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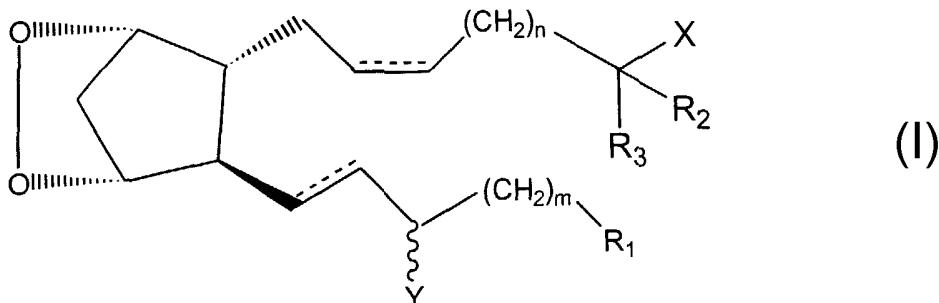
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(54) Title: THE 9, 11-CYCLOENDOPEROXIDE PRO-DRUGS OF PROSTAGLANDIN ANALOGUES FOR TREATMENT OF OCULAR HYPERTENSION AND GLAUCOMA



(I)

(57) Abstract: 9,11-Cycloendoperoxide derivatives of biologically active prostaglandin analogs, and particularly of the ocular hypotensive drugs Bimatoprost, Latanaprost, Unoprostone, Travoprost and prostaglandin H<sub>2</sub> 1-ethanolamide or of structurally closely related analogs, are pro-drugs which hydrolyze under physiological conditions to provide prostaglandin analogues that are capable of providing sustained ocular and other in vivo concentrations of the respective drugs. The compounds of the invention have the formula shown below where the variables have the meaning defined in the specification.

1 THE 9,11-CYCLOENDOPEROXIDE PRO-DRUGS OF PROSTAGLANDIN  
2 ANALOGUES FOR TREATMENT OF OCULAR HYPERTENSION AND  
3 GLAUCOMA

4

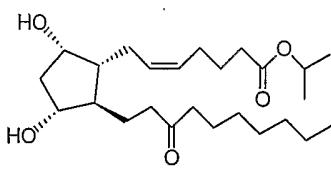
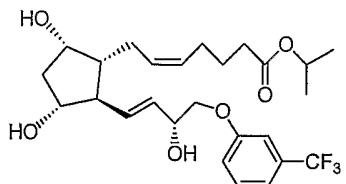
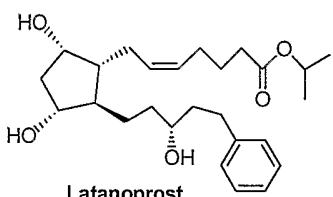
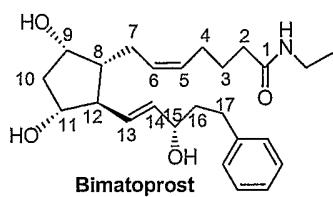
5 BACKGROUND OF THE INVENTION

6 Field of the Invention

7 The present invention is in the field of prostaglandin and analog  
8 drugs. More particularly the present invention is in the field of  
9 prostaglandin analog drugs which are used for treatment of ocular  
10 hypertension, glaucoma or have other useful pharmacological properties.  
11 Still more particularly, the present invention is directed to pro-drugs of  
12 prostaglandin analogs which are used for treatment of ocular hypertension,  
13 glaucoma, have beneficial effects on platelet congregation, gastric  
14 ulceration, blood pressure regulation and inflammation.

15 Background Art

16 Several prostaglandin analogs are presently known for their ability to  
17 reduce intraocular pressure and can be used for treatment of glaucoma and  
18 related diseases of the eye. Among these the drugs known by the names  
19 Bimatoprost (U. S. Patent No. 5,688,819) Latanoprost (U. S. Patent Nos.  
20 4,599,353, 5,296,504, 5,422,368), Unoprostone (U. S. Patent No.  
21 6,329,426) and Travoprost (U. S. Patent Nos. 5,631,287, 5,849,792,  
22 5,889,052, 6,011,062) are mentioned as important ones in current use, and  
23 are shown by chemical structure below. The conventional numbering of  
24 prostaglandins and like structures is indicated in connection with the  
25 formula of Bimatoprost.



1

Travoprost

2

3

4

## 5 SUMMARY OF THE INVENTION

6 In accordance with the present invention 9,11 cycloendoperoxide  
 7 derivatives of biologically active prostaglandin analogs comprise pro-drugs  
 8 which hydrolyze under physiological conditions to provide prostaglandin  
 9 analogues that are capable of providing sustained ocular and other *in vivo*  
 10 concentrations of biologically active prostaglandin analogues. See  
 11 *Fredholm et al.*, Prostaglandins 1976, 11, 507-518 and *Stringfellow et al.*,  
 12 Prostaglandins 1978, 16, 901-910).

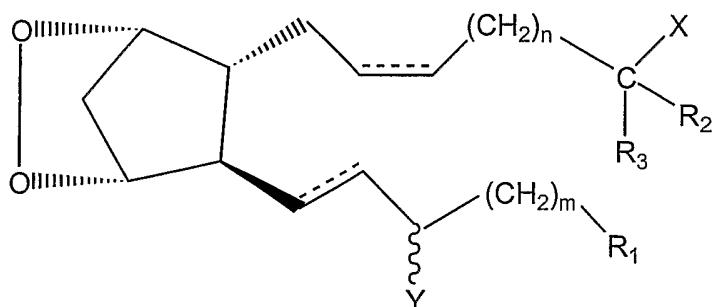
13 The 9,11-cycloendoperoxide analogs of biologically active  
 14 prostaglandins are, generally speaking, chemically stable and are converted  
 15 to the active drugs Bimatoprost, Latanoprost, Unoprostone, Travoprost, and  
 16  $H_2$  1-ethanolamide or to structurally closely related analogs, as well as into  
 17 other biologically active prostaglandins, such as prostaglandins D<sub>2</sub>, E<sub>2</sub>, and  
 18 F<sub>2alpha</sub>, thromboxane and prostacyclin analogs, with ocular hypotensive and  
 19 other biological activity. The thromboxane and prostacyclin analogues  
 20 effect platelet aggregation and are expected to play a crucial role in  
 21 preventing gastric ulceration by inhibiting gastric acid secretion, in blood

- 1 pressure regulation by control of vascular tone, and in inflammation by
- 2 inhibiting protease secretion of polymorphonuclear leucocytes.

3 In addition to being useful as pro-drugs which hydrolyze under  
4 physiologic condition to the corresponding drugs, the 9,11-  
5 cycloendoperoxides of the invention may *per se* have the biological activity  
6 of the corresponding drug into which they hydrolyze, and as such are  
7 expected to provide still better sustained physiological concentration of the  
8 therapeutic agent which they represent.

9        The 9,11-cycloendoperoxide pro-drugs of the present invention, in  
10      addition to undergoing hydrolysis to provide the corresponding  
11      biologically active prostaglandin analogs, also act as substrates to several  
12      naturally occurring enzymes which convert the 9,11-cycloendoperoxide  
13      pro-drugs into other biologically active analogs wherein the molecular  
14      structure is modified. These enzymatic reactions which occur *in vivo* can  
15      also be performed *in vitro* by utilizing the corresponding enzymes isolated  
16      from human or other mammalian organisms, and are illustrated below in  
17      **Reaction Schemes 10 through 20.**

18 The compounds of the invention are generally disclosed by **Formula**  
19 **1**,



Formula 1

22 wherein the dashed lines represent the presence of a bond, or absence of a  
23 bond, wavy lines represent either alpha or beta configuration, solid triangles

1 represent beta configuration and hatched lines represent alpha  
2 configuration;  
3 **n** is an integer having the values of 1 to 6;  
4 **m** is an integer having the values of 1 to 8;  
5 **X** is NH<sub>2</sub>, N(R)<sub>2</sub>, NHR, or OR where R is hydrogen, R<sub>4</sub> or a (CO)R<sub>4</sub> group;  
6 **Y** is =O, =S or OH, OR<sub>5</sub> or --O(CO)R<sub>5</sub> groups, said OH, OR<sub>5</sub> or O(CO)R<sub>5</sub>  
7 groups being attached to the adjacent carbon in alpha or beta configuration;  
8 **R**<sub>1</sub> is H, CH<sub>3</sub>, **R**<sub>7</sub>, OR<sub>7</sub> or SR<sub>7</sub> where **R**<sub>7</sub> is an aliphatic, aromatic or  
9 heteroaromatic ring, said heteroaromatic ring having 1 to 3 heteroatoms  
10 selected from O, S, and N, said aliphatic, aromatic or heteroaromatic ring  
11 being optionally substituted with 1 to 3 **R**<sub>8</sub> groups where **R**<sub>8</sub> is F, Cl, Br, I,  
12 NO<sub>2</sub>, C<sub>1-6</sub> alkyl, C<sub>1-6</sub> fluoro substituted alkyl, COOH, or COOR<sub>9</sub> where R<sub>9</sub>  
13 is alkyl of 1 to 6 carbons or CH<sub>2</sub>OCH<sub>3</sub>;  
14 R<sub>2</sub> and R<sub>3</sub> together represent =O, =S, or independently are hydrogen or  
15 alkyl of 1 to 6 carbon atoms;  
16 R<sub>4</sub> represents (CH<sub>2</sub>)<sub>r</sub>OH, (CH<sub>2</sub>)<sub>r</sub>OCOR<sub>9</sub> or (CH<sub>2</sub>)<sub>r</sub>OR<sub>9</sub> where **r** is an integer  
17 having the values 1 to 6, or R<sub>4</sub> represents saturated or unsaturated acyclic  
18 hydrocarbons having from 1 to 20 carbon atoms, or --(CH<sub>2</sub>)<sub>q</sub>R<sub>6</sub> where q is  
19 0-10 and R<sub>6</sub> is an aliphatic, aromatic or heteroaromatic ring, said  
20 heteroaromatic ring having 1 to 3 heteroatoms selected from O, S, and N,  
21 said aliphatic, aromatic or heteroaromatic ring being optionally substituted  
22 with 1 to **R**<sub>8</sub> groups where **R**<sub>8</sub> is F, Cl, Br, I, NO<sub>2</sub>, C<sub>1-6</sub> alkyl, C<sub>1-6</sub> fluoro  
23 substituted alkyl, COOH, COOR<sub>9</sub> where R<sub>9</sub> is alkyl of 1 to 6 carbons or  
24 CH<sub>2</sub>OCH<sub>3</sub>;  
25 R<sub>5</sub> represents saturated or unsaturated acyclic hydrocarbons having from 1  
26 to 20 carbon atoms, or --(CH<sub>2</sub>)<sub>q</sub>R<sub>6</sub>, or a pharmaceutically acceptable salt of  
27 said compound.  
28  
29

## 1                   DETAILED DESCRIPTION OF THE INVENTION

## 2                   Definitions:

3                   As used herein the term alkyl refers to and covers any and all groups  
4                   which are known as normal alkyl, branched-chain alkyl, cycloalkyl and also  
5                   cycloalkyl-alkyl. The term alkenyl refers to and covers normal alkenyl,  
6                   branch-chained alkenyl and cycloalkenyl groups having one or more sites  
7                   of unsaturation. When referring to saturated or unsaturated acyclic  
8                   hydrocarbons, the term covers normal alkyl, normal alkenyl and normal  
9                   alkynyl groups as well as branch-chained alkyl, branch-chained alkenyl and  
10                  branch-chained alkynyl groups, the normal and branch-chained alkenyl and  
11                  alkynyl groups having one or more sites of unsaturation.

12                  A pharmaceutically acceptable salt may be prepared for any  
13                  compound in this invention having a functionality capable of forming a salt,  
14                  for example an acid or amine functionality. A pharmaceutically acceptable  
15                  salt is any salt which retains the activity of the parent compound and does  
16                  not impart any deleterious or untoward effect on the subject to which it is  
17                  administered and in the context in which it is administered.

18                  Pharmaceutically acceptable salts may be derived from organic or  
19                  inorganic bases. The salt may be a mono or polyvalent ion. Of particular  
20                  interest are the inorganic ions, sodium, potassium, calcium, and  
21                  magnesium. Organic salts may be made with amines, particularly  
22                  ammonium salts such as mono-, di- and trialkyl amines or ethanol amines.  
23                  Salts may also be formed with caffeine, tromethamine and similar  
24                  molecules. Where there is a nitrogen sufficiently basic as to be capable of  
25                  forming acid addition salts, such may be formed with any inorganic or  
26                  organic acids or alkylating agent such as methyl iodide. Preferred salts are  
27                  those formed with inorganic acids such as hydrochloric acid, sulfuric acid  
28                  or phosphoric acid. Any of a number of simple organic acids such as  
29                  mono-, di- or tri- acid may also be used.

1        The compounds of the present invention are capable of existing as  
2    *trans* and *cis* (**E** and **Z**) isomers relative to the five-membered ring shown  
3    in the respective formulas, and relative to olefinic double bonds. Unless  
4    specific orientation of substituents relative to a double bond or the ring is  
5    indicated in the name of the respective compound, and/or by specific  
6    showing in the structural formula of the orientation of the substituents  
7    relative to the double bond or ring, the invention covers *trans* as well as *cis*  
8    isomers relative to each center that gives rise to such isomerism, as well as  
9    mixtures of *trans* and *cis* isomers.

10       The compounds of the present invention also contain one or more  
11    chiral centers and therefore may exist in enantiomeric and diastereomeric  
12    forms. Again, unless the name of a compound or its formula specifically  
13    describes or shows a specific enantiomer or diastereomer, the scope of the  
14    present invention is intended to cover all isomers *per se*, as well as mixtures  
15    of *cis* and *trans* isomers, mixtures of diastereomers and racemic mixtures  
16    of enantiomers (optical isomers) as well.

17       In the presently preferred compounds of the invention the variable **n**  
18    is 3, and the variable **m** is in the range or 1 to 6. The dotted line between  
19    carbons 5 and 6 as the numbering is shown on the structure depicting  
20    Bimatoprost, preferably represents a bond.

21       The variable **Y** preferably represents =O or OH, or O(CO)R<sub>5</sub>, where  
22    R<sub>5</sub> is preferably alkyl of 1 to 6 carbons. Even more preferably **Y** is OH  
23    attached to the adjacent carbon with a bond of alpha orientation.

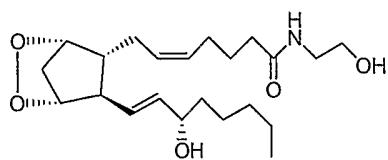
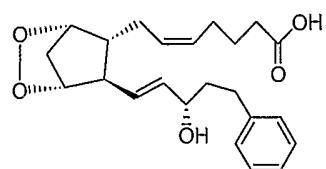
24       In the presently preferred compounds of the invention **R**<sub>1</sub> is methyl,  
25    phenyl, phenyl substituted in the phenyl group in the manner described in  
26    connection with **Formula 1**, or **R**<sub>1</sub> is O-phenyl, or O-phenyl substituted in  
27    the phenyl group in the manner described in connection with **Formula 1**.  
28    When **R**<sub>1</sub> is O-phenyl substituted in the phenyl group then the presently  
29    most preferred substituent is trifluoromethyl.

1       With respect to the group shown as C(X)(R<sub>2</sub>)( R<sub>3</sub>) in **Formula 1** the  
2 R<sub>2</sub> and R<sub>3</sub> groups preferably jointly form an oxo (=O) function, and the  
3 variable X is preferably OH, OR<sub>4</sub> or NHR<sub>4</sub>. R<sub>4</sub> is preferably alkyl of 1 to 6  
4 carbons, or (CH<sub>2</sub>)<sub>r</sub>OH where most preferably r is an integer having the  
5 value 2.

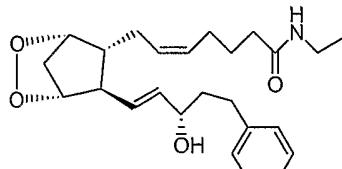
6       The presently most preferred compounds of the invention are the  
7 9,11-cycloendoperoxide pro-drugs structurally closely related to  
8 Bimatoprost, Latanapost, Unoprostone, Travoprost, Bimatoprost acid and  
9 of Prostaglandin H<sub>2</sub> 1-ethanolamide, the chemical structures of which are  
10 provided below. Although these structures show specific examples, they  
11 nevertheless generally show the 9,11-cycloendoperoxide moiety which can  
12 be introduced into the biologically active prostaglandin analogs by the  
13 enzymatic synthetic methods described below in detail. Numbers in  
14 parentheses next to the 9,11-cycloendoperoxides illustrated below refer to  
15 the specific compound numbers which are utilized in the specific  
16 description of examples and corresponding reaction schemes.

17       The enzymatic methods utilize the enzyme human COX-2 which  
18 can be obtained commercially from Cayman Chemical (Ann Arbor, MI). It  
19 was cloned in as described by *Hla et al.* in Proc. Natl. Acad. Sci. USA  
20 1992, 89: 7384-7388, incorporated herein by reference. The enzyme was  
21 prepared by expression of a DNA clone encoding this enzyme in  
22 Baculovirus overexpression system in insect host cells (Sf21 cells).

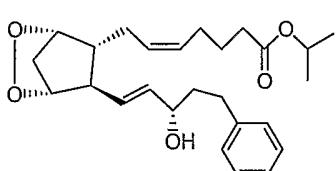
23       The compounds on which the enzymatic syntheses utilizing the  
24 enzyme human COX-2 are performed can be prepared by chemical  
25 reactions and/or a combination of chemical and enzymatic reactions which  
26 are illustrated below, and by such modifications and adaptation of the  
27 chemical and/or enzymatic reactions which are within the skill of the  
28 practicing synthetic chemist in light of the present disclosure combined  
29 with general knowledge and available scientific and patent literature.

Prostaglandin H<sub>2</sub> 1-ethanolamide (17)

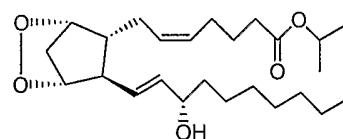
Bimatoprost acid 9,11-cycloendoperoxide (18)



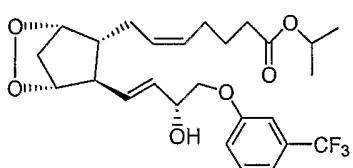
Bimatoprost 9,11-cycloendoperoxide (19)



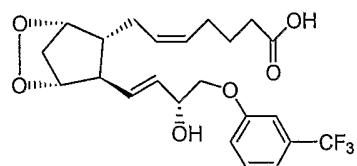
Latanoprost 13,14-dehydro-9,11-cycloendoperoxide (20)



Unoprostone 13,14-dehydro-15-hydroxy-9,11-cycloendoperoxide (21)



1 Travoprost 9,11-cycloendoperoxide (22)



Travoprost acid 9,11-cycloendoperoxide (23)

2

## 1           BIOLOGICAL ACTIVITY, MODES OF ADMINISTRATION

2           The compounds of the invention are primarily active as pro-drugs of  
3           biologically active prostaglandins or prostaglandin analogs. Because the  
4           compounds act primarily as pro-drugs their ultimate biological effect is  
5           substantially the same as that of the corresponding drug. However, because  
6           the compounds of the invention act as pro-drugs, they tend to release the  
7           corresponding drug over a period of time, and therefore are expected to act  
8           as a sustained release drug, capable of maintaining a therapeutically  
9           effective concentration of the corresponding drug for a longer period of  
10           time than the corresponding drug. Still speaking generally, pro-drugs of the  
11           present invention are likely to be administered in the same manner as the  
12           corresponding drug, and in doses comparable to the administration of the  
13           corresponding drug. For specific description of modes of administration  
14           and dosages of the known prostaglandin drugs for which the 9,11-  
15           cycloendoperoxide compounds of the invention serve as pro-drugs,  
16           reference is made to the state of the art and to United States Patent Nos.  
17           5,688,819; 5,296,504; 4,599,353; 5,422,368; 6,329,426, 5,631,287,  
18           5,849,792, 5,889,052 and 6,011,062 the specification of all which is  
19           incorporated herein by reference.

20           The pro-drugs of the present invention may also be administered in  
21           combination with the corresponding drug.

22           An important application of several pro-drugs in accordance with the  
23           present invention is treatment of ocular hypertension or glaucoma. For this  
24           purpose, similarly to the corresponding drug, such as Bimatoprost,  
25           Latanaprost, Unoprostone, Travoprost and prostaglandin H<sub>2</sub> 1-  
26           ethanolamide, the pro-drug is preferably administered as a topical  
27           ophthalmic solution.

28           Still speaking generally, pharmaceutical compositions may be  
29           prepared by combining a therapeutically effective amount of at least one

1 compound according to the present invention, or a pharmaceutically  
2 acceptable salt thereof, as an active ingredient, with conventional  
3 pharmaceutical excipients, and in some cases by preparation of unit dosage  
4 forms suitable for specific use, such as topical ocular use. The  
5 therapeutically efficient amount typically is between about 0.0001 and  
6 about 5% (w/v), preferably about 0.001 to about 1.0% (w/v) in liquid  
7 formulations.

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

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

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

27 For ophthalmic use various buffers and means for adjusting pH may  
28 be used so long as the resulting preparation is ophthalmically acceptable.  
29 Accordingly, buffers include acetate buffers, citrate buffers, phosphate

1 buffers and borate buffers. Acids or bases may be used to adjust the pH of  
2 these formulations as needed.

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

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

11 The ingredients are usually used in the following amounts:

12	Ingredient	Amount (% w/v)
13	active ingredient	about 0.001-5
14	preservative	0-0.10
15	vehicle	0-40
16	tonicity adjustor	1-10
17	buffer	0.01-10
18	pH adjustor	q.s. pH 4.5-7.5
19	antioxidant	as needed
20	surfactant	as needed
21	purified water	as needed to make 100%
22	The actual dose of the active compounds of the present invention depends	
23	on the specific compound, and on the condition to be treated; the selection	
24	of the appropriate dose is well within the knowledge of the skilled artisan.	

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

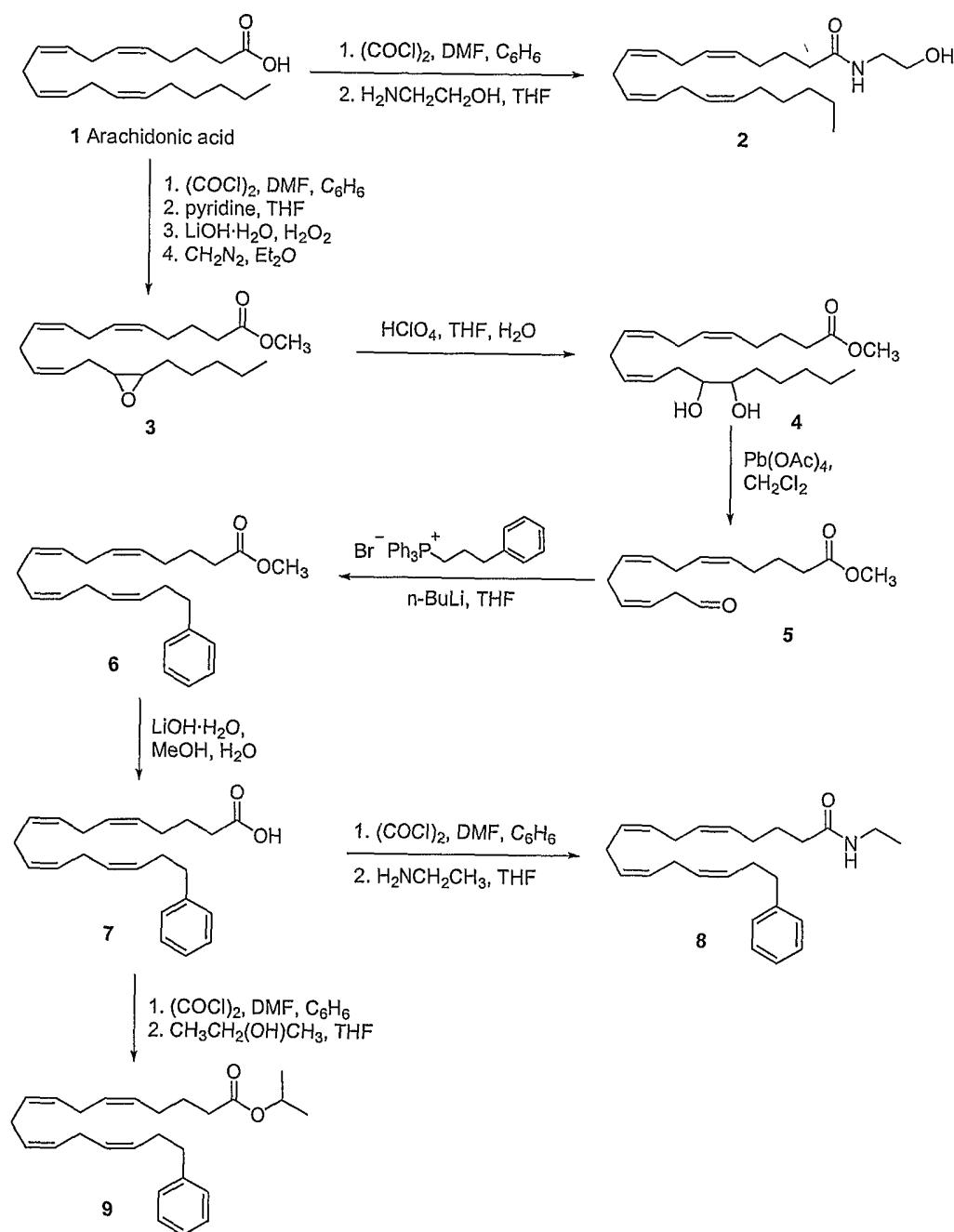
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34

### 3 General Procedure A

#### 4 Chemical synthesis of arachidonyl ethanolamide (**Compound 2**)

5 The chemical synthesis of arachidonyl ethanolamide from  
6 arachidonic acid (**Compound 1**) is illustrated in **Reaction Scheme 1**.  
7 Arachidonyl ethanolamide (**Compound 2**) is synthesized following a  
8 literature procedure of *Abadji et al.*, J. Med. Chem. 1994, 37, 1889-1893,  
9 incorporated herein by reference. To a 0.1 M solution of arachidonic acid  
10 (**Compound 1**, available from Cayman Chemical) in anhydrous benzene at  
11 0 °C is added one equivalent of anhydrous dimethyl formamide and two  
12 equivalents of oxalyl chloride. The reaction is stirred at room temperature  
13 for 1 h and an equal volume of anhydrous tetrahydrofuran (THF) is added.  
14 The mixture is then cooled to 0 °C and a 1 M solution of 10 equivalents 2-  
15 amino-ethanol in anhydrous THF is added. The reaction is stirred at room  
16 temperature until completion. The reaction mixture is then diluted with  
17 chloroform, washed successively with 1 M HCl, 1 M NaOH, brine, dried  
18 over Na<sub>2</sub>SO<sub>4</sub>, and concentrated *in vacuo*. The crude product is purified by  
19 chromatography on silica gel. Arachidonyl ethanolamide (**Compound 2**) is  
20 enzymatically converted into the corresponding 9,11-cycloendoperoxide  
21 derivative as shown in **Reaction Scheme 3** and is described below.



1

## REACTION SCHEME 1

1    Chemical synthesis of Compound 3

2           The chemical synthesis of **Compound 3** from arachidonic acid  
3    (**Compound 1**) is also illustrated in **Reaction Scheme 1**. **Compound 3** is  
4    synthesized by modification of procedures reported by *Ryan et al.* *J. Med.*  
5    *Chem.* 1997, 40, 3617-3625, based on previous work by *Corey et al.*  
6    *Tetrahedron Lett.* 1983, 24, 37-40 and *Manna et al.* *Tetrahedron Lett.* 1983,  
7    24, 33-36. The *Ryan et al.*, *Corey et al.*, and *Manna et al.*  
8    publications are hereby expressly incorporated by reference. To a 0.4 M  
9    solution of arachidonic acid (**Compound 1**) in anhydrous benzene at 0 °C  
10   are added two equivalents of oxalyl chloride. The mixture is stirred for  
11   overnight while allowed to warm to room temperature. The solvent and  
12   excess oxalyl chloride is removed *in vacuo*. The resulting crude acid  
13   chloride is dissolved in anhydrous THF to make a ~ 2 M solution. Half an  
14   equivalent of pyridine is added to the above solution and the mixture is  
15   stirred for 10 min at 0 °C. 0.7 M solution of LiOH•H<sub>2</sub>O in 50% H<sub>2</sub>O<sub>2</sub>  
16   containing one equivalent of LiOH•H<sub>2</sub>O is added and the mixture is stirred  
17   for 20 min. The reaction is quenched with pH 7 buffer and brine and  
18   extracted with CH<sub>2</sub>Cl<sub>2</sub> (×3). The combined organic layer is washed with  
19   brine and dried over Na<sub>2</sub>SO<sub>4</sub>. During this time the epoxy acid is formed  
20   and its formation can be monitored by TLC analysis. Upon completion, the  
21   drying agent is removed by filtration and the solvent is removed *in vacuo*.  
22   The residue is dissolved in anhydrous Et<sub>2</sub>O and treated with excess  
23   diazomethane. After stirring for 15 min, excess diazomethane is  
24   evaporated in a fume hood at room temperature and the solvent is removed  
25   *in vacuo*. The crude product is purified by chromatography on silica gel.  
26

27   Chemical synthesis of Compound 4

28           The chemical synthesis of **Compound 4** from **Compound 3** is also  
29   illustrated in **Reaction Scheme 1**. **Compound 4** is synthesized following

1 procedures reported by *Ryan et al.* *J. Med. Chem.* 1997, 40, 3617-3625. A  
2 0.05 M solution of **Compound 3** in THF-H<sub>2</sub>O (2:1) is treated with five  
3 equivalents of 1.2 M HClO<sub>4</sub> at room temperature for overnight. The  
4 reaction mixture is then quenched with pH 7 buffer and extracted with  
5 EtOAc (×3). The combined organic layer is washed with brine, dried over  
6 Na<sub>2</sub>SO<sub>4</sub>, and concentrated *in vacuo*. The residue is purified by  
7 chromatography on silica gel.

8 Chemical synthesis of Compound 5

9 The chemical synthesis of **Compound 5** from **Compound 4** is also  
10 illustrated in **Reaction Scheme 1**. **Compound 5** is synthesized following  
11 procedures reported by *Ryan et al.* *J. Med. Chem.* 1997, 40, 3617-3625. A  
12 0.2 M solution of **Compound 4** in CH<sub>2</sub>Cl<sub>2</sub> at -20 °C is treated with one  
13 equivalent of lead (IV) tetraacetate (0.2 M solution in CH<sub>2</sub>Cl<sub>2</sub>) for 0.5 h.  
14 The reaction mixture is filtered through a pad of celite-silica gel and  
15 washed with hexane. The solvent is removed *in vacuo* to afford **Compound**  
16 **5** which is unstable and is used immediately in the next reaction.

17

18 General Procedure B

19 Chemical synthesis of Compound 6

20 The chemical synthesis of **Compound 6** from **Compound 5** is also  
21 illustrated in **Reaction Scheme 1**. **Compound 6** is synthesized by Wittig  
22 olefination of **Compound 5** with the ylide triphenyl-(3-phenyl-  
23 propylidene)- δ<sup>5</sup>-phosphane. The ylide is generated by adding one  
24 equivalent of *n*-butyllithium to a 0.3M solution of triphenyl-(3-phenyl-  
25 propyl)phosphonium bromide (available from Lancaster) in THF at -78 °C.  
26 After stirring for 30 min, 0.7 equivalent of **Compound 5** in THF is added  
27 and the reaction is warmed to room temperature and stirred for 1 h. After  
28 completion, the reaction is diluted with hexane, washed successively with

1 pH 7 buffer, brine, dried over  $\text{Na}_2\text{SO}_4$ , and concentrated *in vacuo*. The  
2 crude product is purified by chromatography on silica gel.

3

4 General Procedure C

5 Chemical synthesis of Compound 7

6 The chemical synthesis of **Compound 7** from **Compound 6** is also  
7 illustrated in **Reaction Scheme 1**. A mixture of **Compound 6** and 7  
8 equivalents of lithium hydroxide monohydrate in methanol-water (3:1) is  
9 heated to 50 °C until the reaction is complete by TLC analysis. The  
10 reaction mixture is cooled to room temperature, quenched with aqueous  
11  $\text{NH}_4\text{Cl}$  and extracted with ethyl acetate ( $\times 3$ ). The combined organic layer  
12 is washed with  $\text{H}_2\text{O}$ , brine, dried over  $\text{Na}_2\text{SO}_4$ , and concentrated *in vacuo*.  
13 The crude product is purified by chromatography on silica gel.

14 **Compound 7** is enzymatically converted into the corresponding 9,11-  
15 cycloendoperoxide derivative as shown in **Reaction Scheme 4** and is  
16 described below.

17 Chemical synthesis of Compound 8

18 **Compound 8** is synthesized following General Procedure A, using  
19 ethyl amine instead of 2-amino-ethanol, as illustrated in **Reaction Scheme**  
20 **1**. **Compound 8** is enzymatically converted into the corresponding 9,11-  
21 cycloendoperoxide derivative as shown in **Reaction Scheme 5** and is  
22 described below.

23 Chemical synthesis of Compound 9

24 **Compound 9** is synthesized following General Procedure A, using  
25 isopropyl alcohol instead of 2-amino-ethanol, as illustrated in **Reaction**  
26 **Scheme 1**. **Compound 9** is enzymatically converted into the  
27 corresponding 9,11-cycloendoperoxide derivative as shown in **Reaction**  
28 **Scheme 6** and is described below.

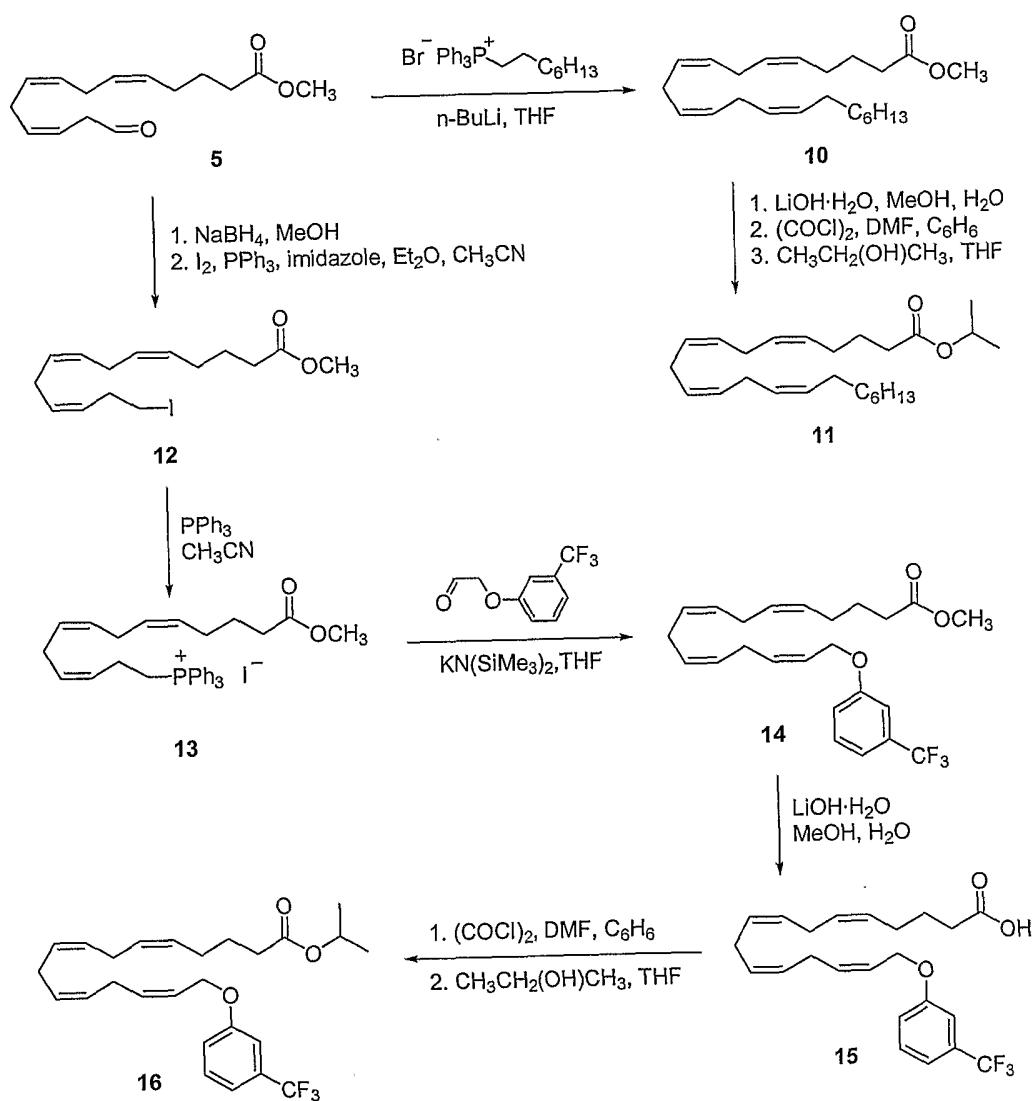
29 Chemical synthesis of Compound 10

1       **Compound 10** is synthesized by Wittig olefination following  
2 General Procedure B, using **Compound 5** and (*n*-  
3 octyl)triphenylphosphonium bromide (available from Lancaster) instead of  
4 triphenyl-(3-phenyl-propyl)phosphonium bromide, as illustrated in  
5 **Reaction Scheme 2**.

6 Chemical synthesis of Compound 11

7       **Compound 11** is synthesized in a three step sequence following  
8 General Procedure C and General Procedure A, using isopropyl alcohol  
9 instead of 2-amino-ethanol as illustrated in **Reaction Scheme 2**.  
10 **Compound 11** is enzymatically converted into the corresponding 9,11-  
11 cycloendoperoxide derivative as shown in **Reaction Scheme 7** and is  
12 described below.

13



## REACTION SCHEME 2

1    Chemical synthesis of Compound 12

2            **Compound 12** is synthesized from **Compound 5** following  
3    procedures reported by Seltzman (*Seltzman et al. J. Med. Chem.* 1997, 40,  
4    3626-3634) and Razdan (*Dasse et al. Tetrahedron* 2000, 56, 9195-9202), as  
5    illustrated in **Reaction Scheme 2**. To a 0.25 M solution of **Compound 5** in  
6    methanol at 0 °C is added 2 equivalents of NaBH<sub>4</sub>. The reaction is warmed  
7    to room temperature and is monitored by TLC analysis. After completion,  
8    the reaction is quenched with aqueous NH<sub>4</sub>Cl and is extracted with EtOAc  
9    (×3). The combined organic layer is washed with H<sub>2</sub>O, brine, dried over  
10   Na<sub>2</sub>SO<sub>4</sub>, and concentrated *in vacuo*. The crude product alcohol is purified  
11   by chromatography on silica gel. This intermediate alcohol is then  
12   converted to **Compound 12** following Razdan's procedures (*Dasse et al.*  
13   *Tetrahedron* 2000, 56, 9195-9202.) 1.1 equivalent of I<sub>2</sub> is added portion-  
14   wise to a solution of 1.1 equivalent of triphenylphosphine and 1.1  
15   equivalent of imidazole in Et<sub>2</sub>O-CH<sub>3</sub>CN (3:1) at 0 °C. The mixture is  
16   stirred at room temperature for 20 min, cooled to 0 °C. To this mixture is  
17   added the intermediate alcohol and the reaction is stirred at room  
18   temperature for 1 h. The reaction is then diluted with pentane-Et<sub>2</sub>O (4:1),  
19   filtered through a pad of silica gel to afford **Compound 12**.

20   Chemical synthesis of Compound 13

21            **Compound 13** is synthesized from **Compound 12** following  
22   procedures reported by Razdan (*Ryan et al. J. Med. Chem.* 1997, 40, 3617-  
23   3625; and *Dasse et al. Tetrahedron* 2000, 56, 9195-9202), as illustrated in  
24   **Reaction Scheme 2**. A 0.2 M solution of Compound 12 and 1.1 equivalent  
25   of triphenylphosphine in CH<sub>3</sub>CN is heated to reflux until completion of the  
26   reaction. The solvent is removed *in vacuo* and the residue is purified by  
27   washing with hexane-benzene (1:1). The product is dried in a vacuum oven  
28   and used directly in the next Wittig reaction.

1

2 Chemical synthesis of Compound 14

3       The chemical synthesis of **Compound 14** is illustrated in **Reaction**  
4 **Scheme 2**. To a 0.3 M solution of **Compound 13** in THF at -78 °C is  
5 added 1 equivalent of potassium bis(trimethylsilyl)amide (available from  
6 Aldrich). The mixture is stirred at -78 °C for 30 min. A solution of 1.5  
7 equivalent of (3-trifluoromethyl-phenoxy)-acetaldehyde (prepared by  
8 reducing 3-(trifluoromethyl)phenoxyacetonitrile (available from Lancaster)  
9 with diisobutylaluminum hydride) in THF is added dropwise to the above  
10 mixture and the reaction is gradually warmed to room temperature. Upon  
11 completion, the reaction is diluted with hexanes, washed with pH 7 buffer,  
12 brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated *in vacuo*. The crude product is  
13 purified by chromatography on silica gel.

14 Chemical synthesis of Compound 15

15       **Compound 15** is synthesized from **Compound 14** following  
16 General Procedure C as illustrated in **Reaction Scheme 2**. **Compound 15**  
17 is enzymatically converted into the corresponding 9,11-cycloendoperoxide  
18 derivative as shown in **Reaction Scheme 8** and is described below.

19 Chemical synthesis of Compound 16

20       **Compound 16** is synthesized from **Compound 14** following  
21 General Procedure A, using isopropyl alcohol instead of 2-aminoethanol, as  
22 illustrated in **Reaction Scheme 2**. **Compound 16** is enzymatically  
23 converted into the corresponding 9,11-cycloendoperoxide derivative as  
24 shown in **Reaction Scheme 9** and is described below.

25

26 General Procedure D

27 Enzymatic synthesis of 9,11-cycloendoperoxide of Prostaglandin H<sub>2</sub> 1-  
28 ethanolamide (Compound 17)

1        The human COX-2 catalyzed biosynthesis of Prostaglandin H<sub>2</sub> 1-  
2    ethanolamide 9,11-cycloendoperoxide (**Compound 17**) from its  
3    arachidonyl ethanolamide (**Compound 2**) is illustrated in **Reaction**  
4    **Scheme 3**. The enzyme human COX-2 was obtained commercially from  
5    Cayman Chemical (Ann Arbor, MI). It was cloned in 1992 (see the  
6    publication by *Hla et al. supra*). The enzyme was prepared by expression  
7    of a DNA clone encoding this enzyme in Baculovirus overexpression  
8    system in insect host cells (Sf21 cells). Ten  $\mu$ M [<sup>3</sup>H]arachidonyl  
9    ethanolamide (**Compound 2**) with a specific activity of 860  $\mu$ Ci/7 mg in 20  
10    $\mu$ l of ethanolic solution was diluted with 960  $\mu$ l of the COX-2 (hCOX-2)  
11   reaction buffer (100 mM Tris-HCl, pH 8.0, containing 2 mM phenol, 5  $\mu$ M  
12   hematin and 1 mM EDTA). One hundred units of hCOX-2 enzyme  
13   preparation in 20  $\mu$ l of hCOX-2 buffer were added to start enzyme reaction.  
14   The total incubation volume was 1 ml. The enzyme reaction was stopped  
15   by adding 1 ml dry ice-cooled stop solution (ether: methanol: 1 M acetic  
16   acid, 30:4:1, v/v) immediately after incubation at 37°C for 2 minutes. The  
17   synthesized products were extracted two times with 3 ml of ethyl acetate  
18   each. The organic phase was collected and dried at room temperature  
19   under nitrogen. The resulting residue was reconstituted in 150  $\mu$ l of  
20   acetonitrile / water (1:1, v/v) for HPLC-Radiometric analysis and  
21   LC/MS/MS analysis. The LC/MS/MS analysis of the synthesized 9,11-  
22   cycloendoperoxide of prostaglandin H<sub>2</sub> 1-ethanolamide eluting at 31.6  
23   minutes indicated that its molecular weight was 395 daltons. The yield of  
24   the synthesis as determined by HPLC-Radiometric analysis was 30%.

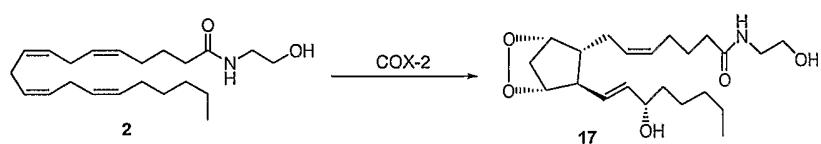
25    Enzymatic synthesis of Bimatoprost acid 9,11-cycloendoperoxide  
26    (**Compound 18**)

27        Bimatoprost acid 9,11-cycloendoperoxide (**Compound 18**) is  
28    synthesized following General Procedure D using **Compound 7**, instead of

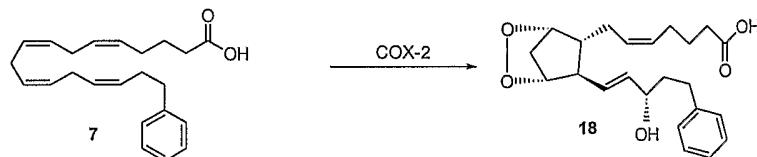
1 arachidonyl ethanolamide (**Compound 2**), as illustrated in **Reaction**  
 2 **Scheme 4.**  
 3 Enzymatic synthesis of Bimatoprost 9,11-cycloendoperoxide (**Compound**  
 4 **19)**

5 Bimatoprost 9,11-cycloendoperoxide (**Compound 19**) is synthesized  
 6 following General Procedure D using **Compound 8** instead of arachidonyl  
 7 ethanolamide (**Compound 2**) as illustrated in **Reaction Scheme 5.**

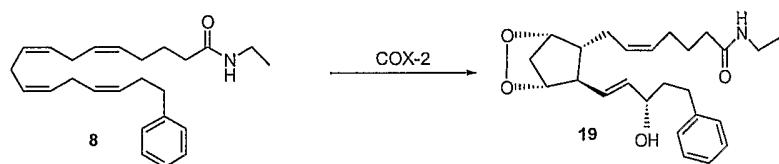
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REACTION SCHEME 3



REACTION SCHEME 4



REACTION SCHEME 5

9

10

11

12 Enzymatic synthesis of Latanoprost 13,14-dehydro-9,11-cycloendoperoxide  
 13 **(Compound 20)**

14 Latanoprost 13,14-dehydro-9,11-cycloendoperoxide (**Compound 20**)  
 15 is synthesized following General Procedure D using **Compound 9** instead

1 of arachidonyl ethanolamide (**Compound 2**), as illustrated in **Reaction**  
2 **Scheme 6.**

3 Enzymatic synthesis of Unoprostone 15-hydroxy-9,11-cycloendoperoxide  
4 (**Compound 21**)

5 Unoprostone 15-hydroxy-9,11-cycloendoperoxide (**Compound 21**)  
6 is synthesized following General Procedure D using **Compound 11** instead  
7 of arachidonyl ethanolamide (**Compound 2**) as illustrated in **Reaction**  
8 **Scheme 7.**

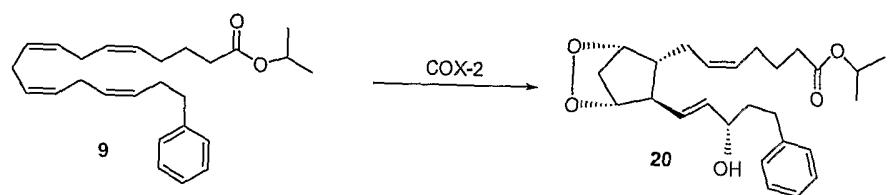
9 Enzymatic synthesis of Travoprost 9,11-cycloendoperoxide (**Compound**  
10 **22**)

11 Travoprost 9,11-cycloendoperoxide (**Compound 22**) is synthesized  
12 following General Procedure D using **Compound 15** instead of arachidonyl  
13 ethanolamide (**Compound 2**) as illustrated in **Reaction Scheme 8.**

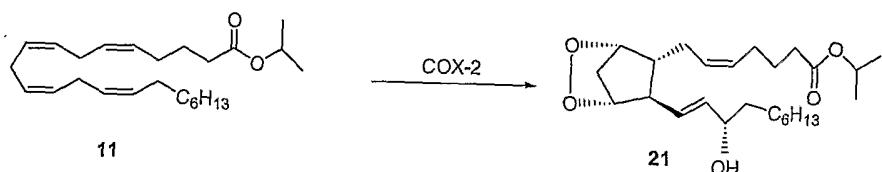
14 Enzymatic synthesis of Travoprost acid 9,11-cycloendoperoxide  
15 (**Compound 23**)

16 Travoprost acid 9,11-cycloendoperoxide (**Compound 23**) is  
17 synthesized following General Procedure D using **Compound 16** instead of  
18 arachidonyl ethanolamide (**Compound 2**) as illustrated in **Reaction**  
19 **Scheme 9.**

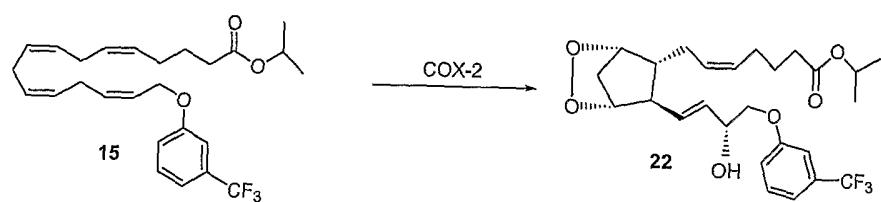
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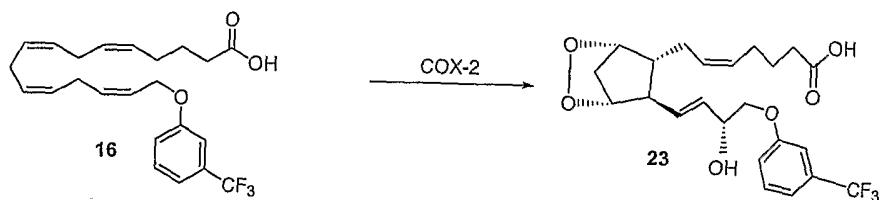
REACTION SCHEME 6



REACTION SCHEME 7



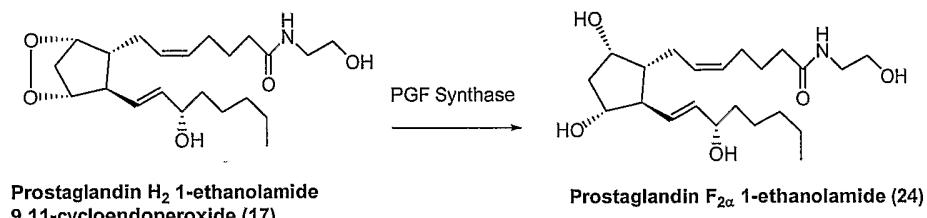
REACTION SCHEME 8

1  
2

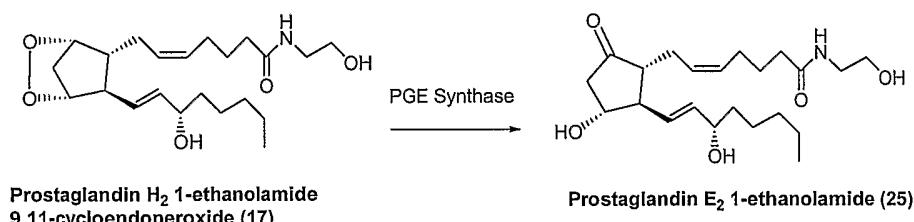
REACTION SCHEME 9

1 As noted above in the Summary section of the present application for  
2 patent, the 9,11-cyclopentadperoxide pro-drugs of the present invention, in  
3 addition to undergoing hydrolysis to provide the corresponding biologically  
4 active prostaglandin analogs, also act as substrates to several naturally  
5 occurring enzymes which convert the 9,11-cyclopentadperoxide pro-drugs  
6 into other biologically active analogs. Several of these enzymatic reactions  
7 which are expected to occur *in vivo* can be performed *in vitro* in  
8 accordance with the present invention resulting in the enzymatic synthesis  
9 from the 9,11-cyclopentadperoxides of the invention of several biologically  
10 active prostaglandin analogs. These enzymatic reactions are illustrated  
11 below in **Reaction Schemes 10 through 20**.

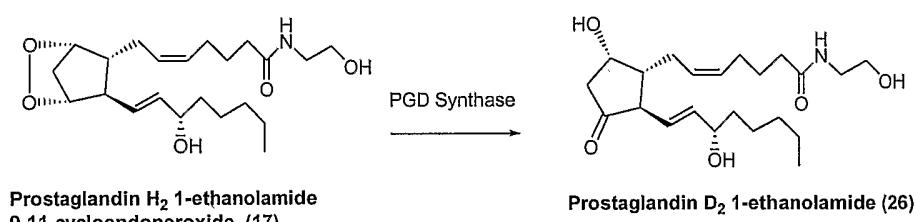
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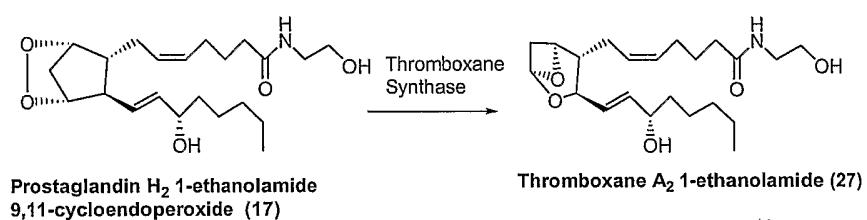
REACTION SCHEME 10



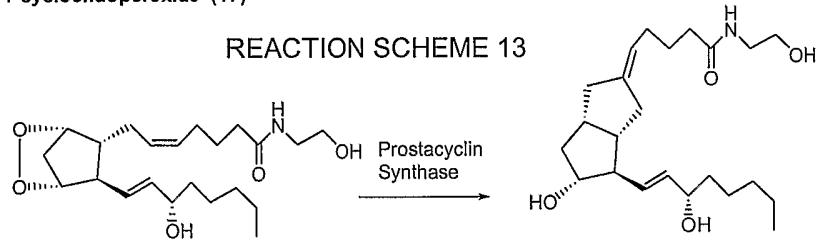
REACTION SCHEME 11



REACTION SCHEME 12



REACTION SCHEME 13



REACTION SCHEME 14

1

2

3

## 1 General Procedure E

2 Enzymatic synthesis of Prostaglandin F<sub>2 $\alpha$</sub>  1-ethanolamide (Compound 24)

3 The human recombinant PGF synthase catalyzed biosynthesis of  
4 Prostaglandin F<sub>2 $\alpha$</sub>  1-ethanolamide from prostaglandin H<sub>2</sub> 1-ethanolamide  
5 9,11-cycloendoperoxide (**Compound 17**) is illustrated above in **Reaction**  
6 **Scheme 10**. 4.5  $\mu$ M [<sup>3</sup>H]Prostaglandin H<sub>2</sub> 1-ethanolamide with a specific  
7 activity of 860 mCi/7 mg reconstituted in 0.6 ml of PGF synthase reaction  
8 buffer was incubated with 100  $\mu$ l of the human recombinant PGF synthase  
9 solution (1.5 mg/ml) at 37°C for 10 minutes. The cDNA clone of human  
10 PGF synthase was isolated from human lung and its enzyme was prepared  
11 by expression of cDNA clones in *E. coli* as described in the publication by  
12 *Suzuki-Yamamoto et al.*, FEBS lett, 1999,462: 335-340, incorporated herein  
13 by reference. The plasmid was transformed into DH5  $\alpha$  *E.coli* strain and  
14 grown in LB/ampicillin medium. The expressed enzyme in *E. coli* was  
15 partially purified to yield a protein concentration of 15 mg per ml. The  
16 pUC8 vectors carrying no PGF synthase DNA insert were also transformed  
17 and prepared with protein concentration of 5 mg per ml as a negative  
18 control. The enzyme reaction was stopped by adding 1 ml dry ice-cooled  
19 stop solution (ether: methanol: 1 M acetic acid, 30:4:1, v/v) immediately  
20 after incubation at 37°C for 10 minutes. The synthesized products were  
21 extracted with 3 ml of ethyl acetate. The organic phase was collected and  
22 dried at room temperature under nitrogen. The resulting residue was  
23 reconstituted in 150  $\mu$ l of acetonitrile / water (1:1, v/v) for HPLC-  
24 Radiometric analysis and LC/MS/MS analysis. The prostaglandin H<sub>2</sub> 1-  
25 ethanolamide 9,11-cycloendoperoxide was completely converted to  
26 prostaglandin F<sub>2 $\alpha$</sub>  1-ethanolamide in 10 minutes. The product ion spectrum  
27 of m/z 398.4 of the biosynthetic product was identical to the standard  
28 prostaglandin F<sub>2 $\alpha$</sub>  1-ethanolamide. They both had the major characteristic  
29 fragment ion at m/z 62, which represents protonated 2-amino ethanol

1 group. There was no conversion by the enzyme preparation from same  
2 DH5<sub>α</sub> cells carrying pUC8 vector without PGF synthase DNA insert. The  
3 yield of the synthesis, as determined by HPLC-Radiometric analysis, was  
4 94%.

5

6 General Procedure F

7 Enzymatic synthesis of Prostaglandin E<sub>2</sub> 1-ethanolamide (Compound 25)

8 Prostaglandin E<sub>2</sub> 1-ethanolamide is synthesized from prostaglandin  
9 H<sub>2</sub> 1-ethanolamide 9,11-cycloendoperoxide (**Compound 17**) following  
10 General Procedure E using the human recombinant PGE synthase obtained  
11 in accordance with the publication of *Jakobsson et al.* Proc. Natl. Acad.  
12 Sci. USA, 1999, 96: 7220-7225, incorporated herein by reference, instead  
13 of the human recombinant PGF synthase, as illustrated in **Reaction**  
14 **Scheme 11.**

15

16 General Procedure G

17 Enzymatic synthesis of Prostaglandin D<sub>2</sub> 1-ethanolamide (Compound 26)

18 Prostaglandin D<sub>2</sub> 1-ethanolamide is synthesized from its  
19 prostaglandin H<sub>2</sub> 1-ethanolamide 9,11-cycloendoperoxide (**Compound 17**)  
20 following General Procedure E using the human recombinant PGD  
21 synthase obtained in accordance with the publication of *Nagata et al.* Proc.  
22 Natl. Acad. Sci. USA, 1991, 88: 4020-4024, incorporated herein by  
23 reference, instead of the human recombinant PGF synthase, as illustrated in  
24 **Reaction Scheme 12.**

25

26 General Procedure H

27 Enzymatic synthesis of Thromboxane A<sub>2</sub> 1-ethanolamide (Compound 27)

28

1           Thromboxane A<sub>2</sub> 1-ethanolamide is synthesized from prostaglandin  
2 H<sub>2</sub> 1-ethanolamide 9,11-cycloendoperoxide (**Compound 17**) following  
3 General Procedure E using the human recombinant thromboxane synthase  
4 obtained in accordance with the publication of *Miyata et al.*, Eur. J.  
5 Biochem, 1994, 224: 273-279, incorporated herein by reference, instead of  
6 the human recombinant PGF synthase, as illustrated in **Reaction Scheme**  
7 **13**.

8

9 General Procedure I

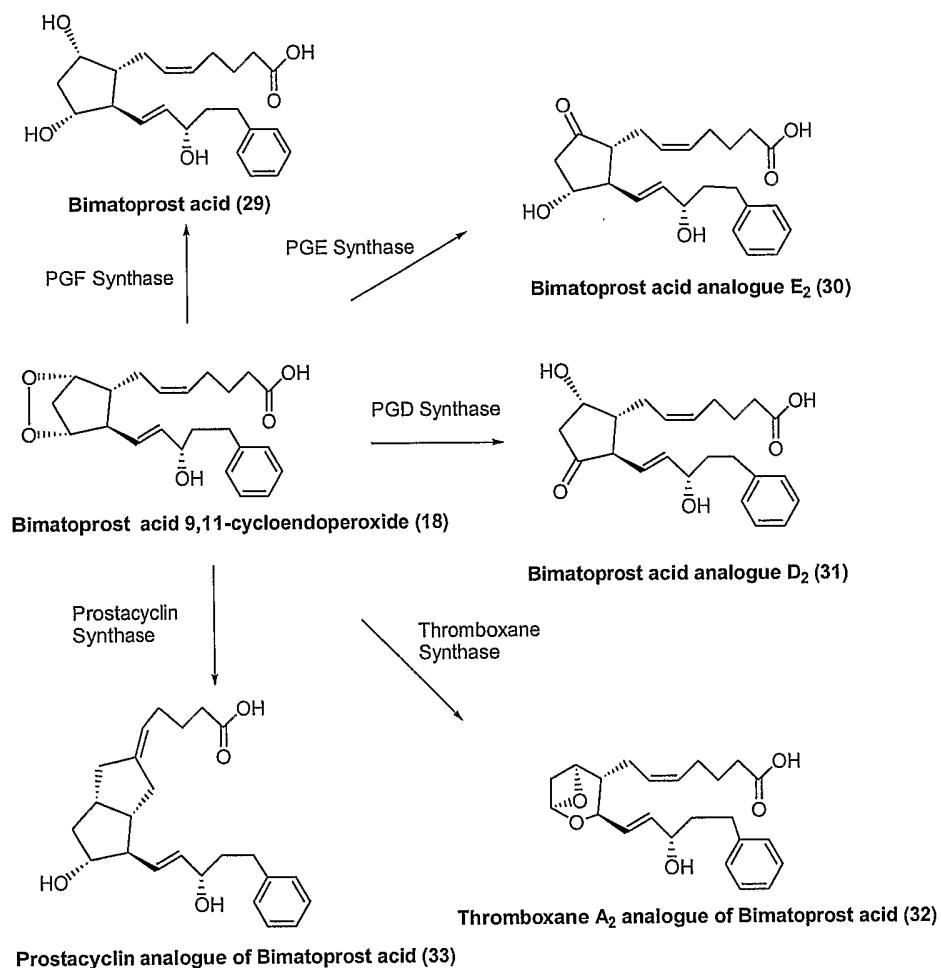
10 Enzymatic synthesis of Prostacyclin 1-ethanolamide (Compound 28)

11           Prostacyclin 1-ethanolamide is synthesized from prostaglandin H<sub>2</sub> 1-  
12 ethanolamide 9,11-cycloendoperoxide (**Compound 17**) following General  
13 Procedure E using the human recombinant prostacyclin synthase obtained  
14 in accordance with the publication of *Miyata et al.*, Biochem. Biophys.  
15 Res. Commun. 1994, 200: 1728-1734, instead of the human recombinant  
16 PGF synthase, as illustrated in **Reaction Scheme 14**.

17 Enzymatic synthesis of Bimatoprost acid (Compound 29), its  
18 prostaglandin analogues E<sub>2</sub> and D<sub>2</sub> (Compounds 30-31), thromboxane  
19 analogue A<sub>2</sub> (Compound 32) and prostacyclin analogue (Compound 33)

20           **Compounds 29-33** are synthesized starting with Bimatoprost acid  
21 9,11-cycloendoperoxide (**Compound 18**), instead of prostaglandin H<sub>2</sub> 1-  
22 ethanolamide (**Compound 17**), following General Procedures E, F, G, H  
23 and I, respectively, as illustrated in **Reaction Scheme 15** below.

24



1

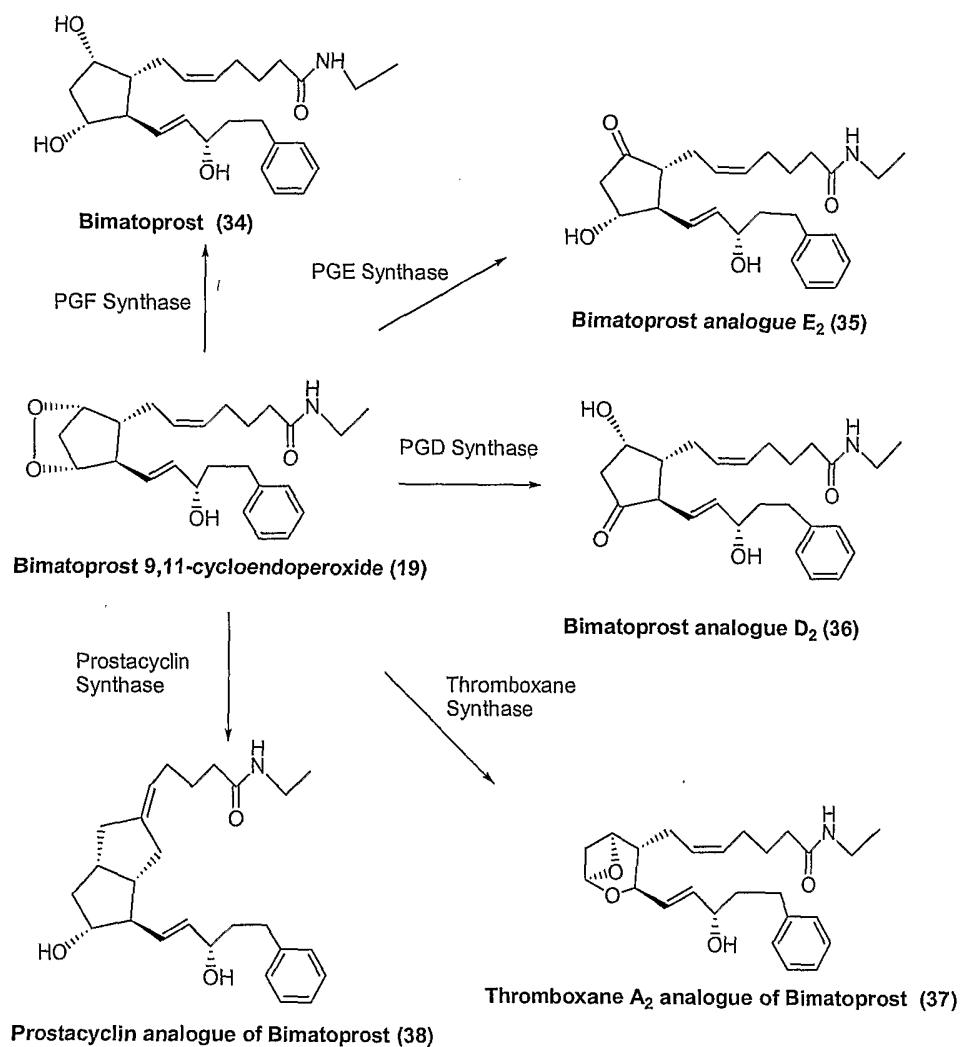
## REACTION SCHEME 15

2

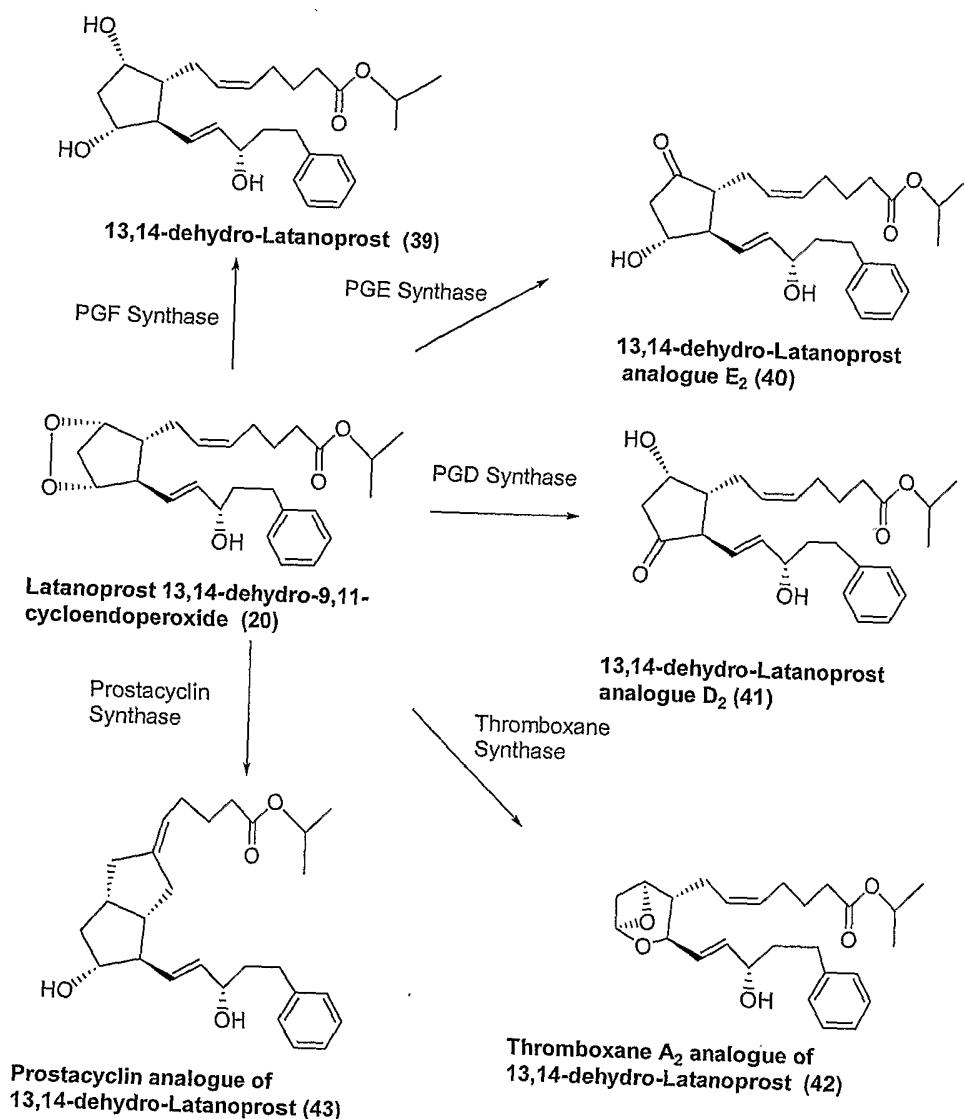
1        Enzymatic synthesis of Bimatoprost (Compound 34), its  
2        prostaglandin analogues E<sub>2</sub> and D<sub>2</sub> (Compounds 35-36), thromboxane  
3        analogue A<sub>2</sub> (Compound 37) and prostacyclin analogue (Compound 38)

4        **Compounds 34-38** are synthesized from Bimatoprost 9,11-  
5        cycloendoperoxide (**Compound 19**), instead of prostaglandin H<sub>2</sub> 1-  
6        ethanamide (**Compound 17**), following General Procedures E, F, G, H  
7        and I, respectively, as illustrated in **Reaction Scheme 16** below.

8



1 Enzymatic synthesis of 13,14-dehydro-Latanoprost (Compound 39), its  
2 prostaglandin analogues E<sub>2</sub> and D<sub>2</sub> (Compounds 40-41), thromboxane  
3 analogue A<sub>2</sub> (Compound 42) and prostacyclin analogue (Compounds 43)  
4 **Compounds 39-43** are synthesized from Latanoprost 13,14-dehydro-  
5 9,11-cycloendoperoxide (**Compound 20**), instead of prostaglandin H<sub>2</sub> 1-  
6 ethanamide, (**Compound 17**) following General Procedures E, F, G, H  
7 and I, respectively, as illustrated in **Reaction Scheme 17** below.  
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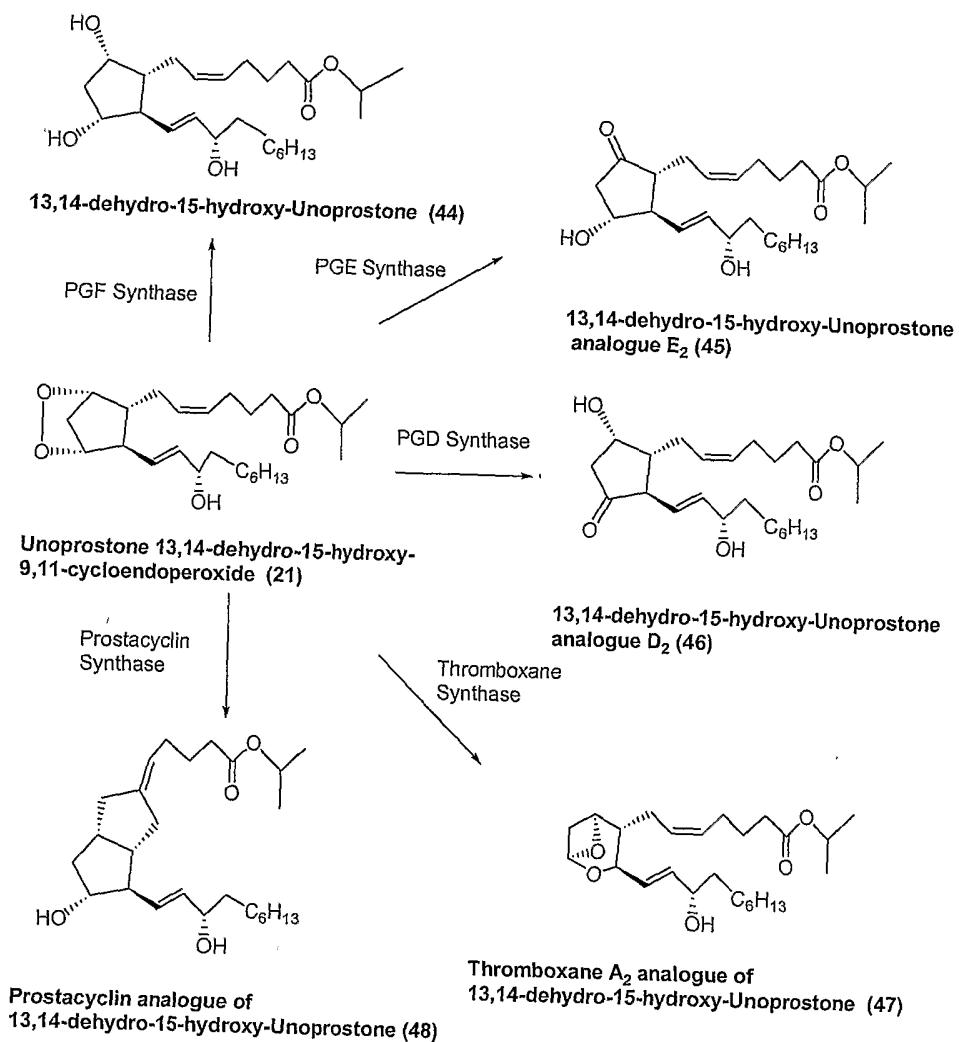
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## REACTION SCHEME 17

1    Enzymatic synthesis of 13,14-dehydro-15-hydro-Unoprostone (Compound  
2    44), its prostaglandin analogues E<sub>2</sub> and D<sub>2</sub> (Compounds 45-46),  
3    thromboxane analogue A<sub>2</sub> (Compound 47) and prostacyclin analogue  
4    **(Compound 48)**

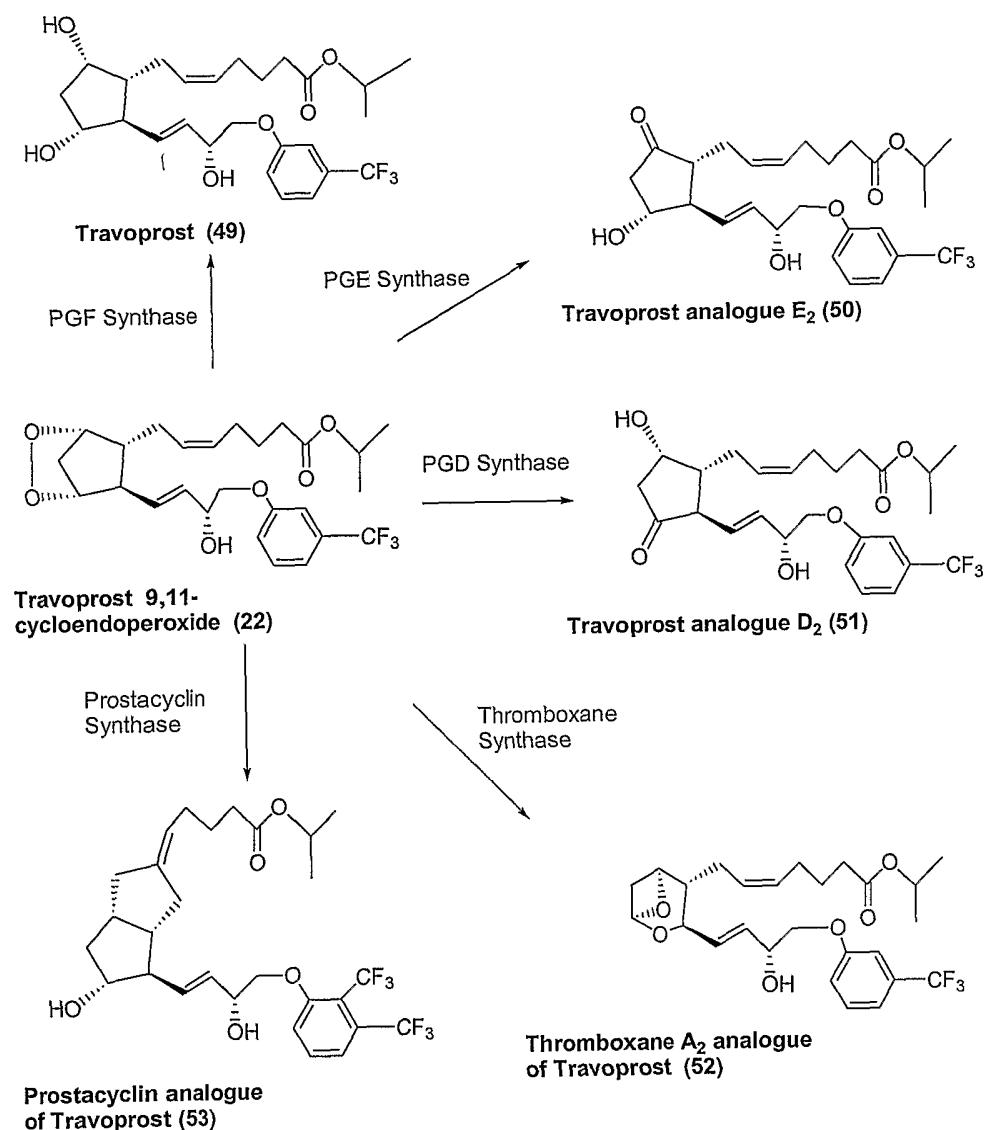
5       **Compounds 44-48** are synthesized from Unoprostone 13,14-  
6    dehydro-15-hydroxy-9,11-cycloendoperoxide (**Compound 21**), instead of  
7    prostaglandin H<sub>2</sub> 1-ethanolamide (**Compound 17**), following General  
8    Procedures E, F, G, H and I, respectively, as illustrated in **Reaction**  
9    **Scheme 18** below.

10



1    Enzymatic synthesis of Travoprost (Compound 49), its prostaglandin  
2    analogues E<sub>2</sub> and D<sub>2</sub> (Compounds 50-51), thromboxane analogue A<sub>2</sub>  
3    (Compound 52) and prostacyclin analogue (Compound 53) Compounds  
4    **49-53** are synthesized from Travoprost 9,11-cycloendoperoxide  
5    (**Compound 22**), instead of prostaglandin H<sub>2</sub> 1-ethanolamide (**Compound**  
6    **17**), following General Procedures E, F, G, H and I, respectively, as  
7    illustrated in **Reaction Scheme 19** below.

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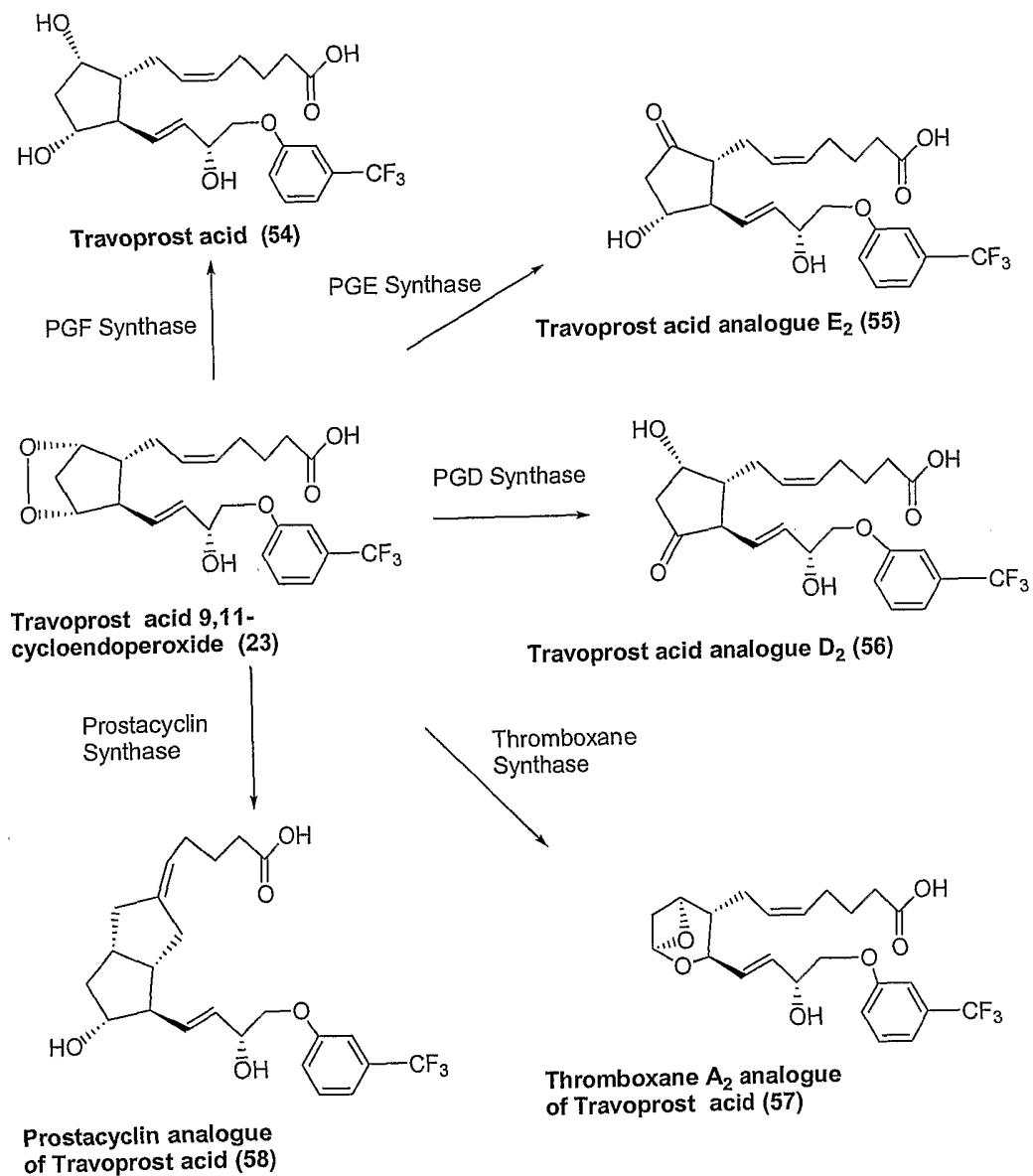


REACTION SCHEME 19

1    Enzymatic synthesis of Travoprost acid (Compound 54), its prostaglandin  
2    analogues E<sub>2</sub> and D<sub>2</sub> (Compounds 55-56), thromboxane analogue A<sub>2</sub>  
3    (Compound 57) and prostacyclin analogue (Compounds 58)

4        Compounds 54-58 are synthesized from Travoprost acid 9,11-  
5    cycloendoperoxide (Compound 23), instead of prostaglandin H<sub>2</sub> 1-  
6    ethanolamide (Compound 17), following General Procedures E, F, G, H  
7    and I, respectively, as illustrated in **Reaction Scheme 20** below.

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## REACTION SCHEME 20

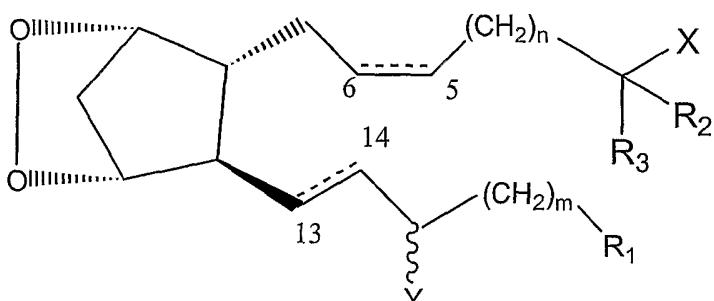
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## WHAT IS CLAIMED IS:

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3       1. A compound of the formula

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7       wherein the dashed lines represent the presence of a bond, or absence of a  
 8       bond, wavy lines represent either alpha or beta configuration, solid triangles  
 9       represent beta configuration and hatched lines represent alpha  
 10      configuration;  
 11      **n** is an integer having the values of 1 to 6;  
 12      **m** is an integer having the values of 1 to 8;  
 13      **X** is NH<sub>2</sub>, N(R)<sub>2</sub>, NHR, or OR where R is hydrogen, R<sub>4</sub> or a --(CO)R<sub>4</sub>  
 14      group;  
 15      **Y** is =O, =S or OH, OR<sub>5</sub> or --O(CO)R<sub>5</sub> groups, said OH, OR<sub>5</sub> or O(CO)R<sub>5</sub>  
 16      groups being attached to the adjacent carbon in alpha or beta configuration;  
 17      **R**<sub>1</sub> is H, CH<sub>3</sub>, **R**<sub>7</sub>, OR<sub>7</sub> or SR<sub>7</sub> where **R**<sub>7</sub> is an aliphatic, aromatic or  
 18      heteroaromatic ring, said heteroaromatic ring having 1 to 3 heteroatoms  
 19      selected from O, S, and N, said aliphatic, aromatic or heteroaromatic ring  
 20      being optionally substituted with 1 to 3 **R**<sub>8</sub> groups where **R**<sub>8</sub> is F, Cl, Br, I,  
 21      NO<sub>2</sub>, C<sub>1-6</sub> alkyl, C<sub>1-6</sub> fluoro substituted alkyl, COOH, or COOR<sub>9</sub> where R<sub>9</sub>  
 22      is alkyl of 1 to 6 carbons or CH<sub>2</sub>OCH<sub>3</sub>;  
 23      R<sub>2</sub> and R<sub>3</sub> together represent =O, =S, or independently are hydrogen or  
 24      alkyl of 1 to 6 carbon atoms;

1     $R_4$  represents  $(CH_2)_rOH$ ,  $(CH_2)_rOCOR_9$  or  $(CH_2)_rOR_9$  where  $r$  is an integer  
2    having the values 1 to 6, or  $R_4$  represents saturated or unsaturated acyclic  
3    hydrocarbons having from 1 to 20 carbon atoms, or  $-(CH_2)_qR_6$  where  $q$  is  
4    0-10 and  $R_6$  is an aliphatic, aromatic or heteroaromatic ring, said  
5    heteroaromatic ring having 1 to 3 heteroatoms selected from O, S, and N,  
6    said aliphatic, aromatic or heteroaromatic ring being optionally substituted  
7    with 1 to  $R_8$  groups where  $R_8$  is F, Cl, Br, I,  $NO_2$ ,  $C_{1-6}$  alkyl,  $C_{1-6}$  fluoro  
8    substituted alkyl,  $COOH$ ,  $COOR_9$  where  $R_9$  is alkyl of 1 to 6 carbons or  
9     $CH_2OCH_3$ ;  
10    $R_5$  represents saturated or unsaturated acyclic hydrocarbons having from 1  
11   to 20 carbon atoms, or  $-(CH_2)_qR_6$ , or a pharmaceutically acceptable salt of  
12   said compound.

13   **2.** A compound in accordance with Claim 1 where  $n$  is 3.

14   **3.** A compound in accordance with Claim 1 where  $m$  is an integer having  
15   the values 1 to 6.

16   **4.** A compound in accordance with Claim 1 where the dotted line between  
17   the carbons designated 13 and 14 represents a bond.

18   **5.** A compound in accordance with Claim 1 where the dotted line between  
19   the carbons designated 13 and 14 represents absence of a bond.

20   **6.** A compound in accordance with Claim 1 where the dotted line between  
21   the carbons designated 5 and 6 represents a bond.

22   **7.** A compound in accordance with Claim 1 where  $Y$  is  $=O$  or  $OH$ , or  
23    $O(CO)R_5$ , where  $R_5$  is alkyl of 1 to 6 carbons.

24   **8.** A compound in accordance with Claim 1 where  $Y$  is  $OH$  attached to the  
25   adjacent carbon by a bond of alpha orientation.

26   **9.** A compound in accordance with Claim 1 where  $R_1$  is methyl, phenyl,  $O$ -  
27   phenyl, phenyl substituted with 1 to 3  $R_8$  groups, or  $O$ -phenyl substituted  
28   with 1 to 3  $R_8$  groups.

29   **10.** A compound in accordance with Claim 1 where  $R_2$  and  $R_3$  jointly form

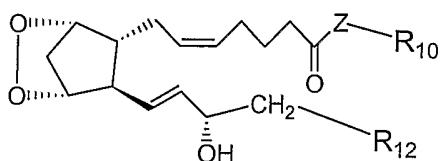
1 an oxo (=O) group.

2 **11.** A compound in accordance with Claim 9 where OH, OR<sub>4</sub> or NHR<sub>4</sub> or  
3 OR<sub>4</sub>.

4 **12.** A compound in accordance with Claim 10 where R<sub>4</sub> is alkyl of 1 to 6  
5 carbons, or (CH<sub>2</sub>)<sub>r</sub>OH.

6 **13.** A compound having the formula

7



10 where Z is O or NH;

11 R<sub>10</sub> is H, R<sub>11</sub> or (CH<sub>2</sub>)<sub>2</sub>OH;

12 R<sub>11</sub> is alkyl of 1 to 3 carbons, and

13 R<sub>12</sub> is selected from the group consisting of *n*-butyl, *n*-hexyl, CH<sub>2</sub>-phenyl  
14 and O-(3-trifluoromethyl)phenyl.

15 **14.** A compound in accordance with Claim 13 where Z is O.

16 **15.** A compound in accordance with Claim 13 where Z is NH.

17 **16.** A compound in accordance with Claim 13 where R<sub>10</sub> is H.

18 **17.** A compound in accordance with Claim 13 where R<sub>10</sub> is ethyl.

19 **18.** A compound in accordance with Claim 13 where R<sub>10</sub> is *iso*-propyl.

20 **19.** A compound in accordance with Claim 13 where R<sub>10</sub> is CH<sub>2</sub>CH<sub>2</sub>OH.

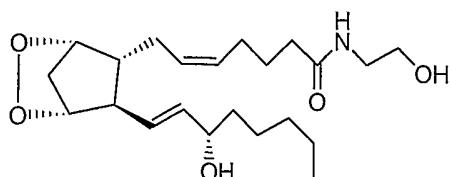
21 **20.** A compound in accordance with Claim 13 where R<sub>12</sub> is *n*-butyl.

22 **21.** A compound in accordance with Claim 13 where R<sub>12</sub> is *n*-hexyl.

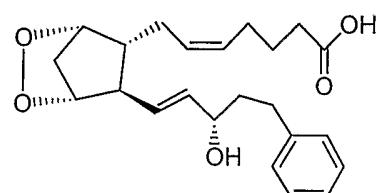
23 **22.** A compound in accordance with Claim 13 where R<sub>12</sub> is CH<sub>2</sub>-phenyl.

24 **23.** A compound in accordance with Claim 13 where R<sub>12</sub> is O-(3-  
25 trifluoromethyl)phenyl.

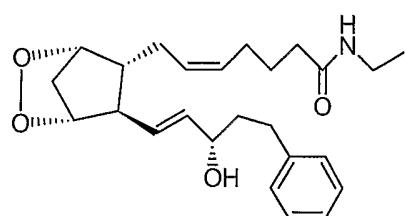
26 **24.** A compound in accordance with Claim 13, having the formula



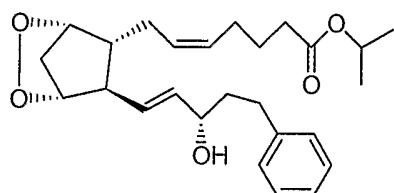
3 **25.** A compound in accordance with Claim 13, having the formula  
4



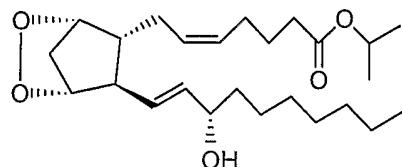
7 **26.** A compound in accordance with Claim 13, having the formula  
8



11 **27.** A compound in accordance with Claim 13, having the formula

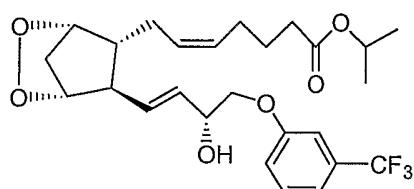


14 **28.** A compound in accordance with Claim 13, having the formula  
15



1 29. A compound in accordance with Claim 13, having the formula

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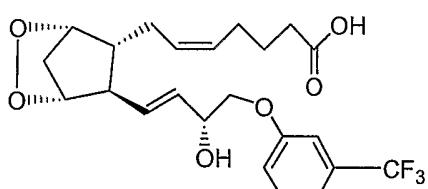


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5 30. A compound in accordance with Claim 13, having the formula

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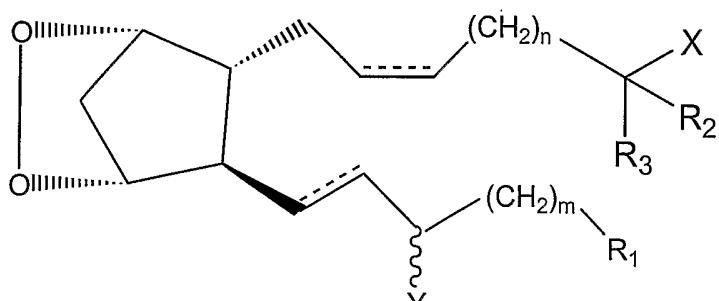


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9 31. A pharmaceutical composition adapted for administration to a  
10 mammal, the composition comprising a pharmaceutically acceptable  
11 excipient and a compound of the formula

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15 wherein the dashed lines represent the presence of a bond, or absence of a  
16 bond, wavy lines represent either alpha or beta configuration, solid triangles  
17 represent beta configuration and hatched lines represent alpha  
18 configuration;

19 **n** is an integer having the values of 1 to 6;

20 **m** is an integer having the values of 1 to 8;

1    X is NH<sub>2</sub>, N(R)<sub>2</sub>, NHR, or OR where R is hydrogen, R<sub>4</sub> or a --(CO)R<sub>4</sub>  
2    group;  
3    Y is =O, =S or OH, OR<sub>5</sub> or --O(CO)R<sub>5</sub> groups, said OH, OR<sub>5</sub> or O(CO)R<sub>5</sub>  
4    groups being attached to the adjacent carbon in alpha or beta configuration;  
5    R<sub>1</sub> is H, CH<sub>3</sub>, R<sub>7</sub>, OR<sub>7</sub> or SR<sub>7</sub> where R<sub>7</sub> is an aliphatic, aromatic or  
6    heteroaromatic ring, said heteroaromatic ring having 1 to 3 heteroatoms  
7    selected from O, S, and N, said aliphatic, aromatic or heteroaromatic ring  
8    being optionally substituted with 1 to 3 R<sub>8</sub> groups where R<sub>8</sub> is F, Cl, Br, I,  
9    NO<sub>2</sub>, C<sub>1-6</sub> alkyl, C<sub>1-6</sub> fluoro substituted alkyl, COOH, or COOR<sub>9</sub> where R<sub>9</sub>  
10   is alkyl of 1 to 6 carbons or CH<sub>2</sub>OCH<sub>3</sub>;  
11   R<sub>2</sub> and R<sub>3</sub> together represent =O, =S, or independently are hydrogen or  
12   alkyl of 1 to 6 carbon atoms;  
13   R<sub>4</sub> represents (CH<sub>2</sub>)<sub>r</sub>OH, (CH<sub>2</sub>)<sub>r</sub>OCOR<sub>9</sub> or (CH<sub>2</sub>)<sub>r</sub>OR<sub>9</sub> where r is an integer  
14   having the values 1 to 6, or R<sub>4</sub> represents saturated or unsaturated acyclic  
15   hydrocarbons having from 1 to 20 carbon atoms, or --(CH<sub>2</sub>)<sub>q</sub>R<sub>6</sub> where q is  
16   0-10 and R<sub>6</sub> is an aliphatic, aromatic or heteroaromatic ring, said  
17   heteroaromatic ring having 1 to 3 heteroatoms selected from O, S, and N,  
18   said aliphatic, aromatic or heteroaromatic ring being optionally substituted  
19   with 1 to R<sub>8</sub> groups where R<sub>8</sub> is F, Cl, Br, I, NO<sub>2</sub>, C<sub>1-6</sub> alkyl, C<sub>1-6</sub> fluoro  
20   substituted alkyl, COOH, COOR<sub>9</sub> where R<sub>9</sub> is alkyl of 1 to 6 carbons or  
21   CH<sub>2</sub>OCH<sub>3</sub>;  
22   R<sub>5</sub> represents saturated or unsaturated acyclic hydrocarbons having from 1  
23   to 20 carbon atoms, or --(CH<sub>2</sub>)<sub>q</sub>R<sub>6</sub>, or a pharmaceutically acceptable salt of  
24   said compound.  
25   **32.** A pharmaceutical composition in accordance with Claim 31 which is  
26   adapted for administration to a mammal to decrease intraocular pressure in  
27   the eye of the mammal.  
28   **33.** A pharmaceutical composition in accordance with Claim 32 wherein

1 the compound is selected from the group consisting of compounds having  
2 the formulas (i) through (vii)  
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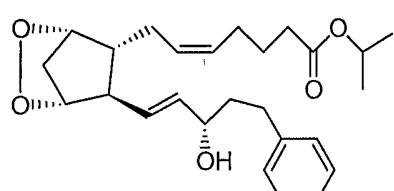
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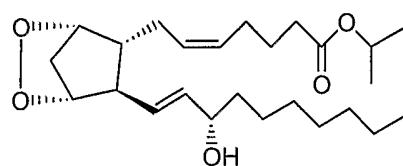
(i)



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(ii)



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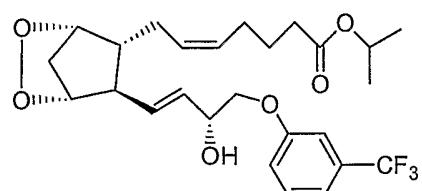
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(iii)

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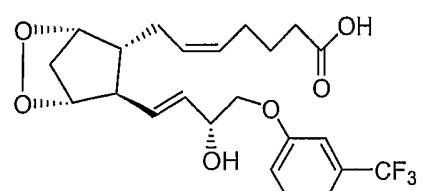


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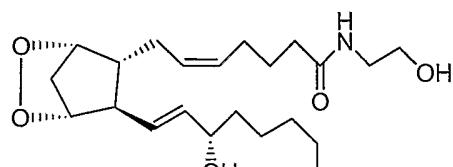


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(v)

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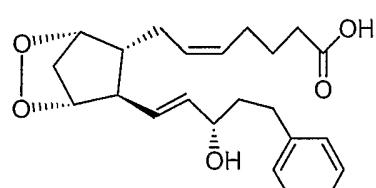


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(vi)

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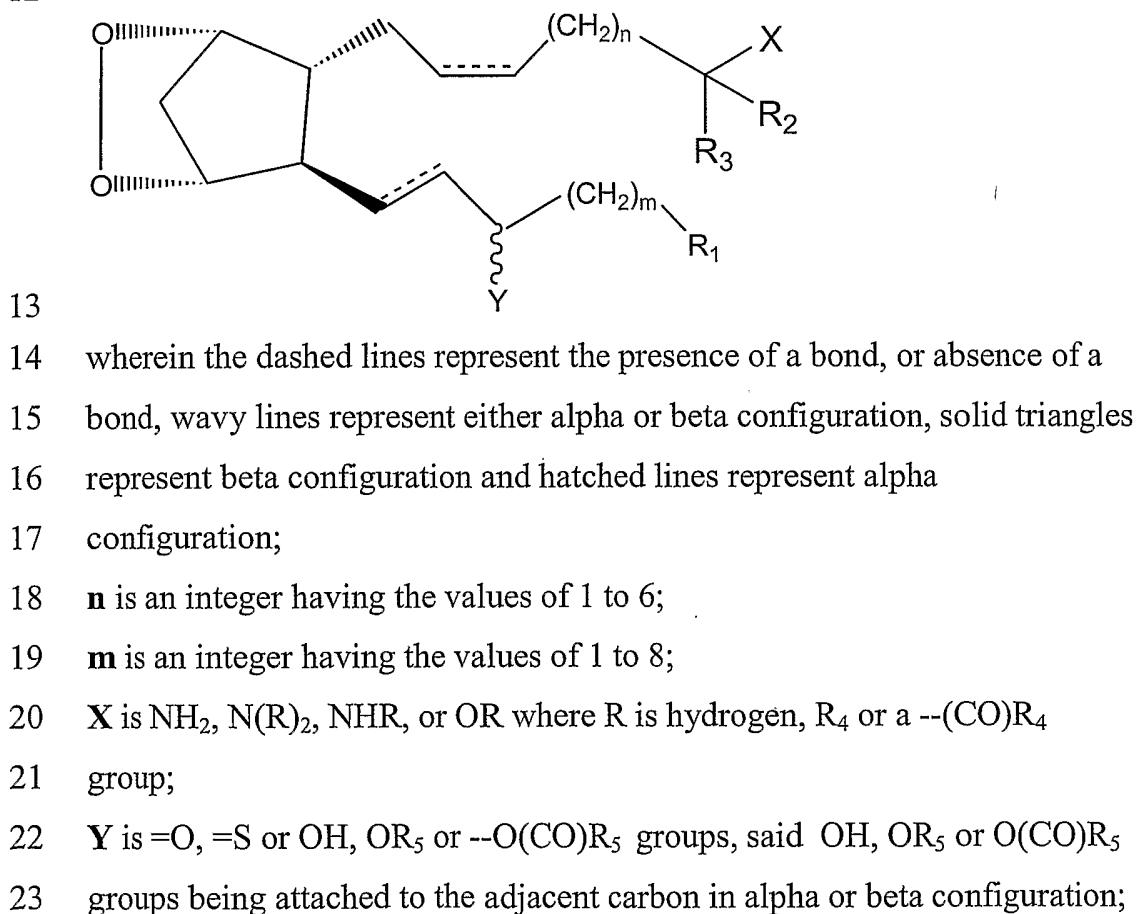


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8 34. A pharmaceutical composition in accordance with Claim 32 adapted  
 9 for topical administration to the mammalian eye.  
 10 35. A method of administering a pro-drug to a mammal in need of such  
 11 administration, the pro-drug comprising a compound of the formula  
 12

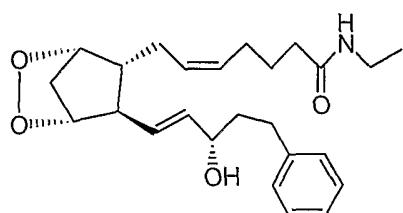


1   **R<sub>1</sub>** is H, CH<sub>3</sub>, **R<sub>7</sub>**, OR<sub>7</sub> or SR<sub>7</sub> where **R<sub>7</sub>** is an aliphatic, aromatic or  
2   heteroaromatic ring, said heteroaromatic ring having 1 to 3 heteroatoms  
3   selected from O, S, and N, said aliphatic, aromatic or heteroaromatic ring  
4   being optionally substituted with 1 to 3 **R<sub>8</sub>** groups where **R<sub>8</sub>** is F, Cl, Br, I,  
5   NO<sub>2</sub>, C<sub>1-6</sub> alkyl, C<sub>1-6</sub> fluoro substituted alkyl, COOH, or COOR<sub>9</sub> where R<sub>9</sub>  
6   is alkyl of 1 to 6 carbons or CH<sub>2</sub>OCH<sub>3</sub>;  
7   R<sub>2</sub> and R<sub>3</sub> together represent =O, =S, or independently are hydrogen or  
8   alkyl of 1 to 6 carbon atoms;  
9   R<sub>4</sub> represents (CH<sub>2</sub>)<sub>r</sub>OH, (CH<sub>2</sub>)<sub>r</sub>OCOR<sub>9</sub> or (CH<sub>2</sub>)<sub>r</sub>OR<sub>9</sub> where r is an integer  
10   having the values 1 to 6, or R<sub>4</sub> represents saturated or unsaturated acyclic  
11   hydrocarbons having from 1 to 20 carbon atoms, or -(CH<sub>2</sub>)<sub>q</sub>R<sub>6</sub> where q is  
12   0-10 and R<sub>6</sub> is an aliphatic, aromatic or heteroaromatic ring, said  
13   heteroaromatic ring having 1 to 3 heteroatoms selected from O, S, and N,  
14   said aliphatic, aromatic or heteroaromatic ring being optionally substituted  
15   with 1 to **R<sub>8</sub>** groups where **R<sub>8</sub>** is F, Cl, Br, I, NO<sub>2</sub>, C<sub>1-6</sub> alkyl, C<sub>1-6</sub> fluoro  
16   substituted alkyl, COOH, COOR<sub>9</sub> where R<sub>9</sub> is alkyl of 1 to 6 carbons or  
17   CH<sub>2</sub>OCH<sub>3</sub>;  
18   R<sub>5</sub> represents saturated or unsaturated acyclic hydrocarbons having from 1  
19   to 20 carbon atoms, or -(CH<sub>2</sub>)<sub>q</sub>R<sub>6</sub>, or a pharmaceutically acceptable salt of  
20   said compound.

1 36. A method in accordance with Claim 35 wherein the pro-drug is  
2 administered to decrease intraocular pressure in the eye of the mammal.

3 37. A method in accordance with Claim 36 wherein the compound is  
4 selected from the group consisting of compounds having the formulas (i)  
5 through (vii)

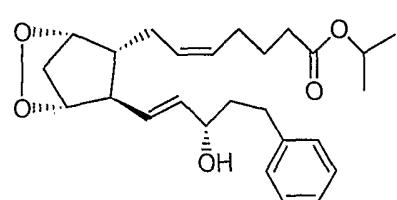
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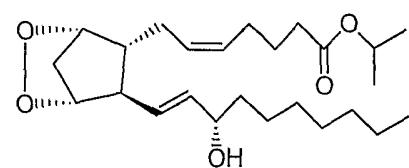
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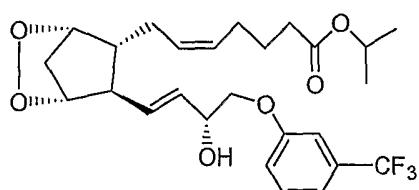
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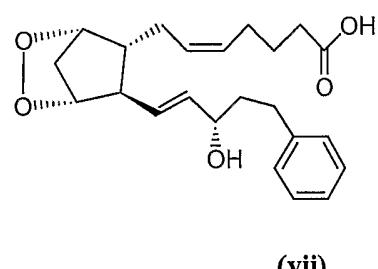
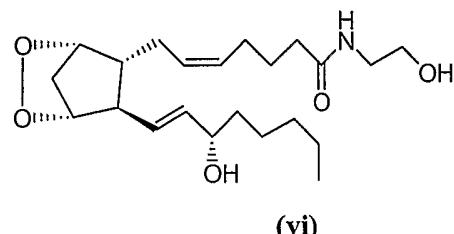
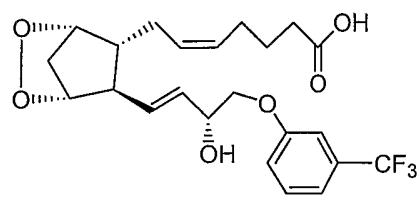
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11 **38.** A method in accordance with Claim 37 wherein the pro-drug is  
12 administered topically to decrease intraocular pressure in the eye of the  
13 mammal.

14

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 03/24305

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D317/06 A61K31/357 A61P27/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category <sup>o</sup>	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RAZ, AMIRAM ET AL: "Endoperoxides and thromboxanes. Structural determinants for platelet aggregation and vasoconstriction" BIOCHIMICA ET BIOPHYSICA ACTA (1977), 488(2), 305-11 , XP008025412 figure 3 ---	1,31
X	WO 02 12445 A (UNIV VANDERBILT) 14 February 2002 (2002-02-14) fig. 7 and 8, PGH2-EA --- -/-	1

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

<sup>o</sup> Special categories of cited documents :

- <sup>\*A\*</sup> document defining the general state of the art which is not considered to be of particular relevance
- <sup>\*E\*</sup> earlier document but published on or after the international filing date
- <sup>\*L\*</sup> document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- <sup>\*O\*</sup> document referring to an oral disclosure, use, exhibition or other means
- <sup>\*P\*</sup> document published prior to the international filing date but later than the priority date claimed

- <sup>\*T\*</sup> later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- <sup>\*X\*</sup> document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- <sup>\*Y\*</sup> document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- <sup>\*&\*</sup> document member of the same patent family

Date of the actual completion of the international search

22 December 2003

Date of mailing of the international search report

08/01/2004

Name and mailing address of the ISA

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Authorized officer

Alfaro Faus, I

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 03/24305

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  

Although claims 35–38 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compounds.
2.  Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 03/24305

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category <sup>o</sup>	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GORMAN R R ET AL: "PROSTAGLANDIS H1 AND H2. CONVENIENT BIOCHEMICAL SYNTHESIS AND ISOLATION. FURTHER BIOLOGICAL AND SPECTROSCOPIC CHARACTERIZATION" PROSTAGLANDINS, BUTTERWORTH, STONEHAM, MA, US, vol. 13, no. 6, June 1977 (1977-06), pages 1043-1053, XP001160973 ISSN: 0090-6980 page 1044 -page 1046; table 1 ---	1
X	GRAFF, G. ET AL: "Identification of 15-keto-9,11-peroxidoprossta-5,13-dienoic acid as a hematin-catalyzed decomposition product of 15-hydroperoxy-9,11-peroxidoprossta-5,13-dienoic acid" LIPIDS (1979), 14(4), 334-42 , XP008025451 figure 7 ---	1
X	DICZFALUSY, ULF ET AL: "Enzymic conversion of C21 endoperoxides to thromboxanes and hydroxy acids" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS (1980), 94(4), 1417-23 , XP008025450 figure 4 ---	1
X	LEDUC, LOUISE E. ET AL: "Analogs of arachidonic acid used to evaluate structural determinants of prostaglandin receptor and enzyme specificities" MOLECULAR PHARMACOLOGY (1981), 19(2), 242-7 , XP008025459 table 2 -----	1

# INTERNATIONAL SEARCH REPORT

on patent family members

International Application No

PCT/US 03/24305

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0212445	A 14-02-2002	AU 8474701 A	18-02-2002
		AU 8475301 A	18-02-2002
		CA 2422306 A1	14-02-2002
		CA 2422803 A1	14-02-2002
		EP 1307585 A1	07-05-2003
		EP 1307538 A1	07-05-2003
		WO 0212549 A1	14-02-2002
		WO 0212445 A1	14-02-2002
		US 2002064804 A1	30-05-2002
		US 2002106707 A1	08-08-2002