tr>
<td>9713 HUVECs</td>
<td>LPS + 15 µg/ml Cpd II</td>
<td>49.8</td>
</tr>
</tbody>
</table>

**EXAMPLE 11:**

[0130] Passage 4 HUVECs, human source lot number 8710 (obtained from ATCC), were put into the wells of a 24-well tissue culture plate at 5,000 cells/well in EGM-2 medium and cultured for 72 hours at 37 °C and 5% CO₂. Then, the medium was replaced with fresh medium, and the following additives were added to the cells: water (vehicle control); 1
μg/ml, 5 μg/ml, 10 μg/ml, 15 μg/ml or 30 μg/ml of the compound of formula II (Cpd II); 15 μg/ml methylphenidate (MP); 10 μM LY 294002; or 10 μM dexamethasone (Dex). After incubation for 1 hour at 37 °C and 5% CO₂, TNFα (Pierce) was added to give a final concentration of 10 ng/ml, and the cells were incubated for an additional 18 hours at 37 °C and 5% CO₂. After this incubation, the supernatants were collected, and the amount of IL-8 in the supernatants determined by ELISA as described in Example 4.

The results are shown in Table 7 below. As can be seen in Table 7, Cpd II decreased IL-8 release stimulated by TNFα in a dose-dependent manner, although there did appear to be some cell death caused by the highest dose (30 μg/ml). Dex and MP slightly decreased IL-8 release and LY 294002 significantly decreased IL-8 release.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean IL-8 (pg/ml)</th>
<th>p value</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>No additives</td>
<td>207.15 ± 66.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 μg/ml Cpd II</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 μg/ml Cpd II</td>
<td>400.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 ng/ml TNFα</td>
<td>34695 ± 301.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 ng/ml TNFα + water</td>
<td>35572 ± 967.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 ng/ml TNFα + 30 μg/ml Cpd II</td>
<td>4829.8 ± 214.13</td>
<td>86.93%</td>
<td></td>
</tr>
<tr>
<td>10 ng/ml TNFα + 15 μg/ml Cpd II</td>
<td>20817 ± 674.63</td>
<td>0.002</td>
<td>41.72%</td>
</tr>
<tr>
<td>10 ng/ml TNFα + 10 μg/ml Cpd II</td>
<td>22050 ± 727.27</td>
<td>0.003</td>
<td>38.24%</td>
</tr>
<tr>
<td>10 ng/ml TNFα + 5 μg/ml Cpd II</td>
<td>34482 ± 2127.22</td>
<td>0.124</td>
<td>3.08%</td>
</tr>
<tr>
<td>10 ng/ml TNFα + 1 μg/ml Cpd II</td>
<td>53657 ± 3935.18</td>
<td>0.011</td>
<td>(-51.1%)</td>
</tr>
<tr>
<td>10 ng/ml TNFα + 15 μg/ml MP</td>
<td>30183 ± 3448.01</td>
<td>0.051</td>
<td>15.24%</td>
</tr>
<tr>
<td>10 ng/ml TNFα + 10 μM LY 294002</td>
<td>9196.1 ± 150.97</td>
<td>0.072</td>
<td>74.58%</td>
</tr>
<tr>
<td>10 ng/ml TNFα + 10 μM Dex</td>
<td>35952 ± 2197.14</td>
<td></td>
<td>6.88%</td>
</tr>
</tbody>
</table>

EXAMPLE 12:

The transcription factor NFκB (nuclear factor κB) is implicated in the regulation of the expression of a wide variety of genes that code for mediators of the immune, acute phase and inflammatory responses. NFκB is a key regulator of cell survival and promoter of carcinogenesis. There are five subunits of the NFκB family in mammals: p50, p65 (RelA), c-Rel, p52 and RelB. The p50/p65 heterodimers and the p50 homodimers are the most common dimers found in the NFκB signaling pathway. NFκB can be activated by a number of stimuli, including components of bacterial cell walls, such as lipopolysaccharide, or inflammatory cytokines, such as TNFα or IL-1β.
[0133] Activator protein-1 (AP-1) is a transcription factor that is activated during the cell cycle to promote cell survival, differentiation and adaptive responses. AP-1 proteins play a role in the expression of many genes involved in proliferation and cell cycle progression. For instance, cell transformation by oncogenes that function in the growth factor signal transduction pathway, such as ras, rasF and mek, results in a high increase in AP-1 component protein expression. Therefore, AP-1 regulated genes support the invasive process observed during malignancy and metastasis. AP-1 belongs to a large family of structurally related transcription factors that includes ATF-4, c-Fos, c-Jun, c-Myc and C/EBP. AP-1 is composed of a mixture of heterodimeric complexes of proteins derived from the Fos and Jun families, including c-Fos, FosB, Fra-1, Fra-2, c-Jun, JunB and JunD. Primarily, AP-1 dimers bind to DNA on a TPA-response element (TRE). AP-1 expression is induced by multiple stimuli such as serum, growth factors, phorbol esters, oncogenes, cytokines of the TGF-β, TNF and interferon families, neuronal depolarization and cellular stress.

[0134] Passage 5 HUVECs, human source lot number 8750, were grown to confluence in 25 cm² flasks in EGM-2 medium. The following additives were added to the flasks (total volume of 5 ml/flask) in EGM-2 medium containing 2% FCS, GA1000 (gentamycin), heparin and ascorbic acid (all from Cambrex): 1 μg/ml of the compound of formula II (Cpd II); 5 μg/ml Cpd II; 15 μg/ml of Cpd II; 15 μg/ml methylphenidate (MP); or 10 μM LY 294002. The flasks were incubated overnight at 37 °C, 5% CO₂. After this incubation, VEGF was added to give a final concentration 10 ng/ml, and the flasks were incubated for an additional 30 minutes.

[0135] Then, the amount of NFκB was determined using a TransAM™ NFκB p65/NFκB p50 Transcription Factor Assay Kit and a Nuclear Extract Kit from Active Motif North America, Carlsbad, CA, according to the manufacturer’s instructions. Briefly, a nuclear extract of the cells was prepared using the Nuclear Extract Kit. Then, the nuclear extract was added to the wells of the 96-well plate of the TransAM™ kit. Oligonucleotide containing an NFκB consensus binding site was immobilized in the wells, and the activated NFκB contained in the nuclear extract was bound to the oligonucleotide. Then, an antibody directed against the NFκB p65 or p50 subunit was added, and the NFκB complex bound to the oligonucleotide was detected. A secondary antibody conjugated to horseradish peroxidase (HRP) was next added to provide a colorimetric readout that was quantified by spectrophotometry (measurement at 450 nm).
The amount of c-Jun was determined using a TransAM™ AP-1 Family Transcription Factor Assay Kit and a Nuclear Extract Kit from Active Motif North America, Carlsbad, CA, according to manufacturer’s instructions. Briefly, a nuclear extract of the cells was prepared using the Nuclear Extract Kit. Then, the nuclear extract was added to the wells of a 96-well plate in which oligonucleotide containing a TPA-responsive element (TRE) was immobilized. Activator protein-1 (AP-1) dimers contained in the nuclear extract were bound to this oligonucleotide and were detected using an antibody specific for c-Jun. A secondary antibody conjugated to horseradish peroxidase (HRP) was next added to provide a colorimetric readout that was quantified by spectrophotometry (measurement at 450 nm).

The results are shown in Tables 8 and 9 below. As can be seen from Table 8, VEGF treatment of HUVECs caused almost a doubling of activated NFκB as detected by the TransAM assay. Cpd II at 15 μg/ml and 5 μg/ml reduced the amount of activated NFκB back to basal levels. As can be seen from Table 9, VEGF treatment of HUVECs caused an increase of c-Jun. Cpd II at 15 μg/ml and 5 μg/ml completely eliminated the increase in the amount of c-Jun.

### Table 8

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean OD 450 nm (NFκB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no additives)</td>
<td>0.070 ± 0.002</td>
</tr>
<tr>
<td>VEGF only</td>
<td>0.111 ± 0.007</td>
</tr>
<tr>
<td>VEGF + 15 μg/ml Cpd II</td>
<td>0.060 ± 0.008</td>
</tr>
<tr>
<td>VEGF + 5 μg/ml Cpd II</td>
<td>0.065 ± 0.010</td>
</tr>
<tr>
<td>VEGF + 1 μg/ml Cpd II</td>
<td>0.097 ± 0.013</td>
</tr>
<tr>
<td>VEGF + 15 μg/ml MP</td>
<td>0.093 ± 0.011</td>
</tr>
<tr>
<td>VEGF + 10 μM LY 294002</td>
<td>0.138 ± 0.008</td>
</tr>
</tbody>
</table>

### Table 9

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean OD 450 nm (c-Jun)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no additives)</td>
<td>0.204 ± 0.016</td>
</tr>
<tr>
<td>VEGF only</td>
<td>0.261 ± 0.013</td>
</tr>
<tr>
<td>VEGF + 15 μg/ml Cpd II</td>
<td>0.204 ± 0.010</td>
</tr>
<tr>
<td>VEGF + 5 μg/ml Cpd II</td>
<td>0.185 ± 0.025</td>
</tr>
<tr>
<td>VEGF + 1 μg/ml Cpd II</td>
<td>0.221 ± 0.008</td>
</tr>
<tr>
<td>VEGF + 15 μg/ml MP</td>
<td>0.230 ± 0.016</td>
</tr>
<tr>
<td>VEGF + 10 μM LY 294002</td>
<td>0.340 ± 0.020</td>
</tr>
</tbody>
</table>
EXAMPLE 13:

Passage 8 (human iliac artery endothelial cells (HIAECs) (obtained from ATCC; catalog no. CC-2545) were grown to confluence in 25 cm² flasks in EGM-2 medium. Eighteen hours prior to the experiment, the medium was replaced with EGM-2 medium containing 0.1% FCS plus heparin, GA1000 (gentamycin) and bovine pituitary extract (all from Cambrex) to place the cells in a resting state. To perform the experiment the medium was aspirated from the flasks, and the following additives were added to the flasks in fresh medium (total volume of 5 ml/flask): 15 μg/ml of the compound of formula II (Cpd II) or 10 μM LY 294002. The flasks were incubated 2 hours at 37 °C, 5% CO₂. After this incubation, VEGF or TNFα was added to give a final concentration 10 ng/ml, and the flasks were incubated for an additional 30 minutes. Then, the amount of NFκB was determined using a TransAM™ NFκB p65/NFκB p50 Transcription Factor Assay Kit and a Nuclear Extract Kit from Active Motif North America, Carlsbad, CA, as described in Example 12.

The results are shown in Table 10 below. As can be seen from Table 10, TNFα treatment of HUVECs caused an extremely large increase in the amount of activated NFκB as detected by the TransAM assay. Cpd II at 15 μg/ml reduced the amount of activated NFκB about 82%. The treatment with VEGF did not result in as large an increase in activated NFκB as achieved with TNFα, but the increased amount was reduced 70% by Cpd. II.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean OD 450 nm (NFκB)</th>
<th>Percent Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no additives)</td>
<td>0.174 ± 0.004</td>
<td></td>
</tr>
<tr>
<td>TNFα only</td>
<td>0.881 ± 0.021</td>
<td></td>
</tr>
<tr>
<td>TNFα + 15 μg/ml Cpd II</td>
<td>0.302 ± 0.003</td>
<td>81.89%</td>
</tr>
<tr>
<td>TNFα + 10 μM LY 294002</td>
<td>0.810 ± 0.007</td>
<td>10.04%</td>
</tr>
<tr>
<td>VEGF only</td>
<td>0.220 ± 0.007</td>
<td></td>
</tr>
<tr>
<td>VEGF + 15 μg/ml Cpd II</td>
<td>0.066 ± 0.005</td>
<td>70.00%</td>
</tr>
</tbody>
</table>

EXAMPLE 14:

Day 18 TRiPS cells, 1x10⁶, were incubated for 30 minutes at 37°C, either with nothing added (“Nil”), with 1 μl CD3/CD28 Dynabeads (Dynal, Oslo, Norway) ("CD3/CD28 beads") per 100,000 cells, or with CD3/CD28 beads and 15 μg/ml of the compound of
formula II (Cpd II). After the incubation, the cells were lysed in Cell-Lytic Mammalian Cell Extraction Reagent (Sigma). After centrifugation to pellet cellular debris, the supernatants (cell extracts) were obtained.

The cell extracts (supernatants) were then analyzed using a Custom AntibodyArray™ manufactured by Hypermatrix Inc., Worcester, MA, following the manufacturer’s instructions. The Custom AntibodyArray™ is a nylon membrane blotted with antibodies to the proteins listed below. Briefly, the cell extracts were incubated with duplicate Custom AntibodyArray™s for 2 hours at room temperature with slow shaking, followed by three washes with Tris buffer (150 mM NaCl, 25 mM Tris, 0.05% Tween-20, pH 7.5). HRP-labeled antibodies specific for phosphorylated-tyrosine, phosphorylated-serine and phosphorylated-threonine in Tris buffer were added, and the arrays incubated for 2 hours. After three more washes with Tris buffer, a peroxidase-reactive luminescent substrate was added. The arrays were visualized by exposure to X-ray film. Densitometry of the X-ray films was measured by scanning and computer analysis. The results are summarized in Table 11 below.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Effect of Cpd II on the protein in CD3/CD28 stimulated TRiPS cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAP1</td>
<td>Activated</td>
</tr>
<tr>
<td>RAP2</td>
<td>Activated</td>
</tr>
<tr>
<td>JAK2</td>
<td>Activated</td>
</tr>
<tr>
<td>STAT4</td>
<td>Activated</td>
</tr>
<tr>
<td>STAT5b</td>
<td>Activated</td>
</tr>
<tr>
<td>PI3kinaseP85</td>
<td>Activated</td>
</tr>
<tr>
<td>MEK1</td>
<td>Decreased level to below basal levels (Nil control)</td>
</tr>
<tr>
<td>JNK1</td>
<td>Decreased level back to basal levels (Nil control)</td>
</tr>
<tr>
<td>JNK2</td>
<td>Decreased level back to basal levels (Nil control)</td>
</tr>
<tr>
<td>JNK3</td>
<td>Decreased level back to basal levels (Nil control)</td>
</tr>
<tr>
<td>MEKK1</td>
<td>Decreased level back to basal levels (Nil control)</td>
</tr>
<tr>
<td>IκB-θ</td>
<td>Decreased level back to basal levels (Nil control)</td>
</tr>
<tr>
<td>IκB-ϕ</td>
<td>Decreased level back to basal levels (Nil control)</td>
</tr>
<tr>
<td>IL-2</td>
<td>Decreased level back to basal levels (Nil control)</td>
</tr>
<tr>
<td>IL-4</td>
<td>Decreased level back to basal levels (Nil control)</td>
</tr>
<tr>
<td>IL-7γ</td>
<td>Decreased level back to basal levels (Nil control)</td>
</tr>
<tr>
<td>Protein</td>
<td>Effect of Cpd II on the protein in CD3/CD28 stimulated TRiPS cells</td>
</tr>
<tr>
<td>---------</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>14-3-3</td>
<td>Slightly decreased the level</td>
</tr>
<tr>
<td>STAT6</td>
<td>Slightly decreased the level</td>
</tr>
<tr>
<td>IκB-ε</td>
<td>Slightly decreased the level</td>
</tr>
<tr>
<td>IκB-α</td>
<td>Slightly decreased the level</td>
</tr>
<tr>
<td>VAV</td>
<td>No effect</td>
</tr>
<tr>
<td>STAT2</td>
<td>No effect</td>
</tr>
</tbody>
</table>

EXAMPLE 15:

[0142] Cells of the MC/9 murine fibroblast cell line (obtained from ATCC, catalog no. CRL-8305) were placed into the wells of a 96-well tissue culture plate at 25,000 cells/well. The culture medium was Delbecco’s Modified Eagle’s Medium (DMEM) (obtained from Cambrex) containing 10% FCS. Nil control wells contained no additives. The remaining wells contained either 25 ng/ml murine nerve growth factor (NGF) (obtained from Upstate Biotechnology, Lake Placid, NY) or 25 ng/ml NGF and 5% TSTIM (a culture supplement prepared from rats and containing concanavalin A which was obtained from BD Biosciences). In addition, the following additives were added to the cells: water (vehicle control); 5 μg/ml of the compound of formula II (Cpd II); 15 μg/ml Cpd II; or 30 μg/ml of Cpd II. After 72 hours of culture at 37 °C and 5% CO₂, cell proliferation was evaluated by the Promega cell titer assay as described in Example 1. The results are shown in Table 12 below.

<table>
<thead>
<tr>
<th>Additive</th>
<th>Mean OD 530 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>No additives</td>
<td>0.058 ± 0.008</td>
</tr>
<tr>
<td>NGF</td>
<td>0.116 ± 0.029</td>
</tr>
<tr>
<td>NGF + water</td>
<td>0.101 ± 0.022</td>
</tr>
<tr>
<td>NGF + 1 μg/ml Cpd II</td>
<td>0.117 ± 0.015</td>
</tr>
<tr>
<td>NGF + 5 μg/ml Cpd II</td>
<td>0.108 ± 0.012</td>
</tr>
<tr>
<td>NGF + 15 μg/ml Cpd II</td>
<td>0.049 ± 0.016</td>
</tr>
<tr>
<td>NGF + TSTIM</td>
<td>0.490 ± 0.047</td>
</tr>
<tr>
<td>NGF + TSTIM + water</td>
<td>0.365 ± 0.026</td>
</tr>
<tr>
<td>NGF + TSTIM + 1 μg/ml Cpd II</td>
<td>0.428 ± 0.027</td>
</tr>
<tr>
<td>NGF + TSTIM + 5 μg/ml Cpd II</td>
<td>0.373 ± 0.016</td>
</tr>
<tr>
<td>NGF + TSTIM + 15 μg/ml Cpd II</td>
<td>0.326 ± 0.024</td>
</tr>
</tbody>
</table>
EXAMPLE 16:

[0143] THP-1 cells were placed in medium (RPMI containing 10% FCS and 8 ng/ml monothioglycerol) at a concentration of 250,000 cells per ml and incubated with 5 µg/ml of compound of formula II (Cpd II) or 15 µg/ml of Cpd II for one hour at 37 °C and 5% CO₂. After 1 hour, lipopolysaccharide (LPS) was added to the cultures to give a final concentration of 200 ng/ml, and the cells were then incubated for an additional 24 hours. After the incubation, the amount of NFkB and c-Jun were determined as described in Example 12. Also, the amount of c-Fos was determined using a TransAM™ AP-1 Family Transcription Factor Assay Kit and a Nuclear Extract Kit from Active Motif North America, Carlsbad, CA, according to manufacturer’s instructions. Briefly, a nuclear extract of the cells was prepared using the Nuclear Extract Kit. Then, the nuclear extract was added to the wells of a 96-well plate in which oligonucleotide containing a TPA-responsive element (TRE) was immobilized. Activator protein-1 (AP-1) dimers contained in the nuclear extract were bound to this oligonucleotide and were detected using an antibody specific for c-Fos. A secondary antibody conjugated to horseradish peroxidase (HRP) was next added to provide a colorimetric readout that was quantified by spectrophotometry (measurement at 450 nm). The results are shown in Figures 9A-B.

EXAMPLE 17:

[0144] Day 10 TRIps cells, 1×10⁶, were incubated with 15 µg/ml of the compound of formula II (Cpd II) for 1 hour at 37°C. Then, the cells were incubated with CD3/CD28 beads (1 µl per 100,000 cells) (obtained from Dynal) for 10 minutes at 37°C. The cells were then lysed with a mild buffer (supplied with Pierce EZ-Detect activation kit described below) to produce cell extracts. Protein concentrations of the resulting extracts were determined by bicinchoninic acid (BCA) assay (Pierce) and placed on ice for immediate use.

[0145] Pulldown assays were performed using Pierce EZ-Detect activation kits according to the manufacturer’s instructions utilizing GST-RAF-1-RBD and GST-RalGDS-RBD for Ras and RAP-1 respectively. Briefly, 400 µg total protein from each extract was combined with recombinant protein and glutathione resin and incubated at 4°C for one hour with gentle shaking. The resin was then washed to remove unbound protein and the activated Ras and RAP-1 proteins were removed by boiling in the presence of SDS-PAGE loading dye containing reducing agent. Ras and RAP-1 western blots were performed to visualize the
proteins using antibodies supplied with the kit. Densitometry of the X-ray films was done by
scanning and computer analysis.

[0146] The results are shown in Table 13. As can be seen from Table 13, incubating
the TRiPS cells with Cpd II resulted in very strong inhibition of Ras protein. Stimulation of
the cells with CD3/CD28 beads did not increase the amount of RAP-1 protein as expected,
but Cpd II also appeared to inhibit RAP-1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Integrated Optical Density for RAS assay</th>
<th>Integrated Optical Density for RAP-1 assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>66.83</td>
<td>259.27</td>
</tr>
<tr>
<td>CD3/CD28 beads only</td>
<td>245.91</td>
<td>213.66</td>
</tr>
<tr>
<td>CD3/CD28 beads + 15 µg/ml Cpd II</td>
<td>84.98</td>
<td>87.26</td>
</tr>
</tbody>
</table>

[0147] The foregoing discussion of the invention has been presented for purposes of
illustration and description. The foregoing is not intended to limit the invention to the form
or forms disclosed herein. Although the description of the invention has included description
of one or more embodiments and certain variations and modifications, other variations and
modifications are within the scope of the invention, e.g., as may be within the skill and
knowledge of those in the art, after understanding the present disclosure. It is intended to
obtain rights which include alternative embodiments to the extent permitted, including
alternate, interchangeable and/or equivalent structures, functions, ranges or steps to those
claimed, whether or not such alternate, interchangeable and/or equivalent structures,
functions, ranges or steps are disclosed herein, and without intending to publicly dedicate any
patentable subject matter.
WHAT IS CLAIMED IS:

1. A method of inhibiting angiogenesis in an animal comprising administering to the animal an effective amount of a compound of formula I:

   \[
   \begin{array}{c}
   (R^1)_n \quad \text{I}
   \end{array}
   \]

   or a salt or a prodrug thereof,

   where

   \[
   n \text{ is an integer from 1 to 5,}
   \]

   each \( R^1 \) is independently aryl, heteroaryl, alkyl, cycloalkyl, alkoxy, aryloxy, acyl, carboxyl, hydroxyl, halogen, amino, nitro, sulfo or sulphydryl, wherein

   each alkyl is optionally substituted with hydroxyl, amino or sulphydryl; and

   \( R^2 \) is hydrogen or lower alkyl.

2. A method of treating an angiogenic disease or condition in an animal comprising administering to the animal an effective amount of a compound of formula I:

   \[
   \begin{array}{c}
   (R^1)_n \quad \text{I}
   \end{array}
   \]
or a salt or a prodrug thereof,

where

\[ n \text{ is an integer from } 1 \text{ to } 5, \]

\[ \text{each } R^1 \text{ is independently aryl, heteroaryl, alkyl, cycloalkyl, alkoxy,} \]

\[ \text{aryloxy, acyl, carboxyl, hydroxyl, halogen, amino, nitro, sulfo or} \]

\[ \text{sulfhydryl, wherein} \]

\[ \text{each alkyl is optionally substituted with hydroxyl, amino or} \]

\[ \text{sulfhydryl; and} \]

\[ R^2 \text{ is hydrogen or lower alkyl.} \]

3. A method of treating an ocular angiogenic disease or condition in an animal comprising administering to the animal an effective amount of a compound of formula I:

\[
\begin{align*}
\text{(R^1)_{n-1}} & \text{-} \text{O} & \text{-} \text{CO} \\
\text{HN} & \text{-} \text{C} & \text{I}
\end{align*}
\]

or a salt or a prodrug thereof,

where

\[ n \text{ is an integer from } 1 \text{ to } 5, \]

\[ \text{each } R^1 \text{ is independently aryl, heteroaryl, alkyl, cycloalkyl, alkoxy,} \]

\[ \text{aryloxy, acyl, carboxyl, hydroxyl, halogen, amino, nitro, sulfo or} \]

\[ \text{sulfhydryl, wherein each alkyl is optionally substituted with hydroxyl,} \]

\[ \text{amino or sulfhydryl; and} \]

\[ R^2 \text{ is hydrogen or lower alkyl.} \]
4. The method of Claim 3 wherein the ocular angiogenic disease or condition is diabetic retinopathy.

5. The method of Claim 3 wherein the ocular angiogenic disease or condition is macular degeneration.

6. The method of Claim 3 wherein the ocular angiogenic disease or condition is retinopathy of prematurity, corneal graft rejection, neovascular glaucoma, retrolental fibroplasias or rubeosis.

7. A method of treating a neoplastic disease in an animal comprising administering to the animal an effective amount of a compound of formula I:

\[
\begin{align*}
\text{(R}^1)_{\text{n}} & \text{C} & \text{O}^\text{R}^2 \\
\text{HN} & \text{C} & \text{C}
\end{align*}
\]

or a salt or a prodrug thereof,

where

\[n \text{ is an integer from 1 to 5,}\]

\[\text{each } R^1 \text{ is independently aryl, heteroaryl, alkyl, cycloalkyl, alkoxy, aryloxy, acyl, carboxyl, hydroxyl, halogen, amino, nitro, sulfo or sulfhydryl, wherein each alkyl is optionally substituted with hydroxyl, amino or sulfhydryl; and}\]

\[R^2 \text{ is hydrogen or lower alkyl.}\]

8. The method of Claim 7 wherein the neoplastic disease is a tumor.

9. The method of Claim 8 wherein the tumor is a malignant tumor.
10. The method of Claim 9 wherein the tumor is a tumor of the bladder, brain, breast, cervix, colon, rectum, kidney, liver, lung, ovary, pancreas, prostate, stomach or uterus.

11. The method of Claim 10 wherein the tumor is a tumor of the brain, breast, colon, liver or pancreas.

12. The method of Claim 11 wherein the tumor is a tumor of the brain.

13. The method of Claim 12 wherein the brain tumor is a glioblastoma.

14. The method of Claim 7 wherein the neoplastic disease is tumor metastasis.

15. A method of treating a proliferative disorder in an animal comprising administering to the animal an effective amount of a compound of formula I:

\[ \text{(R}_1^1)_n \text{O} \text{R}_2 \]

or a salt or a prodrug thereof,

where

\[ n \text{ is an integer from 1 to 5,} \]

each \( R_1 \) is independently aryl, heteroaryl, alkyl, cycloalkyl, alkoxy, aryloxy, acyl, carboxyl, hydroxyl, halogen, amino, nitro, sulf(o or sul)fydryl, wherein each alkyl is optionally substituted with hydroxyl, amino or sulphydryl; and

\[ R_2 \text{ is hydrogen or lower alkyl.} \]

16. The method of Claim 15 wherein the proliferative disorder is a cancer.
17. The method of Claim 16 wherein the cancer is carcinoma, a sarcoma, a lymphoma or a leukemia.

18. The method of Claim 15 wherein the proliferative disorder is a mesangial cell proliferation disorder.

19. The method of Claim 15 wherein the proliferative disorder is a fibrotic disorder.

20. The method of Claim 15 wherein the proliferative disorder is a hyperproliferative skin disorder.

21. The method of Claim 20 wherein the hyperproliferative skin disorder is skin cancer.

22. The method of any one of Claims 1-21 wherein each R^1 is independently aryl, heteroaryl, alkyl, cycloalkyl, alkoxy, hydroxyl, halogen, amino, nitro, sulfo or sulphydryl.

23. The method of any one of Claims 1-21 wherein each R^1 is independently aryl, alkyl, cycloalkyl, alkoxy, aryloxy or acyl.

24. The method of any one of Claims 1-21 wherein each R^1 is independently aryl, alkyl or cycloalkyl.

25. The method of any one of Claims 1-21 wherein each R^1 is independently aryl.

26. The method of any one of Claims 1-21 wherein the compound is:

\[
\text{II}
\]
27. A compound of formula IA:

\[
\begin{array}{c}
\text{(R\textsuperscript{1})\textsubscript{n}} \\
\text{O} \\
\text{HN} \\
\text{IA}
\end{array}
\]

where

5

n is an integer from 1 to 5;

each R\textsuperscript{1} is independently a moiety of the formula –C(O)–R\textsuperscript{8}, –OR\textsuperscript{7} or –C(O)–O–R\textsuperscript{3};

R\textsuperscript{2} is hydrogen or lower alkyl;

R\textsuperscript{3} is hydrogen, alkyl, cycloalkyl or aryl;

10

R\textsuperscript{7} is aryl; and

R\textsuperscript{8} is cycloalkyl or aryl.

28. The compound of Claim 27 wherein R\textsuperscript{1} is a moiety of the formula –OR\textsuperscript{7}.

29. The compound of Claim 28 wherein R\textsuperscript{7} is phenyl.

30. The compound of Claim 27, 28 or 29 wherein n is 1 or 2.
31. A pharmaceutical composition comprising a pharmaceutically-acceptable carrier and compound of formula IA or a salt or a prodrug thereof:

![Chemical Structure](attachment:image.png)

IA

where

n is an integer from 1 to 5;

each $R^1$ is independently a moiety of the formula $-C(O)-R^8$ or $-C(O)-O-$

$R^3$;

$R^2$ is hydrogen or lower alkyl;

$R^3$ is hydrogen, alkyl, cycloalkyl or aryl;

$R^7$ is aryl; and

$R^8$ is cycloalkyl or aryl.

32. The compound of Claim 31 wherein $R^1$ is a moiety of the formula $-OR^7$.

33. The compound of Claim 32 wherein $R^7$ is phenyl.

34. The compound of Claim 31, 32 or 33 wherein n is 1 or 2.
Figure 1A

SUBSTITUTE SHEET (RULE 26)
Figure 1C
Figure 6

Figure 7

SUBSTITUTE SHEET (RULE 26)
PBL IL-8 release

FIGURE 8

C-FOS activation of THP-1 cells

FIGURE 9A

SUBSTITUTE SHEET (RULE 26)
c-JUN and NFkB activation in THP-1 cells

FIGURE 9B