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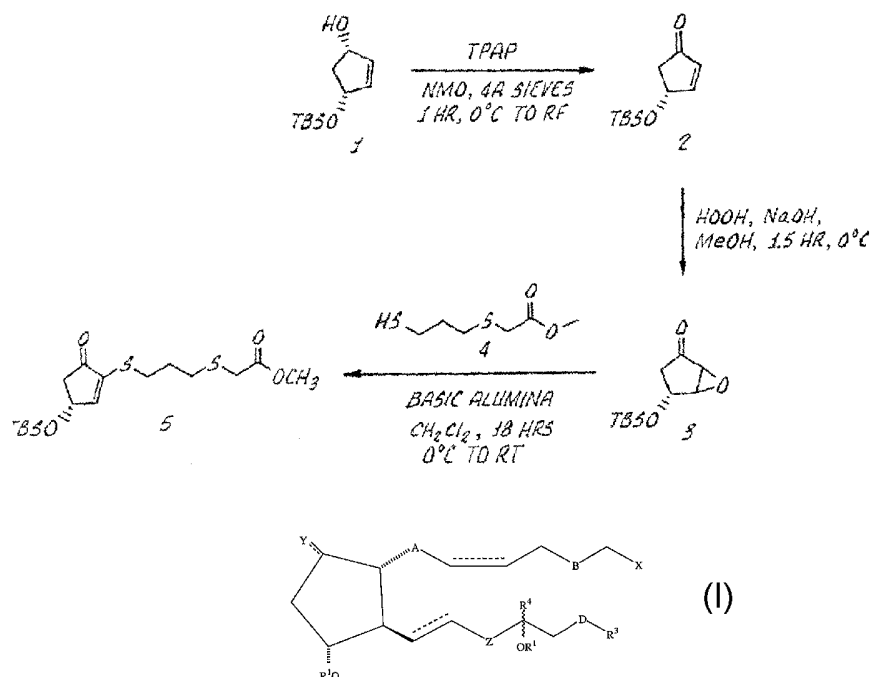
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(54) Title: COMBINATION THERAPY FOR GLAUCOMA TREATMENT



(57) Abstract: A method is disclosed herein comprising administering a compound and a second drug to an eye of a mammal for the treatment of glaucoma or the reduction of intraocular pressure, said compound represented by the general formula I; wherein A, B, D, X, Y, Z, R<sup>1</sup>, R<sup>3</sup> and R<sup>4</sup> are as defined in the specification.

## COMBINATION THERAPY FOR GLAUCOMA TREATMENT

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**By Inventors****Robert M. Burk, Mark Holoboski, Scott M. Whitcup, and Wha-Bin Im**

### **CROSS REFERENCE TO RELATED APPLICATIONS**

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This patent application is a continuation in part of Serial No. 10/672,499, filed September 26, 2003, which is a continuation of 10/346,828, filed January 16, 2003, now United States Patent No. 6,767,920, which is a continuation in part of Serial No. 09/882,720, filed June 14, 2001, now abandoned.

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### **Field of the Invention**

The present invention relates to 3, 7 or 3 and 7 thia or oxa prostanoid acid derivatives for the treatment of glaucoma or elevated intraocular pressure in combination with another drug.

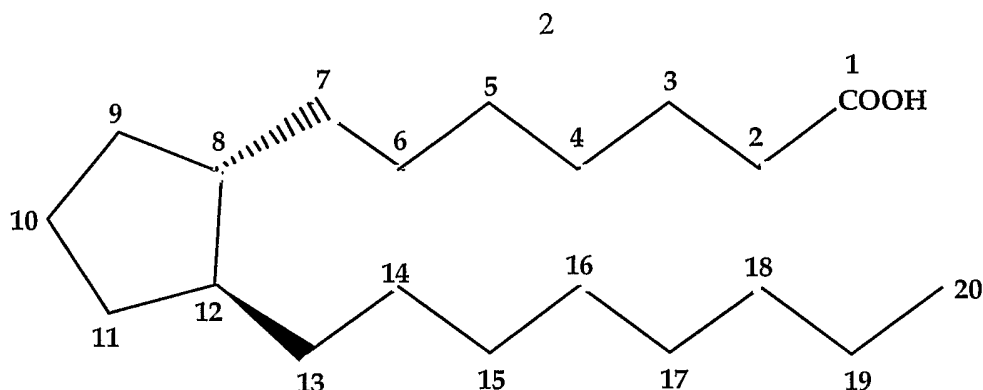
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### **Background of the Invention**

### **Description of Related Art**

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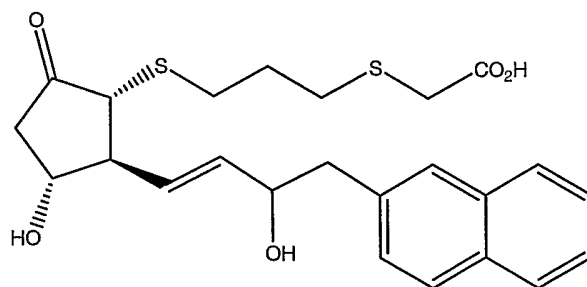
Prostaglandins can be described as derivatives of prostanoid acid which have the following structural formula:



Various types of prostaglandins are known, depending on the structure and substituents carried on the alicyclic ring of the prostanoic acid skeleton. Further classification is based on the number of unsaturated bonds in the side chain indicated by numerical subscripts after the generic type of prostaglandin [e.g. prostaglandin E<sub>1</sub> (PGE<sub>1</sub>), prostaglandin E<sub>2</sub> (PGE<sub>2</sub>)], and on the configuration of the substituents on the alicyclic ring indicated by  $\alpha$  or  $\beta$  [e.g. prostaglandin F<sub>2</sub> $\alpha$  (PGF<sub>2</sub> $\beta$ )].

Prostaglandins are useful for the long-term medical management of glaucoma (see, for example, Bito, L.Z. Biological Protection with Prostaglandins, Cohen, M.M., ed., Boca Raton, Fla, CRC Press Inc., 1985, pp. 231-252; and Bito, L.Z., Applied Pharmacology in the Medical Treatment of Glaucomas Drance, S.M. and Neufeld, A.H. eds., New York, Grune & Stratton, 1984, pp. 477-505. Such prostaglandins include PGF<sub>2</sub> $\alpha$ , PGF<sub>1</sub> $\alpha$ , PGE<sub>2</sub>, and certain lipid-soluble esters, such as C<sub>1</sub> to C<sub>2</sub> alkyl esters, e.g. 1-isopropyl ester, of such compounds.

EP 0 985 663 A1 discloses compounds such as the one shown below.



Inflammatory bowel disease (IBD) is a group of disease characterized by inflammation in the large or small intestines and is manifest in symptoms such as

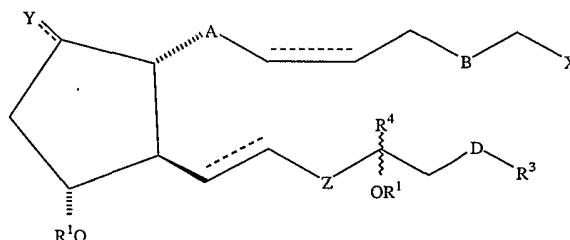
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diarrhea, pain, and weight loss. Nonsteroidal anti-inflammatory drugs have been shown to be associated with the risk of developing IBD, and recently Kabashima and colleagues have disclosed that “EP4 works to keep mucosal integrity, to suppress the innate immunity, and to downregulate the proliferation and activation of CD4+ T cells. These findings have not only elucidated the mechanisms of IBD by NSAIDs, but also indicated the therapeutic potential of EP4-selective agonists in prevention and treatment of IBD.” (Kabashima, et. al., The Journal of Clinical Investigation, April 2002, Vol. 9, 883-893)

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### Summary of the Invention

Methods are disclosed herein comprising administering a compound and a second drug to an eye of a mammal for the treatment of glaucoma or the reduction of intraocular pressure, said compound represented by the general formula I:



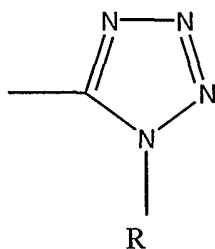
wherein hatched lines represent the  $\alpha$  configuration, a triangle represents the  $\beta$  configuration, a wavy line represents either the  $\alpha$  configuration or the  $\beta$  configuration and a dotted line represents the presence or absence of a double bond;

A and B are independently selected from the group consisting of O, S and CH<sub>2</sub>; provided that at least one of A or B is S;

D represents a covalent bond or CH<sub>2</sub>, O, S or NH;

X is CO<sub>2</sub>R, CONR<sub>2</sub>, CH<sub>2</sub>OR, P(O)(OR)<sub>2</sub>, CONRSO<sub>2</sub>R, SONR<sub>2</sub> or

4



Y is O, OH, OCOR<sup>2</sup>, halogen or cyano;

Z is CH<sub>2</sub> or a covalent bond;

5 R is H or R<sup>2</sup>;

R<sup>1</sup> is H, R<sup>2</sup>, phenyl, or COR<sup>2</sup>;

R<sup>2</sup> is C<sub>1</sub>-C<sub>5</sub> lower alkyl or alkenyl;

R<sup>3</sup> is benzothienyl, benzofuranyl, naphthyl, or substituted derivatives thereof,  
wherein the substituents maybe selected from the group consisting of C<sub>1</sub>-C<sub>5</sub> alkyl,

10 halogen, CF<sub>3</sub>, CN, NO<sub>2</sub>, NR<sub>2</sub>, CO<sub>2</sub>R and OR; and

R<sup>4</sup> is hydrogen or C<sub>1</sub>-C<sub>5</sub> alkyl.

Compositions, medicaments, and dosage forms related thereto are also disclosed.

#### Brief Description of the Drawing Figures

15 FIG. 1 is a schematic of the chemical synthesis of a certain intermediate for  
the compounds of the invention as disclosed in Examples 1 through 3.

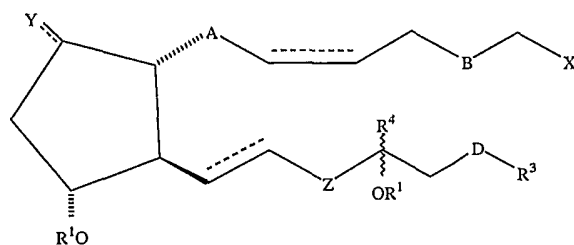
FIG. 2 is a schematic of the chemical synthesis of certain compounds related  
to the compounds of the invention as disclosed in Examples 4 through 7.

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#### Detailed Description of the Invention

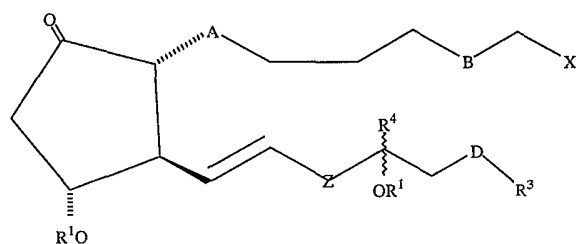
The compounds used for the treatment of glaucoma in combination with other  
therapeutic agents are encompassed by the following structural formula I:

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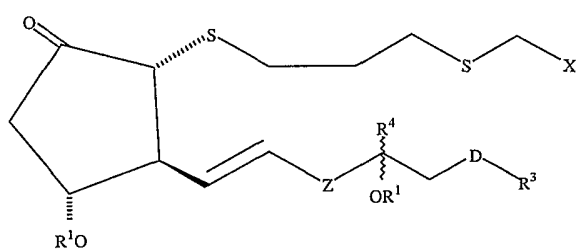


A preferred group of the compounds of the present invention includes compounds that have the following structural formula II:

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Another preferred group includes compounds having the formula III:



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In the above formulae, the substituents and symbols are as hereinabove defined.

In the above formulae:

Preferably A and B are both S.

6

Preferably D represents a covalent bond or is CH<sub>2</sub>; more preferably D is CH<sub>2</sub>.

Preferably Z represents a covalent bond.

Preferably R is H.

Preferably R<sup>1</sup> is H.

5 Preferably R<sup>4</sup> is hydrogen or methyl, most preferably hydrogen.

Preferably Y = O.

Preferably X is CO<sub>2</sub>R and more preferably R is selected from the group consisting of H, methyl, i-propyl and n-propenyl.

10 The above compounds of the present invention may be prepared by methods that are known in the art or according to the working examples below. The compounds, below, are especially preferred representative, of the compounds of the present invention.

15 {3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-5-(naphthyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester,

{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-5-(naphthyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid,

20 {3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-5-(naphthyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid isopropyl ester,

{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-hydroxy-5-(benzothienyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester,

25

{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-hydroxy-5-(benzothienyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid,

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{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-hydroxy-5-(benzothienyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid isopropyl ester,

{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-hydroxy-5-(benzofuranyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester,

{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-hydroxy-5-(benzofuranyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid,

{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-hydroxy-5-(benzofuranyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid isopropyl ester,

{3-[(1R,2S,3R)-3-Hydroxy-2-((E)-3-hydroxy-4-naphthalen-2-yl-but-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester,

15

{3-[(1R,2S,3R)-3-Hydroxy-2-((E)-3-hydroxy-4-naphthalen-2-yl-but-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid,

{3-[(1R,2S,3R)-2-((E)-4-Benzo[b]thiophen-3-yl-3-hydroxybut-1-enyl)-3-

hydroxy-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester,

{3-[(1R,2S,3R)-2-((E)-4-Benzo[b]thiophen-3-yl-3-hydroxybut-1-enyl)-3-hydroxy-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid,

{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-3-(methyl)-5-(naphthyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester,

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{3-[(1R,2S,3R)-3-Hydroxy-2-((E)-3-hydroxy-3-methyl-4-naphthalen-2-yl-but-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester,

{3-[(1R,2S,3R)-3-Hydroxy-2-((E)-3-hydroxy-3-methyl-4-naphthalen-2-yl-but-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid,

{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-3-(methyl)-5-(naphthyl)but-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid,

10 {3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-3-(methyl)-5-(benzothienyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester and

15 {3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-3-(methyl)-5-(benzothienyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester.

Second drugs useful for the treatment of glaucoma or other conditions include, but are not limited to.

For the treatment of glaucoma, combination treatment with the following classes of drugs are contemplated:

20  $\beta$ -Blockers (or  $\beta$ -adrenergic antagonists) including carteolol, levobunolol, metiparanolol, timolol hemihydrate, timolol maleate,  $\beta$ 1-selective antagonists such as betaxolol, and the like, or pharmaceutically acceptable salts or prodrugs thereof; Adrenergic Agonists including

25 non-selective adrenergic agonists such as epinephrine borate, epinephrine hydrochloride, and dipivefrin, and the like, or pharmaceutically acceptable salts or prodrugs thereof; and

- $\alpha_2$ -selective adrenergic agonists such as apraclonidine, brimonidine, and the like, or pharmaceutically acceptable salts or prodrugs thereof;
- Carbonic Anhydrase Inhibitors including acetazolamide, dichlorphenamide, methazolamide, brinzolamide, dorzolamide, and the like, or pharmaceutically acceptable salts or prodrugs thereof;
- 5 Cholinergic Agonists including direct acting cholinergic agonists such as carbachol, pilocarpine hydrochloride, pilocarpine nitrate, pilocarpine, and the like, or pharmaceutically acceptable salts or prodrugs thereof;
- 10 cholinesterase inhibitors such as demecarium, echothiophate, physostigmine, and the like, or pharmaceutically acceptable salts or prodrugs thereof;
- Glutamate Antagonists and other neuroprotective agents such as  $\text{Ca}^{2+}$  channel blockers such as memantine, amantadine, rimantadine, nitroglycerin, dextrophan, detromethorphan, CGS-19755, dihydropyridines, verapamil, emopamil,
- 15 benzothiazepines, bepridil, diphenylbutylpiperidines, diphenylpiperazines, HOE 166 and related drugs, fluspirilene, eliprotil, ifenprodil, CP-101,606, tibalosine, 2309BT, and 840S, flunarizine, nicardipine, nifedimpine, nimodipine, barnidipine, verapamil, lidoflazine, prenylamine lactate, amiloride, and the like, or pharmaceutically acceptable salts or prodrugs thereof;
- 20 Prostamides such as bimatoprost, or pharmaceutically acceptable salts or prodrugs thereof;
- Prostaglandins including travoprost, UFO-21, chloprostenol, fluprostenol, 13,14-dihydro-chloprostenol, latanoprost and the like;
- isopropyl unoprostone; and
- 25 Cannabinoids including CB1 agonists such as WIN-55212-2 and CP-55940 and the like, or pharmaceutically acceptable salts or prodrugs thereof.

For treatment of diseases affecting the eye including glaucoma, these compounds can be administered topically, periocularly, intraocularly, or by any other effective means known in the art. The compounds disclosed herein may be

administered topically, periocularly, or by intraocular injection. Delivery may be by sustained release. For example, the drug may be delivered via a sustained release polymer, where the drug is released over time by diffusion of the drug from the polymer or degradation of the polymer. The polymer might be injected or implanted  
5 anywhere in or around the eye, including the subconjunctival or subtenons space.

Pharmaceutical compositions may be prepared by combining a therapeutically effective amount of at least one compound according to the present invention, or a pharmaceutically acceptable acid addition salt thereof, as an active ingredient, with conventional ophthalmically acceptable pharmaceutical excipients, and by  
10 preparation of unit dosage forms suitable for topical ocular use. The therapeutically efficient amount typically is between about 0.0001 and about 5% (w/v), preferably about 0.001 to about 1.0% (w/v) in liquid formulations.

For topical ophthalmic application, preferably solutions are prepared using a physiological saline solution as a major vehicle. The pH of such ophthalmic solutions  
15 should preferably be maintained between 6.5 and 7.2 with an appropriate buffer system. The formulations may also contain conventional, pharmaceutically acceptable preservatives, stabilizers and surfactants.

Preferred preservatives that may be used in the pharmaceutical compositions of the present invention include, but are not limited to, benzalkonium chloride, chlorobutanol, thimerosal, phenylmercuric acetate and phenylmercuric nitrate. A  
20 preferred surfactant is, for example, Tween 80. Likewise, various preferred vehicles may be used in the ophthalmic preparations of the present invention. These vehicles include, but are not limited to, polyvinyl alcohol, povidone, hydroxypropyl methyl cellulose, poloxamers, carboxymethyl cellulose, hydroxyethyl cellulose and purified  
25 water.

Tonicity adjustors may be added as needed or convenient. They include, but are not limited to, salts, particularly sodium chloride, potassium chloride, mannitol and glycerin, or any other suitable ophthalmically acceptable tonicity adjustor.

Various buffers and means for adjusting pH may be used so long as the resulting preparation is ophthalmically acceptable. Accordingly, buffers include acetate buffers, citrate buffers, phosphate buffers and borate buffers. Acids or bases may be used to adjust the pH of these formulations as needed.

5 In a similar vein, an ophthalmically acceptable antioxidant for use in the present invention includes, but is not limited to, sodium metabisulfite, sodium thiosulfate, acetylcysteine, butylated hydroxyanisole and butylated hydroxytoluene.

Other excipient components which may be included in the ophthalmic preparations are chelating agents. The preferred chelating agent is edentate disodium, 10 although other chelating agents may also be used in place or in conjunction with it.

The ingredients are usually used in the following amounts:

|    | <u>Ingredient</u> | <u>Amount (% w/v)</u>  |
|----|-------------------|------------------------|
|    | active ingredient | about 0.001-5          |
|    | preservative      | 0-0.10                 |
| 15 | vehicle           | 0-40                   |
|    | tonicity adjustor | 1-10                   |
|    | buffer            | 0.01-10                |
|    | pH adjustor       | q.s. pH 4.5-7.5        |
|    | antioxidant       | as needed              |
| 20 | surfactant        | as needed              |
|    | purified water    | as needed to make 100% |

The actual dose of the active compounds of the present invention depends on the specific compound, and on the condition to be treated; the selection of the 25 appropriate dose is well within the knowledge of the skilled artisan.

The ophthalmic formulations of the present invention are conveniently packaged in forms suitable for metered application, such as in containers equipped with a dropper, to facilitate the application to the eye. Containers suitable for dropwise application are usually made of suitable inert, non-toxic plastic material, 30 and generally contain between about 0.5 and about 15 ml solution.

Those skilled in the art will readily understand that for oral or rectal administration the compounds of the invention are admixed with pharmaceutically acceptable excipients which per se are well known in the art. Specifically, a drug to

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be administered systemically, it may be confectioned as a powder, pill, tablet or the like, or as a syrup or elixir suitable for oral administration. Description of the substances normally used to prepare tablets, powders, pills, syrups and elixirs can be found in several books and treatise well known in the art, for example in Remington's

5 Pharmaceutical Science, Edition 17, Mack Publishing Company, Easton, Pa.

Parenteral administration is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for dissolving or suspending in liquid prior to injection, or as emulsions. Descriptions of substances and methods normally used to prepare

10 formulations for parenteral administration can be found in several treatises and books well known in the art such as, Handbook On Injectable Drugs (11th edition), edited by Lawrence A. Trissel, (Chicago: Login Brothers Book Company; January 15, 2001).

The invention is further illustrated by the following non-limiting Examples, which

15 are summarized in the reaction schemes of Figures 1 and 2 wherein the compounds are identified by the same designator in both the Examples and the Figures.

#### Example 1

#### **(R)-4-(*tert*-Butyldimethylsilanyloxy)cyclopent-2-enone (2).**

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Tetrapropylammonium perruthenate (9.4 mg, 0.027 mmol) was added to a mixture of (1S, 4R)-4-(*tert*-butyldimethylsilanyloxy)cyclopent-2-enol prepared, according to *Tetrahedron Letters*, Vol. 37, No. 18, 1996, pp. 3083-6, (118.6 mg, 0.54 mmol), 4-methylmorpholine N-oxide (94.9 mg, 0.81 mmol) and crushed 4Å

25 sieves (270 mg) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL). The mixture was stirred for 30 min and was passed through a plug of silica gel with CH<sub>2</sub>Cl<sub>2</sub>. The filtrate was concentrated *in vacuo* to give 100 mg (86%) of the above titled compound.

#### Example 2

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**(R)-4-(tert-Butyldimethylsilyloxy)-6-oxabicyclo[3.1.0]hexan-2-one (3).**

Hydrogen peroxide (4.5 mL, 46.3 mmol, 30% wt. % solution in water) and 1N NaOH (46  $\mu$ L, 0.046 mmol) were added to a solution of enone **2** (2.5 g, 11.5 mmol) in MeOH (30 mL) at 0° C. After stirring 1.5 h at 0° C the mixture was concentrated *in vacuo*, washed with saturated aqueous NH<sub>4</sub>Cl and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3X). The combined organics were washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated *in vacuo* to afford the above titled compound.

10

Example 3**({3-[(R)-3-(tert-Butyldimethylsilyloxy)-5-oxocyclopent-1-enylsulfanyl]propylsulfanyl}acetic acid methyl ester (5).**

15

The epoxide **3** prepared above was diluted with CH<sub>2</sub>Cl<sub>2</sub> (30 mL), (3-mercaptopropylsulfanyl) acetic acid methyl ester **4** (1.93 g, 10.7 mmol), prepared according to *Chem. Pharm. Bull.* 28 (2), 1980, 558-566, was added and the solution was cooled to 0° C. Basic alumina (11.9 g) was added and the reaction mixture was warmed to room temperature. After stirring for 18 h the mixture was filtered through celite and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, 6:1 hex/EtOAc) to yield 3.6 g (80 %) of the above titled compound.

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Example 4

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**(3-[(1R,2S,3R)-3-(tert-Butyldimethylsilyloxy)-2-[(S)-(E)-3-(tert-butyldimethylsilyloxy)oct-1-enyl]-5-oxocyclopentylsulfanyl]propylsulfanyl)acetic acid methyl ester (7).**

14

*tert*-Butyllithium (1.47 mL of a 1.7M solution in pentane, 2.5 mmol) was added dropwise to a solution of *tert*-butyl[(S)-1-((E)-2-iodovinyl)hexyloxy]dimethylsilane **6** (462.5 mg, 1.25 mmol) in Et<sub>2</sub>O (6.0 mL) at -78° C. After stirring for 30 min lithium 2-thienylcyanocuprate (6.0 mL of a 0.25M solution in THF, 1.5 mmol) was added and the reaction was stirred an additional 30 min at -78° C. A solution of enone **5** (430 mg, 1.1 mmol) in Et<sub>2</sub>O (1 mL) was added and stirring was continued for an additional 1 h. The reaction mixture was then quickly poured into saturated aqueous NH<sub>4</sub>Cl cooled to 0° C. The mixture was extracted with EtOAc and the organic portion was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated *in vacuo*. The residue was quickly purified by flash column chromatography (silica gel, 100% hexane followed by 8:1 hex/EtOAc) to afford 270 mg (39%) of the above titled compound.

#### Example 5

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{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-hydroxyoct-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester (**8**).

Hydrogen fluoride-pyridine (220 µL) was added to a solution of *bis*-TBDMS ether **7** (70 mg, 0.11 mmol) in CH<sub>3</sub>CN (2.0 mL) at 0° C. The reaction was warmed to room temperature, stirred 1 h, and re-cooled to 0° C. The reaction was quenched with saturated aqueous NaHCO<sub>3</sub> until gas evolution ceased. The mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (4X). The combined organics were washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated *in vacuo*. Purification of the residue by flash column chromatography (silica gel, 100% CH<sub>2</sub>Cl<sub>2</sub> followed by 30:1 CH<sub>2</sub>Cl<sub>2</sub>:MeOH) provided 40 mg (90%) of the above titled compound.

#### Example 6

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{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-hydroxyoct-1-enyl)-5-oxocyclopentyl-sulfanyl]propylsulfanyl}acetic acid (9).

Methyl ester **8** (50 mg, 0.124 mmol) was dissolved in CH<sub>3</sub>CN (10 mL) and  
5 pH 7.2 phosphate buffer (3.0 mL) was added. The mixture was treated with PLE  
(400 µL, 1.34 mol/L) and stirred for 16 h at 23 °C. The reaction mixture was  
extracted with EtOAc (3X). The combined organics were washed with brine, dried  
(Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated *in vacuo*. Purification of the residue by flash  
column chromatography (silica gel, 100% EtOAc) gave 5.3 mg (11%) of the above  
10 titled compound.

#### Example 7

{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-hydroxyoct-1-enyl)-5-oxocyclopentyl-  
15 sulfanyl]propylsulfanyl}acetic acid isopropyl ester (10).

Isopropyl-*p*-tolyltriazene (200 µL ) was added dropwise to a solution of  
carboxylic acid **9** (10.5 mg, 0.026 mmol) in acetone (5.0 mL) at 23 °C. After stirring  
for 1 h the reaction was quenched with 1N HCl and the solvent was removed *in*  
20 *vacuo*. The residue was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2X). The combined organics were  
dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated *in vacuo*. Purification of the residue by  
flash column chromatography (silica gel, 4:1 hex/EtOAc) gave 4.3 mg (38%) of the  
above titled compound.

25

#### Example 8

16

(3-[(1R,2S,3R)-3-(*tert*-Butyldimethylsilanyloxy)-2-[(S)-(E)-3-(*tert*-butyldimethylsilanoxy)-5-(naphthyl)pent-1-enyl]-5-oxocyclopentylsulfanyl] propylsulfanyl)acetic acid methyl ester (H).

- 5 (3-[(1R,2S,3R)-3-(*tert*-Butyldimethylsilanyloxy)-2-[(S)-(E)-3-(*tert*-butyldimethylsilanoxy)-5-(naphthyl)pent-1-enyl]-5-oxocyclopentylsulfanyl] propylsulfanyl)acetic acid methyl ester (L).

The named compound is prepared by substituting *tert*-butyl-[(E)-3-iodo-1-(2-naphthalen-2-yl-ethyl)allyloxy]dimethylsilane for *tert*-butyl[(S)-1-((E)-2-iodovinyl)hexyloxy]dimethylsilane in the method of Example 4. FCC gives a higher R<sub>f</sub> compound and a lower R<sub>f</sub> compound, designated as H and L, respectively.

15 Example 9(H)

{3-[(1R,2S,3R)-3-Hydroxy-2-[(S)-(E)-3-(hydroxy)-5-(naphthyl)pent-1-enyl]-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester (H).

- 20 The named compound is prepared by repeating the method of Example 5 with the named compound of Example 8 (H) rather than the named compound of Example 4.

Example 9 (L)

- 25 {3-[(1R,2S,3R)-3-Hydroxy-2-[(S)-(E)-3-(hydroxy)-5-(naphthyl)pent-1-enyl]-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester (L).

The named compound is prepared by repeating the method of Example 5 with the named compound of Example 8 (L) rather than the named compound of Example

4.

5

Example 10 (H)

**{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-5-(naphthyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid (H).**

10

The named compound is prepared by repeating the method of Example 6 with the named compound of Example 9 (H) rather than the named compound of Example 5.

Example 10 (L)

15

**{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-5-(naphthyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid (L).**

20

The named compound is prepared by repeating the method of Example 6 with the named compound of Example 9 (L) rather than the named compound of Example 5.

Example 11

25

**{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-5-(naphthyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid isopropyl ester.**

The named compound is prepared by repeating the method of Example 7 with the named compound of Example 10 rather than the named compound of Example 6.

5

Example 12

(3-[(1R,2S,3R)-3-(*tert*-Butyldimethylsilanyloxy)-2-[(S)-(E)-3-(*tert*-butyldimethylsilanoxy)-5-(benzothienyl)pent-1-enyl]-5-oxocyclopentylsulfanyl] propylsulfanyl)acetic acid methyl ester (H).

10

(3-[(1R,2S,3R)-3-(*tert*-Butyldimethylsilanyloxy)-2-[(S)-(E)-3-(*tert*-butyldimethylsilanoxy)-5-(benzothienyl)pent-1-enyl]-5-oxocyclopentylsulfanyl] propylsulfanyl)acetic acid methyl ester (L).

15

The named compound is prepared by substituting [(E)-1-(2-Benzo[*b*]thiophen-2-yl-ethyl)-3-iodoallyloxy]-*tert*-butyldimethylsilane for *tert*-butyl[(S)-1-((E)-2-iodovinyl)hexyloxy]dimethylsilane in the method of Example 4. FCC gives a higher R<sub>f</sub> compound and a lower R<sub>f</sub> compound, designated as H and L, respectively.

20

Example 13(H)

{3-[(1R,2S,3R)-3-Hydroxy-2-[(S)-(E)-3-hydroxy-5-(benzothienyl)pent-1-enyl]-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester (H).

25

The named compound is prepared by repeating the method of Example 5 with the named compound of Example 12 (H) rather than the named compound of Example 4.

5

Example 13(L)

**{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-hydroxy-5-(benzothienyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester (L).**

10

The named compound is prepared by repeating the method of Example 5 with the named compound of Example 12 (H) rather than the named compound of Example 4.

Example 14(H)

15

**{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-hydroxy-5-(benzothienyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid (H).**

20

The named compound is prepared by repeating the method of Example 6 with the named compound of Example 13 (H) rather than the named compound of Example 5.

Example 14(L)

25

**{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-hydroxy-5-(benzothienyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid (L).**

20

The named compound is prepared by repeating the method of Example 6 with the named compound of Example 13 (L) rather than the named compound of Example 5.

5

Example 15

**{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-hydroxy-5-(benzothienyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid isopropyl ester.**

10

The named compound is prepared by repeating the method of Example 7 with the named compound of Example 14 rather than the named compound of Example 6.

15

Example 16

**(3-[(1R,2S,3R)-3-(*tert*-Butyldimethylsilanyloxy)-2-[(S)-(E)-3-(*tert*-butyldimethylsilanyloxy)-5-(benzofuranyl)pent-1-enyl]-5-oxocyclopentylsulfanyl]propylsulfanyl)acetic acid methyl ester.**

20

The named compound is prepared by substituting [(E)-1-(2-Benzo[*b*]furan-2-yl-ethyl)-3-iodoallyloxy]-*tert*-butyldimethylsilane for *tert*-butyl[(S)-1-((E)-2-iodovinyl) hexyloxy]dimethylsilane in the method of Example 4.

25

Example 17

**{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-hydroxy-5-(benzofuranyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester.**

The named compound is prepared by repeating the method of Example 5 with the named compound of Example 16 rather than the named compound of Example 4.

5

Example 18

**{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-hydroxy-5-(benzofuranyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid.**

10

The named compound is prepared by repeating the method of Example 6 with the named compound of Example 17 rather than the named compound of Example 5.

15

Example 19

**{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-hydroxy-5-(benzofuranyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid isopropyl ester.**

20

The named compound is prepared by repeating the method of Example 7 with the named compound of Example 18 rather than the named compound of Example 6.

Example 20

**(3-[(1R,2S,3R)-3-(*tert*-Butyldimethylsilanyloxy)-2-[(E)-3-(*tert*-butyldimethylsilanoxy)-4-naphthalen-2-yl-but-1-enyl]-5-oxocyclopentylsulfanyl] propylsulfanyl)acetic acid methyl ester (H).**

25

**(3-((1R,2S,3R)-3-(*tert*-Butyldimethylsilanyloxy)-2-[(*E*)-3-(*tert*-butyldimethylsilanoxy)-4-naphthalen-2-yl-but-1-enyl]-5-oxocyclopentylsulfanyl) propylsulfanyl)acetic acid methyl ester (L).**

5 The named compound is prepared by substituting *tert*-butyl-((E)-3-iodo-1-naphthalen-2-yl-methylallyloxy)dimethylsilane for *tert*-butyl[(S)-1-((E)-2-iodovinyl) hexyloxy]dimethylsilane in the method of Example 4. FCC gives a higher R<sub>f</sub> compound and a lower R<sub>f</sub> compound, designated as H and L, respectively.

10 Example 21 (H)

**{3-[(1R,2S,3R)-3-Hydroxy-2-((E)-3-hydroxy-4-naphthalen-2-yl-but-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester (H).**

15           The named compound is prepared by repeating the method of Example 5 with  
the named compound of Example 20 (H) rather than the named compound of  
Example 4.

### Example 21(L)

20 {3-[(1R,2S,3R)-3-Hydroxy-2-((E)-3-hydroxy-4-naphthalen-2-yl-but-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester (L).

The named compound is prepared by repeating the method of Example 5 with the named compound of Example 20 (H) rather than the named compound of Example 4.

25

Example 22(H)

**{3-[(1R,2S,3R)-3-Hydroxy-2-((E)-3-hydroxy-4-naphthalen-2-yl-but-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid (H).**

The named compound is prepared by repeating the method of Example 6 with the named compound of Example 21 (H) rather than the named compound of Example 5.

5

Example 22(L)

10 {3-[(1R,2S,3R)-3-Hydroxy-2-((E)-3-hydroxy-4-naphthalen-2-yl-but-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid (L).

The named compound is prepared by repeating the method of Example 6 with the named compound of Example 21 (H) rather than the named compound of

15 Example 5.

Example 23

{3-[(1R,2S,3R)-2-[(E)-4-Benzo[b]thiophen-3-yl-3-(*tert*-  
20 butyldimethylsilanyloxy)but-1-enyl]-3-(*tert*-butyldimethylsilanyloxy)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester (H).

{3-[(1R,2S,3R)-2-[(E)-4-Benzo[b]thiophen-3-yl-3-(*tert*-  
butyldimethylsilanyloxy)but-1-enyl]-3-(*tert*-butyldimethylsilanyloxy)-5-  
25 oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester (L).

The named compound is prepared by substituting ((E)-1-Benzo[b]thiophen-3-ylmethyl-3-iodo-allyloxy)-*tert*-butyldimethylsilane for

24

*tert*-butyl[(S)-1-((E)-2-iodovinyl)hexyloxy]dimethylsilane in the method of Example 4. FCC gives a higher R<sub>f</sub> compound and a lower R<sub>f</sub> compound, designated as H and L respectively.

5

Example 24(H)

10 {3-[(1R,2S,3R)-2-((E)-4-Benzo[b]thiophen-3-yl-3-hydroxybut-1-enyl)-3-hydroxy-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester (H).

The named compound is prepared by repeating the method of Example 5 with  
15 the named compound of Example 23 (H) rather than the named compound of Example 4.

Example 24(L)

{3-[(1R,2S,3R)-2-((E)-4-Benzo[b]thiophen-3-yl-3-hydroxybut-1-enyl)-3-  
20 hydroxy-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester (L).

The named compound is prepared by repeating the method of Example 5 with  
the named compound of Example 23 (H) rather than the named compound of  
25 Example 4.

Example 25(H)

25

{3-[(1R,2S,3R)-2-((E)-4-Benzo[b]thiophen-3-yl-3-hydroxybut-1-enyl)-3-hydroxy-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid (H).

The named compound is prepared by repeating the method of  
5 Example 6 with the named compound of Example 24 (H) rather than the  
named compound of Example 5.

Example 25(L)

10 {3-[(1R,2S,3R)-2-((E)-4-Benzo[b]thiophen-3-yl-3-hydroxybut-1-enyl)-3-  
hydroxy-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid (L).

The named compound is prepared by repeating the method of Example 6 with  
the named compound of Example 24 (H) rather than the named compound of  
15 Example 5.

Example 26

(3-[(1R,2S,3R)-3-(*tert*-Butyldimethylsilanyloxy)-2-[(S)-(E)-3-(*tert*-  
butyldimethylsilanoxy)-3-(methyl)-5-(naphthyl)pent-1-enyl]-5-  
20 oxocyclopentylsulfanyl] propylsulfanyl)acetic acid methyl ester (H).

(3-[(1R,2S,3R)-3-(*tert*-Butyldimethylsilanyloxy)-2-[(S)-(E)-3-(*tert*-  
butyldimethylsilanoxy)-3-(methyl)-5-(naphthyl)pent-1-enyl]-5-  
oxocyclopentylsulfanyl] propylsulfanyl)acetic acid methyl ester (L).

25

The named compound is prepared by substituting *tert*-Butyl-[(E)-3-iodo-1-  
methyl-1-(2-naphthalen-2-yl-ethyl)allyloxy]dimethylsilane for *tert*-butyl[(S)-1-((E)-  
2-iodovinyl)hexyloxy]dimethylsilane in the method of Example 4. FCC gives a

26

higher R<sub>f</sub> compound and a lower R<sub>f</sub> compound, designated as H and L, respectively.

Example 27(H)

5 {3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-3-(methyl)-5-(naphthyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester (H).

The named compound is prepared by repeating the method of Example 5 with  
10 the named compound of Example 26 (H) rather than the named compound of Example 4.

Example 27(L)

15 {3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-3-(methyl)-5-(naphthyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester (L).

The named compound is prepared by repeating the method of Example 5 with  
the named compound of Example 26 (H) rather than the named compound of  
20 Example 4.

Example 28(H)

25 {3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-3-(methyl)-5-(naphthyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid (H).

27

The named compound is prepared by repeating the method of Example 6 with the named compound of Example 27 (H) rather than the named compound of Example 5.

Example 28(L)

5

**{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-3-(methyl)-5-(naphthyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid (L).**

10

The named compound is prepared by repeating the method of Example 6 with the named compound of Example 27(L) rather than the named compound of Example 5.

Example 29

15

**(3-[(1R,2S,3R)-3-(*tert*-Butyldimethylsilanyloxy)-2-[(E)-3-(*tert*-butyldimethylsilanoxy)-3-methyl-4-naphthalen-2-yl-but-1-enyl]-5-oxocyclopentylsulfanyl] propylsulfanyl)acetic acid methyl ester (H).**

20

**(3-[(1R,2S,3R)-3-(*tert*-Butyldimethylsilanyloxy)-2-[(E)-3-(*tert*-butyldimethylsilanoxy)-3-methyl-4-naphthalen-2-yl-but-1-enyl]-5-oxocyclopentylsulfanyl] propylsulfanyl)acetic acid methyl ester (L).**

25

The named compound is prepared by substituting *tert*-butyl-[(E)-3-iodo-1-methyl-1-(2-naphthalen-2-yl-methyl)allyloxy]dimethylsilane for *tert*-butyl[(S)-1-((E)-2-iodovinyl) hexyloxy]dimethylsilane in the method of Example 4. FCC gives a higher R<sub>f</sub> compound and a lower R<sub>f</sub> compound, designated as H and L, respectively.

Example 30(H)

28

**{3-[ (1R,2S,3R)-3-Hydroxy-2-((E)-3-hydroxy-3-methyl-4-naphthalen-2-yl-but-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester (H).**

5           The named compound is prepared by repeating the method of Example 5 with  
the named compound of Example 29 (H) rather than the named compound of  
Example 4.

10 Example 30(L)

**{3-[(1R,2S,3R)-3-Hydroxy-2-((E)-3-hydroxy-3-methyl-4-naphthalen-2-yl-but-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester (L).**

15           The named compound is prepared by repeating the method of Example 5 with  
the named compound of Example 29 (L) rather than the named compound of  
Example 4.

Example 31(H)

20

**{3-[(1R,2S,3R)-3-Hydroxy-2-((E)-3-hydroxy-3-methyl-4-naphthalen-2-yl-but-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid (H).**

The named compound is prepared by repeating the method of Example 6 with  
25 the named compound of Example 30 (H) rather than the named compound of  
Example 5.

Example 31(L)

29

{3-[(1R,2S,3R)-3-Hydroxy-2-((E)-3-hydroxy-3-methyl-4-naphthalen-2-yl-but-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid (L).

The named compound is prepared by repeating the method of Example 6 with  
5 the named compound of Example 30 (L) rather than the named compound of Example 5.

10

Example 32

(3-[(1R,2S,3R)-3-(*tert*-Butyldimethylsilanyloxy)-2-[(S)-(E)-3-(*tert*-butyldimethylsilanoxy)-3-(methyl)-5-(benzylthienyl)pent-1-enyl]-5-oxocyclopentylsulfanyl] propylsulfanyl)acetic acid methyl ester (H).

15

(3-[(1R,2S,3R)-3-(*tert*-Butyldimethylsilanyloxy)-2-[(S)-(E)-3-(*tert*-butyldimethylsilanoxy)-3-(methyl)-5-(benzothienyl)pent-1-enyl]-5-oxocyclopentylsulfanyl] propylsulfanyl)acetic acid methyl ester (L).

The named compound is prepared by [(E)-1-(2-Benzo[*b*]thiophen-2-yl-ethyl)-3-iodo-1-methylallyloxy]-*tert*-butyldimethylsilane for *tert*-butyl[(S)-1-((E)-2-iodovinyl) hexyloxy]dimethylsilane in the method of Example 4. FCC gives a  
20 higher R<sub>f</sub> compound and a lower R<sub>f</sub> compound, designated as H and L, respectively.

25

Example 33(H)

{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-3-(methyl)-5-(benzothienyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester (H).

The named compound is prepared by repeating the method of Example 5 with the named compound of Example 32 (H) rather than the named compound of Example 4.

Example 33(L)

- 5 {3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-3-(methyl)-5-(benzothienyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester (L).

10 The named compound is prepared by repeating the method of Example 5 with the named compound of Example 32 (L) rather than the named compound of Example 4.

Example 34(H)

- 15 {3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-3-(methyl)-5-(benzothienyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid (H).

20 The named compound is prepared by repeating the method of Example 6 with the named compound of Example 33 (H) rather than the named compound of Example 5.

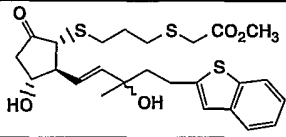
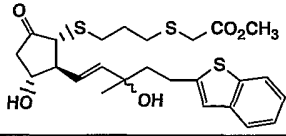
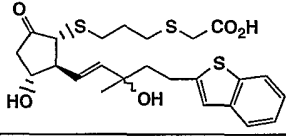
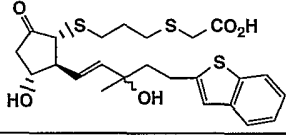
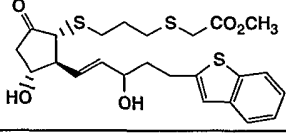
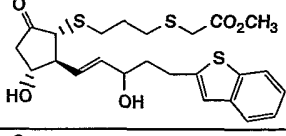
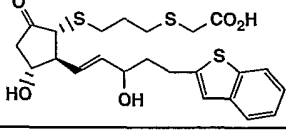
Example 34(L)

- 25 {3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-3-(methyl)-5-(benzothienyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid (L).

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The named compound is prepared by repeating the method of Example 6 with the named compound of Example 33L rather than the named compound of Example 5.

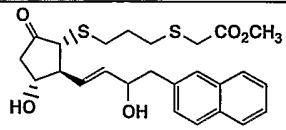
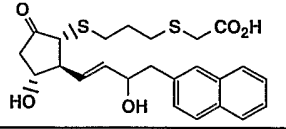
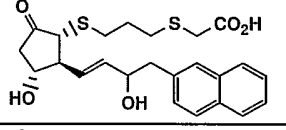
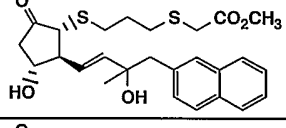
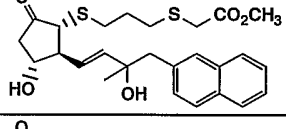
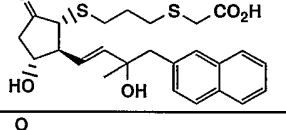
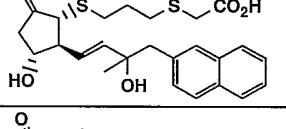
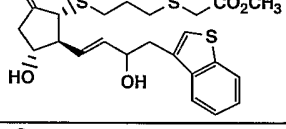
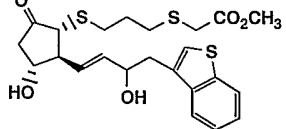
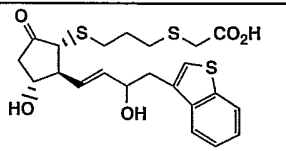
The compounds of the Examples are subject to *in vitro* testing as described below. The results are reported in the table as IC50s in nM.

| Example No. |                                                                                     | hEP <sub>2</sub>  | hEP <sub>3</sub> | hEP <sub>4</sub> |
|-------------|-------------------------------------------------------------------------------------|-------------------|------------------|------------------|
| 33H         |    | NA                | NA               | 200              |
| 33L         |    | NA                | NA               | 300              |
| 34H         |   | >>10 <sup>4</sup> | >10 <sup>4</sup> | 32               |
| 34L         |  | NA                | >10 <sup>4</sup> | 68               |
| 13H         |  | NA                | NA               | 91               |
| 13L         |  | >>10 <sup>4</sup> | 7200             | 93               |
| 14H         |  | >>10 <sup>4</sup> | >10 <sup>4</sup> | 27               |
| Example No. |                                                                                     | hEP <sub>2</sub>  | hEP <sub>3</sub> | hEP <sub>4</sub> |

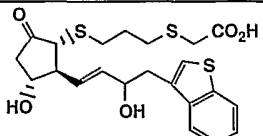
32

|             |  |                  |                  |                  |
|-------------|--|------------------|------------------|------------------|
| 14L         |  | $10^4$           | $>10^4$          | 13               |
| 9H          |  | NA               | NA               | 40               |
| 9L          |  | NA               | $>10^4$          | 40               |
| 10H         |  | $>>10^4$         | $>10^4$          | 450              |
| 10L         |  | $>10^4$          | 8300             | 19.5             |
| 27H         |  | NA               | NA               | 500              |
| 27L         |  | NA               | NA               | 3400             |
| 28H         |  | NA               | $>10^4$          | 1700             |
| 28L         |  | NA               | $>10^4$          | 1500             |
| 21H         |  | NA               | $>10^4$          | 100              |
|             |  |                  |                  |                  |
| Example No. |  | hEP <sub>2</sub> | hEP <sub>3</sub> | hEP <sub>4</sub> |

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|             |                                                                                     |                  |                  |                  |
|-------------|-------------------------------------------------------------------------------------|------------------|------------------|------------------|
| 21L         |    | NA               | $>10^4$          | 13               |
| 22H         |    | NA               | $>10^4$          | 32               |
| 22L         |    | $>>10^4$         | $>10^4$          | 6.2              |
| 30H         |    | NA               | $>10^4$          | 3100             |
| 30L         |   | NA               | NA               | 3200             |
| 31H         |  | NA               | 8100             | 300              |
| 31L         |  | NA               | 9300             | 900              |
| 24H         |  | NA               | NA               | 200              |
| 24L         |  | 9300             | $>10^4$          | 30               |
| 25H         |  | $>10^4$          | NA               | 69               |
|             |                                                                                     |                  |                  |                  |
| Example No. |                                                                                     | hEP <sub>2</sub> | hEP <sub>3</sub> | hEP <sub>4</sub> |

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|     |                                                                                   |      |         |   |
|-----|-----------------------------------------------------------------------------------|------|---------|---|
| 25L |  | 2200 | $>10^4$ | 5 |
|-----|-----------------------------------------------------------------------------------|------|---------|---|

### HUMAN RECOMBINANT EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>4</sub>, AND FP RECEPTORS: STABLE TRANSFECTANTS.

HEK-293 cells stably expressing the human or feline FP receptor, or EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>3</sub>, or EP<sub>4</sub> receptors were washed with TME buffer, scraped from the bottom of the flasks, and homogenized for 30 sec using a Brinkman PT 10/35 polytron. TME buffer was added to achieve a final 40 ml volume in the centrifuge tubes (the composition of TME is 100 mM TRIS base, 20 mM MgCl<sub>2</sub>, 2M EDTA; 10N HCl is added to achieve a pH of 7.4).

The cell homogenate was centrifuged at 19000 r.p.m. for 20 min at 4° C using a Beckman Ti-60 rotor. The resultant pellet was resuspended in TME buffer to give a final 1 mg/ml protein concentration, as determined by Biorad assay. Radioligand binding competition assays vs. [<sup>3</sup>H]-17  $\beta$ -phenyl PGF<sub>2 $\alpha$</sub>  (5 nM) were performed in a 100 $\mu$ l volume for 60 min. Binding reactions were started by adding plasma membrane fraction. The reaction was terminated by the addition of 4 ml ice-cold TRIS-HCl buffer and rapid filtration through glass fiber GF/B filters using a Brandel cell harvester. The filters were washed 3 times with ice-cold buffer and oven dried for one hour. Non-specific binding was determined with 10  $\mu$ M unlabeled 17  $\beta$ -phenyl PGF<sub>2 $\alpha$</sub> .

[<sup>3</sup>H]-PGE<sub>2</sub> (5 nM; specific activity 180 Ci mmol) was used as the radioligand for EP receptors. Binding studies employing EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>3</sub>, EP<sub>4</sub> were performed in duplicate in at least three separate experiments. A 200 $\mu$ l assay volume was used. Incubations were for 60 min at 25°C and were terminated by the addition of 4 ml of ice-cold 50 mM TRIS-HCl, followed by rapid filtration through Whatman GF/B filters and three additional 4 ml washes in a cell harvester (Brandel). Non-specific binding determined with 10<sup>-5</sup>M of unlabeled PGE<sub>2</sub>.

Plasmids encoding the human EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>4</sub>, and FP receptors were prepared by cloning the respective coding sequences into the eukaryotic expression vector pCEP4 (Invitrogen). The pCEP4 vector contains an Epstein Barr virus (EBV) origin of replication, which permits episomal replication in primate cell lines expressing EBV nuclear antigen (EBNA-1). Similarly, competitive experiments were carried out using [3H]17-phenylPGF<sub>2α</sub> at 5 nM in the presence of test ligands at various concentrations. Also non-specific binding was determined in the presence of excess unlabeled PGF<sub>2α</sub> (10<sup>-5</sup> M).

It also contains a hygromycin resistance gene that is used for eukaryotic selection. The cells employed for stable transfection were human embryonic kidney cells (HEK-293) that were transfected with and express the EBNA-1 protein. These HEK-293-EBNA cells (Invitrogen) were grown in medium containing Geneticin (G418) to maintain expression of the EBNA-1 protein. HEK-293 cells were grown in DMEM with 10% fetal bovine serum (FBS), 250 µg ml<sup>-1</sup> G418 (Life Technologies) and 200 µg ml<sup>-1</sup> gentamicin or penicillin/streptomycin. Selection of stable transfectants was achieved with 200 µg ml<sup>-1</sup> hygromycin, the optimal concentration being determined by previous hygromycin kill curve studies.

For transfection, the cells were grown to 50-60% confluency on 10 cm plates. The plasmid pCEP4 incorporating cDNA inserts for the respective human prostanoid receptor (20 µg) was added to 500 µl of 250 mM CaCl<sub>2</sub>. HEPES buffered saline x 2 (2 x HBS, 280 mM NaCl, 20 mM HEPES acid, 1.5 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 7.05 – 7.12) was then added dropwise to a total of 500 µl, with continuous vortexing at room temperature. After 30 min, 9 ml DMEM were added to the mixture. The DNA/DMEM/calcium phosphate mixture was then added to the cells, which had been previously rinsed with 10 ml PBS. The cells were then incubated for 5 hr at 37° C in humidified 95% air/5% CO<sub>2</sub>. The calcium phosphate solution was then removed and the cells were treated with 10% glycerol in DMEM for 2 min. The glycerol solution was then replaced by DMEM with 10% FBS. The

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cells were incubated overnight and the medium was replaced by DMEM/10% FBS containing  $250 \mu\text{g ml}^{-1}$  G418 and penicillin/streptomycin. The following day hygromycin B was added to a final concentration of  $200 \mu\text{g ml}^{-1}$ .

5 Ten days after transfection, hygromycin B resistant clones were individually selected and transferred to a separate well on a 24 well plate. At confluence each clone was transferred to one well of a 6 well plate, and then expanded in a 10 cm dish. Cells were maintained under continuous hygromycin selection until use.

#### 10 HUMAN RECOMBINANT EP<sub>3</sub> AND TP RECEPTORS: TRANSIENT TRANSFECTANTS.

Plasmids encoding the human EP<sub>3</sub> (D isoform) or TP receptor were prepared by cloning the respective coding sequences into a pcDNA<sub>3</sub> vector (Invitrogen). COS-7 cells were transfected with pcDNA<sub>3</sub> containing cDNA encoding the EP<sub>3</sub> or  
15 TP receptor by employing the lipofectin method, according to the manufacturers instructions (Gibco). For radioligand binding studies, cells were harvested two days after transfection.

#### 20 RADIOLIGAND BINDING

Radioligand binding studies on plasma membrane fractions prepared from cells were performed as follows. Cells washed with TME buffer were scraped from the bottom of the plates and homogenized for 30 sec using a Brinkman PT 10/35 polytron. TME buffer was added as necessary to achieve a 40 ml volume in the centrifuge tubes. TME is  
25 comprised of 50 mM TRIS base, 10 mM MgCl<sub>2</sub>, 1mM EDTA; pH 7.4 is achieved by adding 1 N HCl. The cell homogenate was centrifuged at 19,000 rpm for 20-25 min at 4°C using a Beckman Ti-60 or Ti-70 rotor. The pellet was then resuspended in TME buffer to provide a final protein concentration of 1 mg/ml, as determined by Bio-Rad assay. Radioligand binding assays were performed in a 100  $\mu\text{l}$  or 200  $\mu\text{l}$  volume.

30 The binding of [<sup>3</sup>H] PGE<sub>2</sub> (specific activity 165 Ci/mmol) was determined in duplicate and in at least 3 separate experiments. Incubations were for 60 min at 25° C and were terminated by the addition of 4 ml of ice-cold 50 mM TRIS-HCl

followed by rapid filtration through Whatman GF/B filters and three additional 4 ml washes in a cell harvester (Brandel). Competition studies were performed using a final concentration of 2.5 or 5 nM [ $^3\text{H}$ ] PGE<sub>2</sub> and non-specific binding was determined with  $10^{-5}$  M unlabelled PGE<sub>2</sub>.

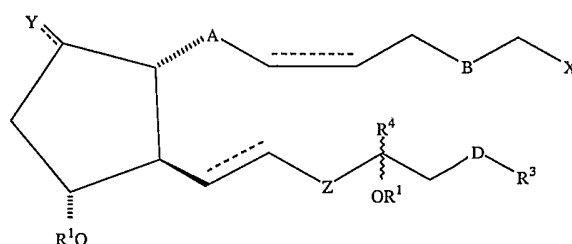
5

The binding of [ $^3\text{H}$ ]-SQ29548 (specific activity 41.5 Ci mmol<sup>-1</sup>) at TP receptors were determined in duplicate in at least three separate experiments. Radiolabeled SQ29548 was purchased from New England Nuclear. Incubations were for 60 min at 25°C and were terminated by the addition of 4 ml of ice-cold 50 mM TRIS-HCl, followed by rapid filtration through Whatman GF/B filters and three additional 4 ml washes in a cell harvester (Brandel). Competition studies were performed using a final concentration of 10 nM [ $^3\text{H}$ ]-SQ 29548 and non-specific binding determined with 10  $\mu\text{M}$  of the unlabeled prostanoid. For all radioligand binding studies, the criteria for inclusion were >50% specific binding and between 15 500 and 1000 displaceable counts or better.

The foregoing description details specific methods and compositions that can be employed to practice the present invention, and represents the best mode contemplated. However, it is apparent for one of ordinary skill in the art that further compounds with the desired pharmacological properties can be prepared in an analogous manner, and that the disclosed compounds can also be obtained from 20 different starting compounds via different chemical reactions. Similarly, different pharmaceutical compositions may be prepared and used with substantially the same result. Thus, however detailed the foregoing may appear in text, it should not be construed as limiting the overall scope hereof; rather, the ambit of the present 25 invention is to be governed only by the lawful construction of the appended claims.

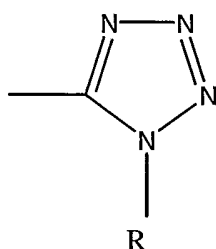
## CLAIMS

1. A method comprising administering a compound and a second drug to an eye of a mammal for the treatment of glaucoma or the reduction of intraocular pressure, said compound represented by the general formula I;



- wherein hatched lines represent the  $\alpha$  configuration, a triangle represents the  $\beta$  configuration, a wavy line represents either the  $\alpha$  configuration or the  $\beta$  configuration and a dotted line represents the presence or absence of a double bond;
- 10 A and B are independently selected from the group consisting of O, S and  $\text{CH}_2$ , provided that at least one of A or B is S;
- D represents a covalent bond or  $\text{CH}_2$ , O, S or  $\text{NH}$ ;
- X is  $\text{CO}_2\text{R}$ ,  $\text{CONR}_2$ ,  $\text{CH}_2\text{OR}$ ,  $\text{P}(\text{O})(\text{OR})_2$ ,  $\text{CONR}\text{SO}_2\text{R}$ ,  $\text{SONR}_2$  or

15



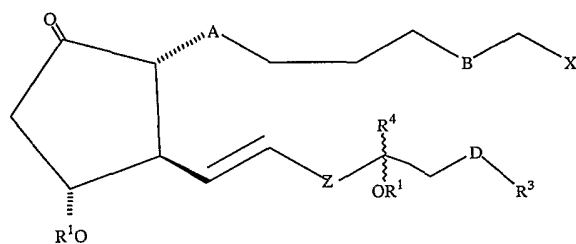
Y is O, OH,  $\text{OCOR}^2$ , halogen or cyano;

Z is  $\text{CH}_2$  or a covalent bond;

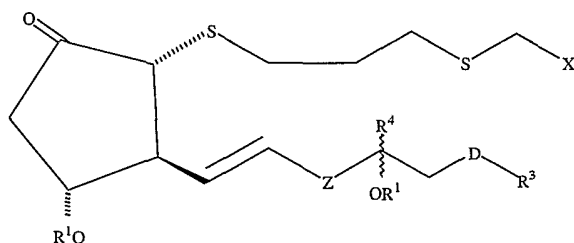
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- R is H or R<sup>2</sup>;  
 R<sup>1</sup> is H, R<sup>2</sup>, phenyl, or COR<sup>2</sup>;  
 R<sup>2</sup> is C<sub>1</sub>-C<sub>5</sub> lower alkyl or alkenyl;  
 R<sub>3</sub> is benzothienyl, benzofuranyl, naphthyl, or substituted derivatives thereof,  
 5 wherein the substituents may be selected from the group consisting of C<sub>1</sub>-C<sub>5</sub> alkyl, halogen, CF<sub>3</sub>, CN, NO<sub>2</sub>, NR<sub>2</sub>, CO<sub>2</sub>R and OR and R<sup>4</sup> is hydrogen or C<sub>1</sub>-C<sub>5</sub> lower alkyl.

2. The method according to claim 1 wherein said compound is represented by  
 10 the general formula II;



3. The method according to claim 2 wherein said compound is represented by  
 the general formula III;



15

4. The method of claim 1 wherein Z represents a covalent bond.  
 5. The method of claim 1 wherein D represents a covalent bond or is CH<sub>2</sub>.

6. The method of claim 1 wherein X is CO<sub>2</sub> R.
7. The method of claim 6 wherein R is selected from the group consisting of H, methyl, i-propyl, and n-propenyl.
8. The method of claim 1 wherein R is H, or n-propenyl.
- 5 9. The method of claim 1 wherein R<sub>1</sub> is H.
10. The method of claim 1 wherein D is CH<sub>2</sub>.
11. The method of claim 10 wherein R<sup>3</sup> is benzo[b]thienyl, 3-chlorobenzo[b]thienyl or naphthyl.
12. The method of claim 1 wherein said compound is selected from the group
- 10 consisting of

{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-5-(naphthyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester,

- 15 {3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-5-(naphthyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid,

{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-5-(naphthyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid isopropyl ester,

20

{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-hydroxy-5-(benzothienyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester,

- {3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-hydroxy-5-(benzothienyl)pent-1-enyl)-
- 25 5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid,

{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-hydroxy-5-(benzothienyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid isopropyl ester,

{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-hydroxy-5-(benzofuranyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester,

- 5 {3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-hydroxy-5-(benzofuranyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid,

{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-hydroxy-5-(benzofuranyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid isopropyl ester,

10

{3-[(1R,2S,3R)-3-Hydroxy-2-((E)-3-hydroxy-4-naphthalen-2-yl-but-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester,

- 15 {3-[(1R,2S,3R)-3-Hydroxy-2-((E)-3-hydroxy-4-naphthalen-2-yl-but-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid,

{3-[(1R,2S,3R)-2-((E)-4-Benzo[b]thiophen-3-yl-3-hydroxybut-1-enyl)-3-hydroxy-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester,

- 20 {3-[(1R,2S,3R)-2-((E)-4-Benzo[b]thiophen-3-yl-3-hydroxybut-1-enyl)-3-hydroxy-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid,

{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-3-(methyl)-5-(naphthyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester,

25

{3-[(1R,2S,3R)-3-Hydroxy-2-((E)-3-hydroxy-3-methyl-4-naphthalen-2-yl-but-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester,

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{3-[(1R,2S,3R)-3-Hydroxy-2-((E)-3-hydroxy-3-methyl-4-naphthalen-2-yl-but-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid,

5 {3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-3-(methyl)-5-(naphthyl)but-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid,

{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-3-(methyl)-5-(benzothienyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester and

10

{3-[(1R,2S,3R)-3-Hydroxy-2-((S)-(E)-3-(hydroxy)-3-(methyl)-5-(benzothienyl)pent-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester.

15 13. The method of claim 1 wherein D is CH<sub>2</sub> and Z represents a covalent bond.

14. The method of claim 1 wherein R<sup>4</sup> is hydrogen or methyl.

15. The method of claim 1 wherein R<sup>4</sup> is hydrogen.

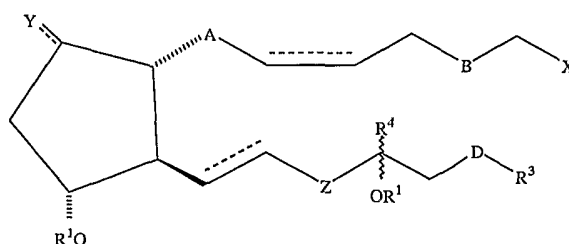
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16. The method of claim 1 wherein said second drug is selected from the group consisting of  $\beta$ -blockers, adrenergic agonists,  $\alpha_2$ -selective adrenergic agonists, carbonic anhydrase inhibitors, cholinergic agonists, glutamate antagonists, prostamides, prostaglandins, cannabinoids, and combinations thereof.

25

17. A composition comprising a compound and a second drug, said compound having a structure according to Formula I

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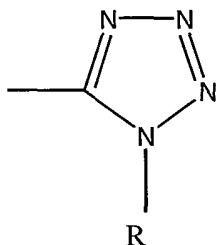
wherein hatched lines represent the  $\alpha$  configuration, a triangle represents the  $\beta$  configuration, a wavy line represents either the  $\alpha$  configuration or the  $\beta$

- 5 configuration and a dotted line represents the presence or absence of a double bond;  
A and B are independently selected from the group consisting of O, S and  $\text{CH}_2$ ,  
provided that at least one of A or B is S;

D represents a covalent bond or  $\text{CH}_2$ , O, S or NH;

X is  $\text{CO}_2\text{R}$ ,  $\text{CONR}_2$ ,  $\text{CH}_2\text{OR}$ ,  $\text{P}(\text{O})(\text{OR})_2$ ,  $\text{CONRSO}_2\text{R}$ ,  $\text{SONR}_2$  or

10



Y is O, OH,  $\text{OCOR}^2$ , halogen or cyano;

Z is  $\text{CH}_2$  or a covalent bond;

- 15 R is H or  $\text{R}^2$ ;

$\text{R}^1$  is H,  $\text{R}^2$ , phenyl, or  $\text{COR}^2$ ;

$\text{R}^2$  is  $\text{C}_1$ - $\text{C}_5$  lower alkyl or alkenyl;

$\text{R}_3$  is benzothienyl, benzofuranyl, naphthyl, or substituted derivatives thereof,

wherein the substituents maybe selected from the group consisting of  $\text{C}_1$ - $\text{C}_5$  alkyl,

halogen,  $\text{CF}_3$ , CN,  $\text{NO}_2$ ,  $\text{NR}_2$ ,  $\text{CO}_2\text{R}$  and OR and  $\text{R}^4$  is hydrogen or  $\text{C}_1$ - $\text{C}_5$  lower alkyl.

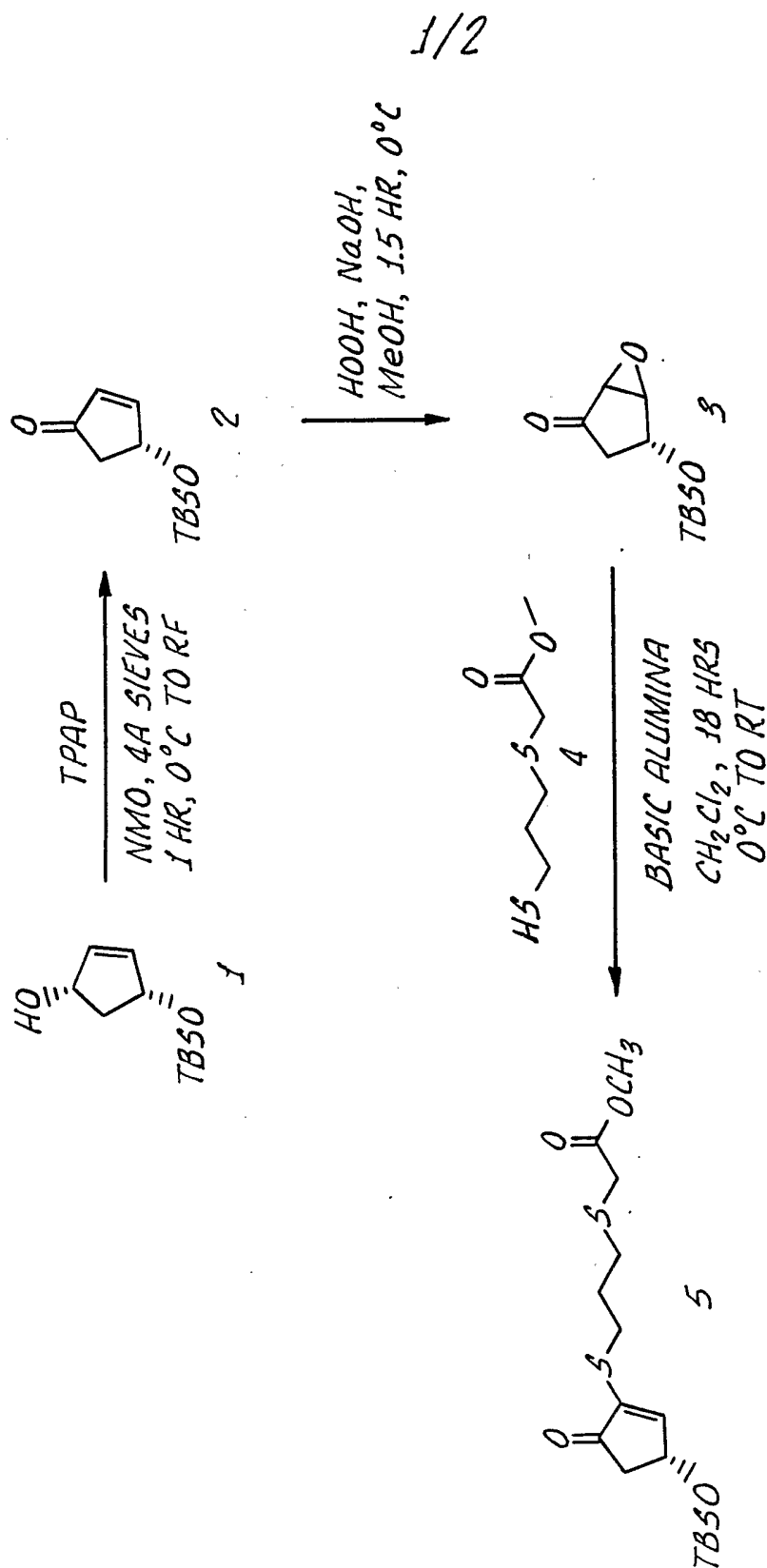


**Fig. 1.**  
(SCHEME 1)

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