



(72) BRYANT, Henry Uhlman, US

(72) DODGE, Jeffrey Alan, US

(72) LUGAR, Charles Willis, III, US

(71) ELI LILLY AND COMPANY, US

(51) Int.Cl.<sup>6</sup> C07D 295/088, C07C 49/84, C07C 309/65, C07C 69/035,  
A61K 31/55, A61K 31/535, A61K 31/445, A61K 31/40, C07C 43/23,  
A61K 31/21, C07C 217/18, A61K 31/135, A61K 31/12

(30) 1996/09/26 (60/026,715) US

(54) **NAPHTOFLUORENES, INTERMEDIAIRES, COMPOSITIONS  
ET METHODES**

(54) **NAPHTHOFLUORENE COMPOUNDS, INTERMEDIATES,  
COMPOSITIONS AND METHODS**

(57) Naphtofluorènes, intermédiaires, formulations, procédés et méthodes pour prévenir la perte ou la résorption de matière osseuse, notamment l'ostéoporose, ainsi que les troubles pathologiques d'origine cardiovasculaire, comme l'hyperlipidémie, et les cancers associés à l'oestrogène.

(57) The invention provides naphthofluorene compounds, intermediates, formulations, processes, and methods of inhibiting bone loss or bone resorption, particularly osteoporosis, cardiovascular-related pathological conditions, including hyperlipidemia, and estrogen-dependent cancer.

**Abstract**

The invention provides naphthofluorene compounds, intermediates, formulations, processes, and methods of  
5 inhibiting bone loss or bone resorption, particularly osteoporosis, cardiovascular-related pathological conditions, including hyperlipidemia, and estrogen-dependent cancer.

NAPHTHOFLUORENE COMPOUNDS, INTERMEDIATES,  
COMPOSITIONS, AND METHODS

Osteoporosis describes a group of diseases which arises  
5 from diverse etiologies, but which are characterized by the  
net loss of bone mass per unit volume. The consequence of  
this loss of bone mass and resulting bone fracture is the  
failure of the skeleton to provide adequate support for the  
body. One of the most common types of osteoporosis is  
10 associated with menopause. Most women lose from about 20%  
to about 60% of the bone mass in the trabecular compartment  
of the bone within 3 to 6 years after the cessation of  
menses. This rapid loss is generally associated with an  
increase of bone resorption and formation. However, the  
15 resorptive cycle is more dominant and the result is a net  
loss of bone mass. Osteoporosis is a common and serious  
disease among postmenopausal women.

There are an estimated 25 million women in the United  
States alone who are afflicted with this disease. The  
20 results of osteoporosis are personally harmful, and also  
account for a large economic loss due to its chronicity and  
the need for extensive and long term support  
(hospitalization and nursing home care) from the disease  
sequelae. This is especially true in more elderly patients.

25 Additionally, although osteoporosis is generally not  
thought of as a life threatening condition, a 20% to 30%  
mortality rate is related to hip fractures in elderly women.  
A large percentage of this mortality rate can be directly  
associated with postmenopausal osteoporosis.

30 The most vulnerable tissue in the bone to the effects  
of postmenopausal osteoporosis is the trabecular bone. This  
tissue is often referred to as spongy or cancellous bone and  
is particularly concentrated near the ends of the bone (near  
the joints) and in the vertebrae of the spine. The  
35 trabecular tissue is characterized by small osteoid  
structures which interconnect with each other, as well as  
the more solid and dense cortical tissue which makes up the

outer surface and central shaft of the bone. This interconnected network of trabeculae gives lateral support to the outer cortical structure and is critical to the biomechanical strength of the overall structure. In postmenopausal osteoporosis, it is primarily the net resorption and loss of the trabeculae which leads to the failure and fracture of bone. In light of the loss of the trabeculae in the postmenopausal woman, it is not surprising that the most common fractures are those associated with bones which are highly dependent on trabecular support, for example, the vertebrae, the neck of the weight-bearing bones such as the femur and the forearm. Indeed, hip fracture, collicles fractures, and vertebral crush fractures are hallmarks of postmenopausal osteoporosis.

The most generally accepted method for the treatment of postmenopausal osteoporosis is estrogen replacement therapy. Although therapy is generally successful, patient compliance with the therapy is low, primarily because estrogen treatment frequently produces undesirable side effects. An additional method of treatment would be the administration of a bisphosphonate compound, such as, for example, Fosamax<sup>®</sup> (Merck & Co., Inc.).

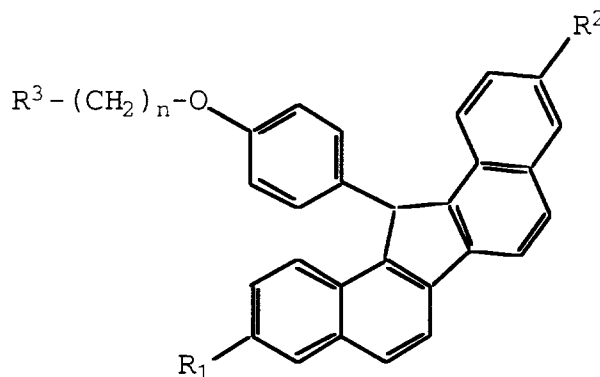
Throughout premenopausal time, most women have less incidence of cardiovascular disease than men of the same age. Following menopause, however, the rate of cardiovascular disease in women slowly increases to match the rate seen in men. This loss of protection has been linked to the loss of estrogen and, in particular, to the loss of estrogen's ability to regulate the levels of serum lipids. The nature of estrogen's ability to regulate serum lipids is not well understood, but evidence to date indicates that estrogen can up regulate the low density lipid (LDL) receptors in the liver to remove excess cholesterol. Additionally, estrogen appears to have some effect on the biosynthesis of cholesterol, and other beneficial effects on cardiovascular health.

It has been reported in the literature that serum lipid levels in postmenopausal women having estrogen replacement therapy return to concentrations found in the premenopausal state. Thus, estrogen would appear to be a reasonable  
5 treatment for this condition. However, the side effects of estrogen replacement therapy are not acceptable to many women, thus limiting the use of this therapy. An ideal therapy for this condition would be an agent which regulates serum lipid levels in a manner analogous to estrogen, but  
10 which is devoid of the side effects and risks associated with estrogen therapy.

Estrogen dependent cancers are major diseases effecting both women and to a lesser extent men. Cancer cells of this type are dependent on a source of estrogen to maintain the  
15 original tumor as well as to proliferate and metastasize to other locations. The most common forms of estrogen dependent cancer are breast and uterine carcinomas. Current chemotherapy of these diseases relies primarily on the use of anti-estrogens, predominately tamoxifen. The use of  
20 tamoxifen, although efficacious, is not without undesirable side-effects, for example, estrogen agonist properties, such as uterine hypertrophy and carcinogenic potential. Compounds of the current invention while showing the same or better potential for anti-cancer activity, also demonstrate  
25 a lower potential for estrogen agonist activity.

In response to the clear need for new pharmaceutical agents which are capable of alleviating the symptoms of, *inter alia*, postmenopausal syndrome, the instant invention provides novel naphthofluorene compounds, pharmaceutical  
30 formulations thereof, and methods of using such compounds for the treatment of postmenopausal syndrome and other estrogen-related pathological conditions.

The instant invention relates to compounds of formula I:



5

I

wherein:

$R^1$  is -H, -OH, -O(C<sub>1</sub>-C<sub>4</sub> alkyl),  
 -OCO(C<sub>1</sub>-C<sub>6</sub> alkyl), -O(CO)O(C<sub>1</sub>-C<sub>6</sub> alkyl), -OCOAr, -O(CO)OAr,  
 10 where Ar is phenyl or optionally substituted phenyl, or -  
 OSO<sub>2</sub>(C<sub>2</sub>-C<sub>6</sub> alkyl);

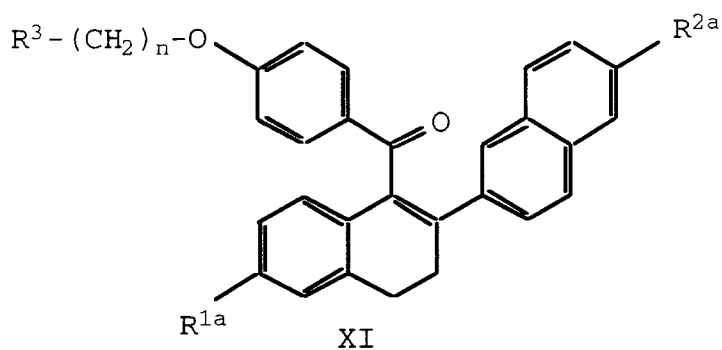
$R^2$  is -H, -OH, -O(C<sub>1</sub>-C<sub>4</sub> alkyl),  
 -OCO(C<sub>1</sub>-C<sub>6</sub> alkyl), -O(CO)O(C<sub>1</sub>-C<sub>6</sub> alkyl), -OCOAr, -O(CO)OAr,  
 where Ar is phenyl or optionally substituted phenyl, or -  
 15 OSO<sub>2</sub>(C<sub>2</sub>-C<sub>6</sub> alkyl);

$R^3$  is 1-piperidinyl, 1-pyrrolidinyl, methyl-1-pyrrolidinyl, dimethyl-1-pyrrolidinyl, 4-morpholino, dimethylamino, diethylamino, or 1-hexamethyleneimino; and  
 n is 2 or 3;

20 or a pharmaceutically acceptable salt or solvate thereof.

In addition to providing compounds of formula I, the instant invention also relates to compounds of formula XI, which are useful as intermediates for the synthesis of  
 25 compounds of formula I:

5



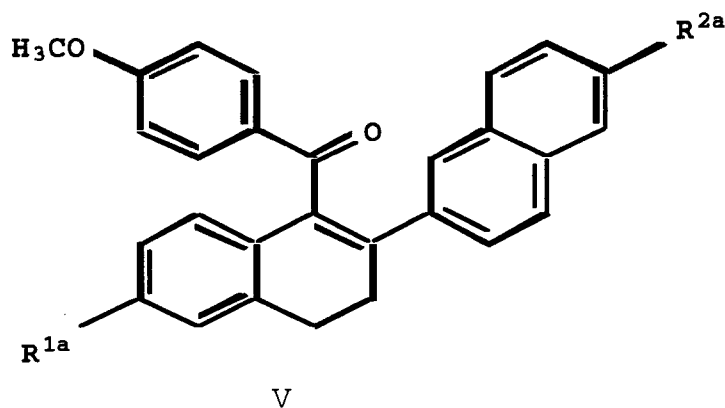
wherein:

5  $R^{1a}$  is -H or  $-OR^5$ , wherein  $R^5$  is a hydroxy protecting group;

$R^{2a}$  is -H,  $-OR^6$ , wherein  $R^6$  is a hydroxy protecting group, or halo; and

$R^3$  and  $n$  are as previously defined.

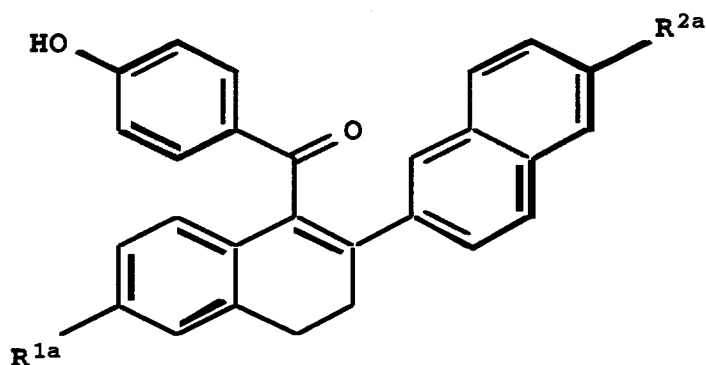
10 The instant invention also relates to compounds of formula V, which are useful as intermediates for the synthesis of compounds of formula I:



15 wherein  $R^{1a}$  and  $R^{2a}$  are as previously defined.

The instant invention also relates to compounds of formula VI, which are useful as intermediates for the synthesis of compounds of formula I:

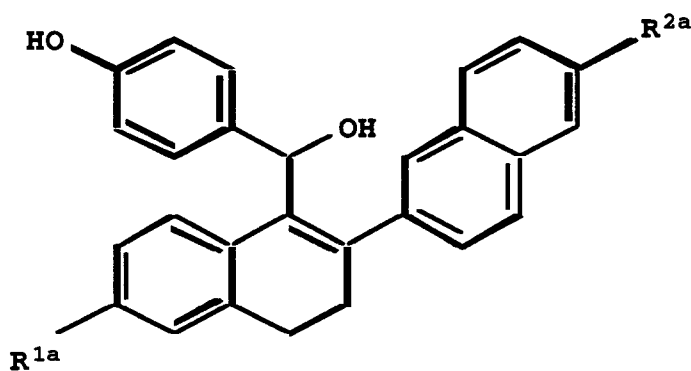
6



VI

wherein R<sup>1a</sup> and R<sup>2a</sup> are as previously defined.

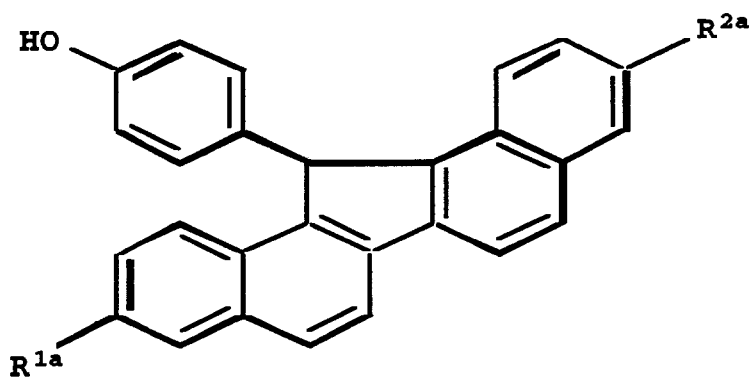
The instant invention also relates to compounds of  
 5 formula VII, which are useful as intermediates for the  
 synthesis of compounds of formula I:



VII

10 wherein R<sup>1a</sup> and R<sup>2a</sup> are as previously defined.

The instant invention also relates to compounds of  
 formula II, which are useful as intermediates for the  
 synthesis of compounds of formula I:



II

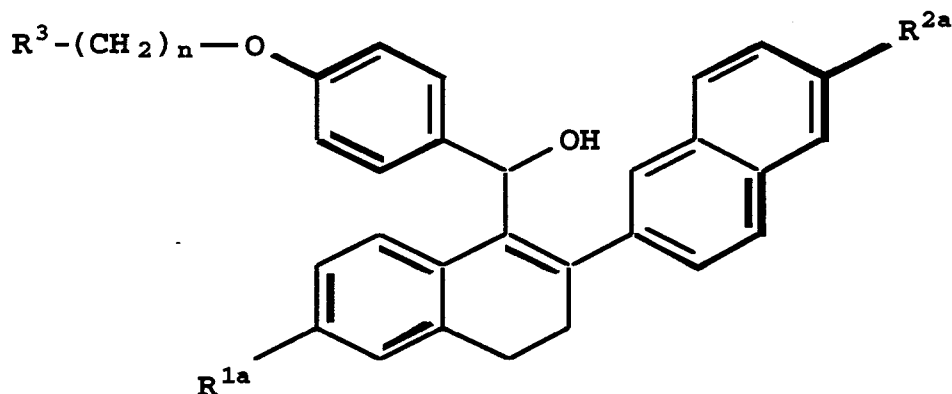
15



wherein R<sup>1a</sup> and R<sup>2a</sup> are as previously defined.

The instant invention also relates to compounds of formula XII, which are useful as intermediates for the synthesis of compounds of formula I:

5



XII

wherein R<sup>1a</sup>, R<sup>2a</sup>, R<sup>3</sup>, and n are as previously defined.

The instant invention further relates to pharmaceutical formulations containing compounds of formula I, and the use of said compounds at least for the inhibition of bone loss or bone resorption, particularly osteoporosis, cardiovascular-related pathological conditions, including hyperlipidemia, and estrogen-dependent cancer.

15 General terms used in the description of compounds herein described bear their usual meanings. For example, "C<sub>1</sub>-C<sub>6</sub> alkyl" refers to straight or branched aliphatic chains of 1 to 6 carbon atoms including methyl, ethyl, propyl, isopropyl, butyl, n-butyl, pentyl, isopentyl, hexyl, 20 isohexyl, and the like. Similarly, the term "-OC<sub>1</sub>-C<sub>4</sub> alkyl" represents a C<sub>1</sub>-C<sub>4</sub> alkyl group attached through an oxygen such as, for example, methoxy, ethoxy, n-propoxy, isopropoxy, and the like. Of these C<sub>1</sub>-C<sub>4</sub> alkoxy groups, methoxy is highly preferred.

25 The term "substituted phenyl" refers to a phenyl group having one or more substituents selected from the group consisting of C<sub>1</sub>-C<sub>4</sub> alkyl, -OC<sub>1</sub>-C<sub>4</sub> alkyl, hydroxy, nitro, chloro, fluoro, or tri(chloro or fluoro)methyl.

The term "hydroxy protecting group" contemplates numerous functionalities used in the literature to protect a hydroxyl function during a chemical sequence and which can be removed to yield the phenol. Included within this group would be acyls, mesylates, tosylates, benzyl, 5 alkylsilyloxys, -OC<sub>1</sub>-C<sub>4</sub> alkyls, and the like. Numerous reactions for the formation and removal of such protecting groups are described in a number of standard works including, for example, *Protective Groups in Organic* 10 *Chemistry*, Plenum Press (London and New York, 1973); Green, T.W., *Protective Groups in Organic Synthesis*, Wiley, (New York, 1981); and *The Peptides*, Vol. I, Schrooder and Lubke, Academic Press (London and New York, 1965). Methods for removing preferred R<sup>7</sup> hydroxy protecting groups, 15 particularly methyl and alkylsilyloxy, are essentially as described in the Examples, *infra*.

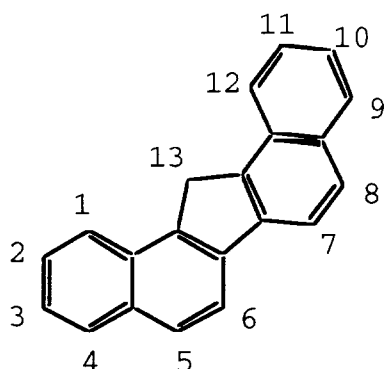
The term "leaving group" means a chemical entity which is capable of being displaced by an amino function via an SN<sub>2</sub> reaction. Such reactions are well known in the art and 20 such groups would include halogens, mesylates, tosylates, and the like. A preferred leaving group would be bromo.

The term "solvate" represents an aggregate that comprises one or more molecules of the solute, such as a formula I compound, with one or more molecules of solvent.

25 The term "inhibit" includes its generally accepted meaning which includes prohibiting, preventing, restraining, and slowing, stopping, or reversing progression, severity, or ameliorating a resultant symptom or effect.

The compounds of the instant invention are named and 30 numbered according to the Ring Index, The American Chemical Society, as follows:

9



Compounds of formula I would include, but not be limited to:

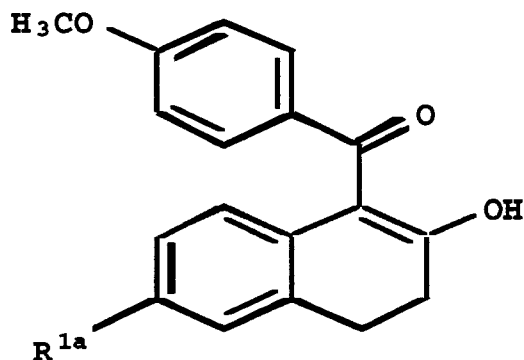
3-Hydroxy-13-[4-(2-piperidin-1-ylethoxy)phenyl]-13H-naphtho[a]fluorene,

3-Methoxy-13-[4-(2-piperidin-1-ylethoxy)phenyl]-13H-naphtho[a]fluorene,

3-Hydroxy-13-[4-(2-piperidin-1-ylethoxy)phenyl]-13H-10-hydroxynaphtho[a]fluorene,

3-Hydroxy-13-[4-(2-pyrrolidin-1-ylethoxy)phenyl]-13H-naphtho[a]fluorene, and the like.

The starting material for preparing compounds of the present invention is a compound of formula III



III

wherein  $R^{1a}$  is  $-H$  or  $-OR^5$  in which  $R^5$  is a hydroxy protecting group.

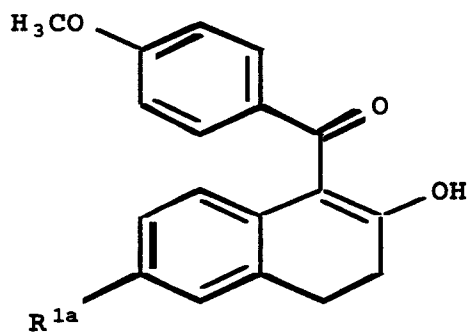
Compounds of formula III are well known in the art and are prepared essentially as described by Jones *et al.* in

U.S. Pat. No. 4,400,543 and Jones *et al.* in U.S. Pat. No. 5,147,880, and Bryant *et al.* U.S. Pat. No. 5,484,797, the disclosures of which are herein incorporated by reference. See also, Jones *et al.*, *J. Med. Chem.*, 35:931-8 (1992) and  
5 Jones *et al.*, *J. Med. Chem.*, 22:962 (1979).

In preparing compounds of the present invention, generally, a 1-acylated-2-tetralone of formula III is treated with a base to form its corresponding anion, which is reacted with diphenylchlorophosphate, providing an enol  
10 phosphate derivative of formula IV. The formula IV compound undergoes formal addition-elimination when treated with a naphthyl Grignard reagent, which results in substitution of the 2-phosphate substituent by the naphthyl moiety, thereby producing a compound of formula V.

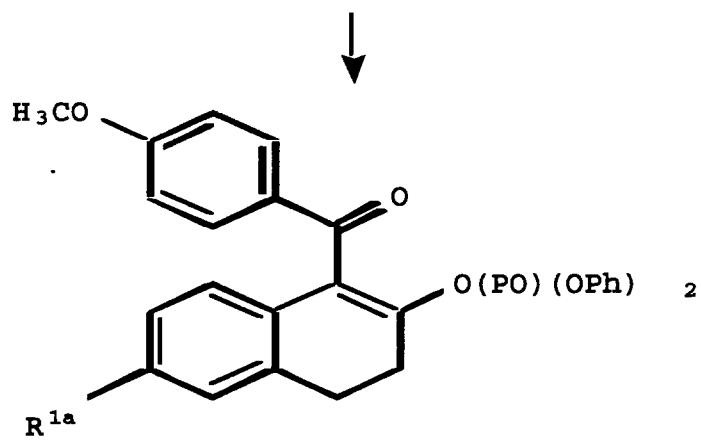
15 Dealkylation of a formula V compound by a thiolate anion demethylation reagent selectively dealkylates the group which is located para to the electron-withdrawing carbonyl group. The result of such selective dealkylation is a phenolic compound of formula VI, which can be reduced under the  
20 influence of hydride reducing agents to produce an allylic alcohol derivative of formula VII. The allylic alcohol is then cyclized under the influence of an acid catalyst to provide a naphthofluorene derivative of formula II. A compound of formula II serves as an intermediate to the  
25 compounds of this invention. This synthetic route is as shown below in Scheme I, and R<sup>1a</sup> and R<sup>2a</sup> are as defined above.

Scheme I

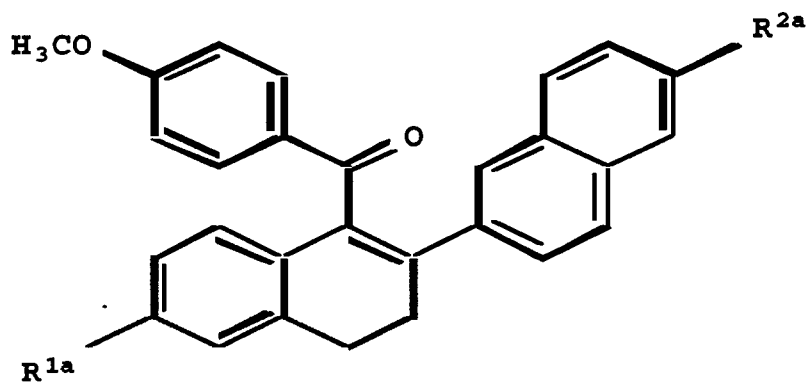


III

11

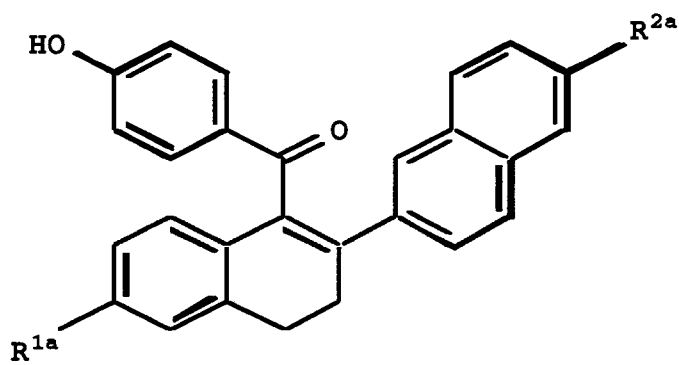


IV



5

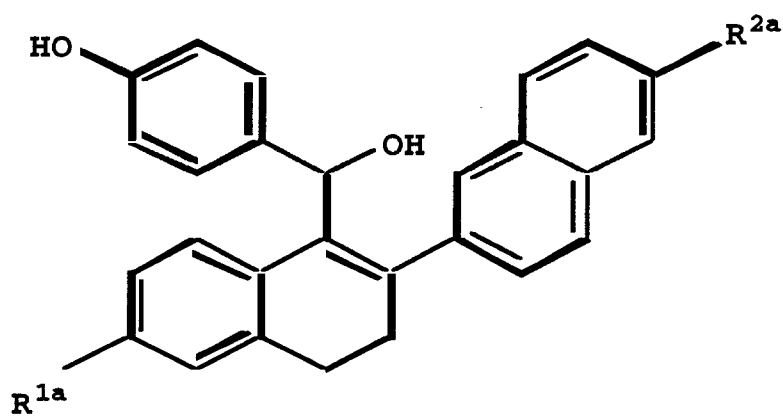
V



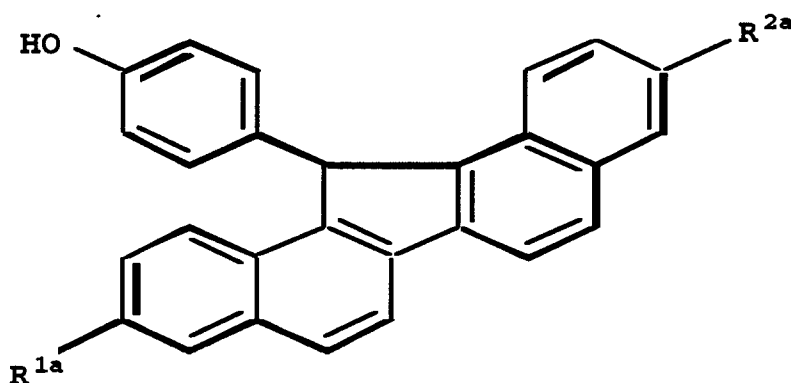
VI

10

12



VII



II

5

In the first step of the present process, a compound of formula III is converted to a dihydronaphthalene derivative of formula V via a two-step protocol, essentially as described by Jones *et al.*, *J. Med. Chem.*, 35: 931-8 (1992). In essence, a formula III enolic compound is phosphorylated by one or more equivalents of a phosphorylating reagent which is a diarylchloro- or diarylbromo-phosphate and preferably diphenylchlorophosphate. This reaction may be carried out in a variety of inert solvents including ethers, THF, dioxane, ethyl acetate, toluene, and acetonitrile and in the presence of an acid scavenger such as an alkali metal hydride, alkali metal hydroxide, or alkali metal carbonate or a trialkyl amine such as triethyl amine. The alkali metal base or tertiary amine may also act as a basic

catalyst in the phosphorylation process. Although it is preferable to run the reaction at ice bath temperature so as to avoid unwanted side products, elevated temperatures can also be used, but they are usually unnecessary to complete  
5 the phosphorylation reaction. The product of the phosphorylation reaction, an enol phosphate derivative of formula IV may be isolated by usual techniques, such as chromatography. However, it is most convenient to generate the enolphosphate using a solvent/ acid scavenger  
10 combination which is compatible with the next step of the reaction (addition of a Grignard Reagent). Thus, the combination of sodium hydride in THF under a nitrogen atmosphere is preferred, and provides a rapid phosphorylation leading to a compound of formula IV.

15 The intermediate enol phosphate, either isolated or generated in situ, may then be reacted with one or more equivalents of a naphthyl Grignard reagent or a naphthyl lithium organocuprate reagent. One to two equivalents of a naphthyl magnesium bromide is preferred, and 4-  
20 methoxynaphthyl magnesium bromide is particularly preferred. The reaction is typically conducted at ice bath temperature to minimize side reactions, but elevated temperatures can be used to increase the rate of the reaction. The addition of the naphthyl moiety, followed by the elimination of the  
25 phosphate leaving group (formally an addition, elimination process) gives rise to a dihydronaphthalene derivative of formula V, which can be isolated by conventional techniques such as crystallization or chromatography.

The resulting dihydronaphthalene derivative of formula  
30 V is then demethylated to provide an intermediate of formula VI. In order to accomplish regioselective demethylation at the methoxy group para to the carbonyl, a nucleophilic demethylation reagent is used, and alkali metal thiolates (alkali metal salt of an organic thiol) are preferred.  
35 Especially preferred are lithium thioethylate or lithium thiomethylate, in excess to the extent of 1.2 or more equivalents of the demethylation reagent over the substrate.

The reaction is conducted under an inert atmosphere to preserve the demethylation reagent and in a solvent which is practically inert to the nucleophilic nature of the thiolate reagent. Suitable solvents for the demethylation are those which are most conducive to bimolecular nucleophilic displacement reactions, and these include dimethylsulfoxide dimethylformamide, dimethylacetamide, and THF. Anhydrous dimethylformamide is preferred. In order to simultaneously achieve a satisfactory reaction rate and also obtain good control of the selectivity for demethylation at the site para to the carbonyl group, it is important to carefully control the temperature of the reaction. Although the demethylation process will take place in the range of temperatures from 60 °C to 120 °C, it is advantageous to use a temperature in the range of 80- 90 °C to optimize the yield of the desired product. A temperature of 80 °C is particularly preferred. Under the preferred reaction conditions, the transformation from a formula V compound to a formula VI compound is complete after heating for about 2 to 4 hours at the indicated temperature.

A formula VI compound is added to an appropriate solvent and reacted with a reducing agent such as, for example, lithium aluminum hydride (LAH).

The amount of reducing agent used in this reaction is an amount sufficient to reduce the carbonyl group of formula VI to form the carbinol compounds of formula VII. Generally, a liberal excess of the reducing agent per equivalent of the substrate is used.

Appropriate solvents include any solvent or mixture of solvents which will remain inert under reducing conditions. Suitable solvents include diethyl ether, dioxane, and tetrahydrofuran (THF). The anhydrous form of these solvents is preferred, and anhydrous THF is especially preferred.

The temperature employed in this step is that which is sufficient to effect completion of the reduction reaction. Ambient temperature, in the range from about 17° C to about 25° C, generally is adequate.



The length of time for this step is that amount necessary for the reaction to occur. Typically, this reaction takes from about 1 hour to about 20 hours. The optimal time can be determined by monitoring the progress of the reaction via conventional chromatographic techniques.

In the final transformation shown in Scheme I, the allylic alcohol derivative of formula VI undergoes a cyclization-dehydration process which produces the naphthofluorene derivative of formula II. This process is acid-catalyzed and a variety of mineral acids, Lewis acids, and organic acids stronger than acetic acid may be used. Among these catalysts are alkylsulfonic acids, aryl sulfonic acids, sulfuric acid, hydrochloric acid, hydrobromic acid, polyphosphoric acid, and boron trifluoride etherate. Hydrochloric acid is preferred. The cyclization reaction typically proceeds at room temperature, but higher temperatures may be advantageous in speeding up the reaction rate.

Under the preferred reaction conditions, the transformation from a formula VII compound to a formula II compound is complete after stirring for about 5 minutes to about 2 hours at 50 °C temperature or by stirring for overnight at ambient temperature.

Compounds of formula II are useful for the preparation of pharmaceutically active compounds of formula I of the present invention.

Upon preparation of a formula II compound, it is reacted with a compound of formula VIII



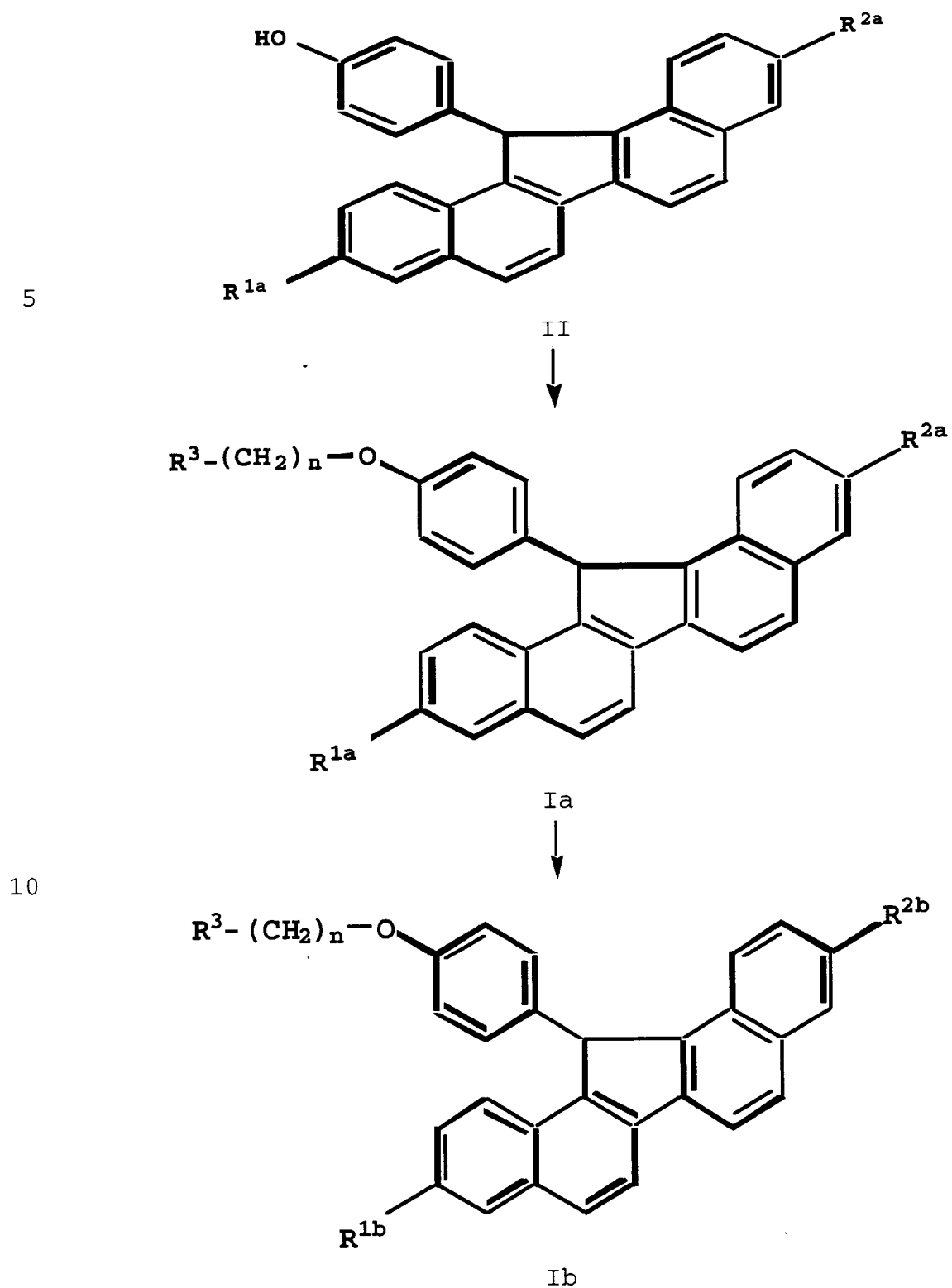
wherein  $\text{R}^3$  and  $n$  are as defined above and  $\text{Q}$  is a bromo or, preferably, a chloro moiety, to form a compound of formula Ia. The formula Ia compound is then deprotected, when  $\text{R}^5$  and/or  $\text{R}^6$  hydroxy protecting groups are present, to form a

X-10293

16

compound of formula Ib. These process steps are shown in Scheme II below.

Scheme II



wherein:

R<sup>1a</sup>, R<sup>2a</sup>, R<sup>3</sup>, and n are as defined above;

R<sup>1b</sup> is -H or -OH; and

R<sup>2b</sup> is -H, -OH, or halo;

5 or a pharmaceutically acceptable salt thereof.

In the first step of the process shown in Scheme II, the alkylation is carried out via standard procedures. Compounds of formula VIII are commercially available or are prepared by means well known to one of ordinary skill in the  
10 art. Preferably, the hydrochloride salt of a formula VIII compound, particularly 2-chloroethylpiperidine hydrochloride, is used.

Generally, at least about 1 equivalent of formula II substrate are reacted with 2 equivalents of a formula VIII  
15 compound in the presence of at least about 4 equivalents of an alkali metal carbonate, preferably cesium carbonate or potassium carbonate, and an appropriate solvent.

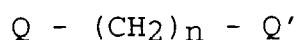
Solvents for this reaction are those solvents or mixture of solvents which remain inert throughout the  
20 reaction. N,N-dimethylformamide, especially the anhydrous form thereof, is preferred.

The temperature employed in this step should be sufficient to effect completion of this alkylation reaction. Often, ambient temperature is sufficient and preferred, but  
25 in certain cases, higher temperatures may be required.

The present reaction preferably is run under an inert atmosphere, particularly nitrogen.

Under the preferred reaction conditions, this reaction will run to completion in about 16 to about 20 hours. Of  
30 course, the progress of the reaction can be monitored via standard chromatographic techniques.

As an alternative for preparing compounds of formula Ia, a formula II compound is reacted with an excess of an  
35 alkylating agent of the formula



wherein Q and Q' each are the same or different leaving group, in an alkali solution. Appropriate leaving groups include the sulfonates such as methanesulfonate, 4-bromobenzenesulfonate, toluenesulfonate, ethanesulfonate, 5 isopropylsulfonate, 4-methoxybenzenesulfonate, 4-nitrobenzenesulfonate, 2-chlorobenzenesulfonate, triflate, and the like, halogens such as bromo, chloro, and iodo, and other related leaving groups. Halogens are preferred leaving groups and bromo is especially preferred.

10 A preferred alkali solution for this alkylation reaction contains potassium carbonate in an inert solvent such as, for example, methylethyl ketone (MEK) or DMF. In this solution, the 4-hydroxy group of the phenolic moiety of a formula II compound exists as a phenoxide ion which 15 displaces one of the leaving groups of the alkylating agent.

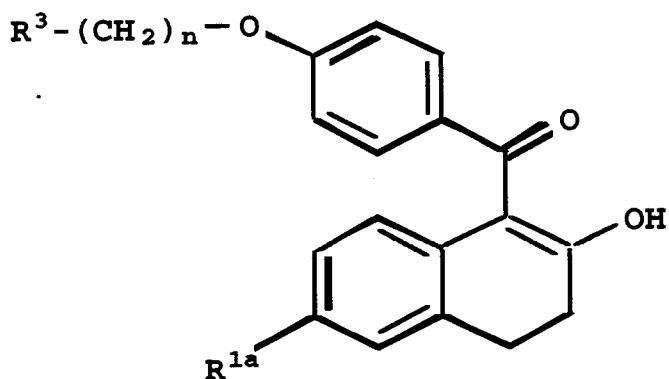
This reaction is best when the alkali solution containing the reactants and reagents is brought to reflux and allowed to run to completion. When using MEK as the preferred solvent, reaction times run from about 6 hours to 20 about 20 hours.

The reaction product from this step is then reacted with 1-piperidine, 1-pyrrolidine, methyl-1-pyrrolidine, dimethyl-1-pyrrolidine, 4-morpholine, dimethylamine, diethylamine, or 1-hexamethyleneimine, or other secondary 25 amines, via standard techniques, to form compounds of formula Ia. Preferably, the hydrochloride salt of piperidine is reacted with the alkylated compound of formula IIb in an inert solvent, such as anhydrous DMF, and heated to a temperature in the range from about 60° C to about 110° 30 C. When the mixture is heated to a preferred temperature of about 90° C, the reaction only takes about 30 minutes to about 1 hour. However, changes in the reaction conditions will influence the amount of time this reaction needs to be run to completion. Of course, the progress of this reaction 35 step can be monitored via standard chromatographic techniques.

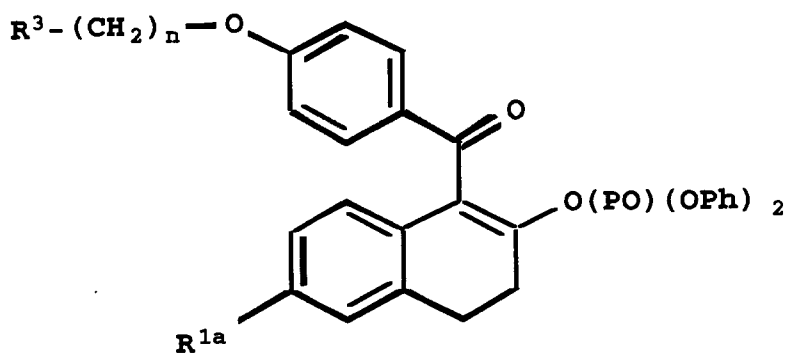
An alternative route for preparing compounds of the present invention is as depicted in Scheme III, in which  $R^{1a}$ ,  $R^{2a}$  and  $R^3$  are as defined above.

5

Scheme III

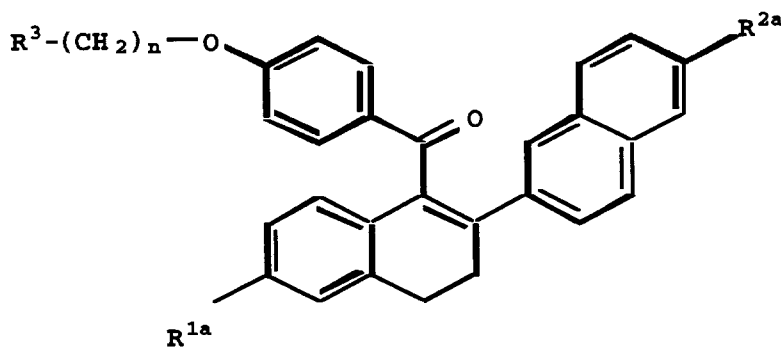


IX



10

X

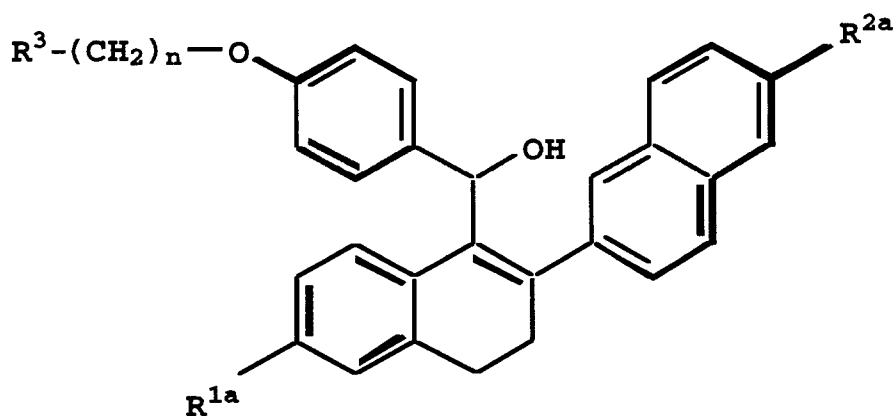


XI

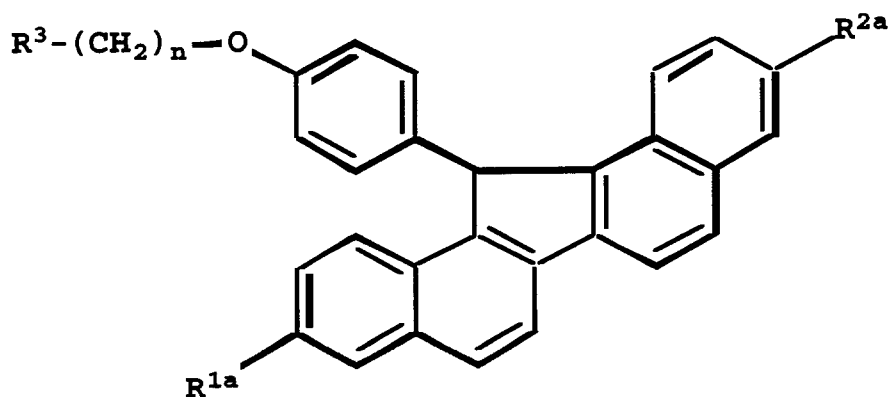


15

20



XII



Ia

5

In this alternative, the starting material, a 1-acylated-2-tetralone of formula IX already includes the basic side chain moiety. The compound of formula IX treated with a base to form its corresponding anion, which is reacted with diphenylchlorophosphate, providing an enol phosphate derivative of formula X. The formula X compound undergoes formal addition-elimination when treated with a naphthyl Grignard reagent, which results in substitution of the 2-phosphate substituent by the naphthyl moiety, thereby producing a naphthalene compound of formula XI. Reduction of the carbonyl group to produce a benzylic alcohol of formula XII and subsequent cyclization under acidic conditions provides a formula Ia compound of this invention

In essence, a formula IX enolic compound which already bears the basic side chain is phosphorylated by one or more equivalents of a phosphorylating reagent which is a diarylchloro- or diarylbromo-phosphate and preferably  
5 diphenylchlorophosphate. This reaction, may be carried out in a variety of inert solvents including ethers, THF, dioxane, ethyl acetate, toluene, and acetonitrile and in the presence of an acid scavenger such as an alkali metal hydride, alkali metal hydroxide, or alkali metal carbonate  
10 or a trialkyl amine such as triethyl amine. The alkali metal base or tertiary amine may also act as a basic catalyst in the phosphorylation process. Although it is preferable to run the reaction at ice bath temperature so as to avoid unwanted side products, elevated temperatures can  
15 also be used, but they are usually unnecessary to complete the phosphorylation reaction. The product of the phosphorylation reaction, an enol phosphate derivative of formula X may be isolated by usual techniques, such as chromatography. However, it is most convenient to generate  
20 the enolphosphate using a solvent/ acid scavenger combination which is compatible with the next step of the reaction (addition of a Grignard Reagent). Thus, the combination of sodium hydride in THF under a nitrogen atmosphere is preferred, and leads to a rapid  
25 phosphorylation leading to a compound of formula X.

The intermediate enol phosphate of formula X is either isolated or generated in situ, and is then reacted with one or more equivalents of a naphthyl Grignard reagent or a naphthyl lithium organocuprate reagent. One to two  
30 equivalents of a naphthyl magnesium bromide is preferred, and naphthyl magnesium bromide or 4-methoxynaphthyl magnesium bromide is particularly preferred. The reaction is typically conducted at ice bath temperature to minimize side reactions, but elevated temperatures can be used to  
35 increase the rate of the reaction. The addition of the naphthyl moiety, followed by the elimination of the phosphate leaving group (formally an addition, elimination

process) gives rise directly to a dihydronaphthalene derivative of formula XI, which can be isolated by conventional techniques such as crystallization of the free base or salts or chromatography of the former.

5       The reduction of the carbonyl group of a compound of formula XI to the carbinol of formula XII is effected in the same manner as provided above for Scheme II.

10       In the final transformation shown in Scheme III, the dihydronaphthalene derivative of formula XII undergoes a cyclization-dehydration process which produces the naphthofluorene derivative of formula Ia. This process is acid-catalyzed and a variety of mineral acids, Lewis acids, and organic acids may be used. Among these catalysts are alkylsulfonic acids, aryl sulfonic acids, sulfuric acid, 15 hydrochloric acid, hydrobromic acid, polyphosphoric acid, and boron trifluoride etherate. Hydrochloric acid is preferred. The cyclization reaction typically proceeds at room temperature, but higher temperatures may be advantageous in speeding up the reaction rate.

20       Under the preferred reaction conditions, the transformation from a formula XII compound to a formula Ia compound is complete after stirring for about 5 minutes to about 2 hours at 50 °C temperature or by stirring for overnight at ambient temperature.

25       Compounds of formula Ia, in which R<sup>5</sup> and/or R<sup>6</sup>, when present, are C<sub>1</sub>-C<sub>4</sub> alkyl, preferably methyl, are pharmaceutically active for the methods herein described and are encompassed by the definition herein of compounds of formula I. Both isomers and mixtures of isomers generated 30 at the 13-position are contemplated by, and within the scope of, the compounds of formula I.

35       Preferred compounds of formula I are obtained by cleaving, when present, the R<sup>5</sup> and R<sup>6</sup> hydroxy protecting groups of formula Ia compounds via well known procedures. Numerous reactions for the formation and removal of such protecting groups are described in a number of standard works including, for example, *Protective Groups in Organic*



Chemistry, Plenum Press (London and New York, 1973); Green, T.W., *Protective Groups in Organic Synthesis*, Wiley, (New York, 1981); and *The Peptides*, Vol. I, Schrooder and Lubke, Academic Press (London and New York, 1965). Methods for  
5 removing preferred R<sup>5</sup> and/or R<sup>6</sup> hydroxy protecting groups, particularly methyl, are essentially as described in the Examples, *infra*.

Alternatively, compounds such as those represented by formula Ia are prepared from a compound of formula XI in a  
10 novel one-pot reaction in which a compound of formula XI is treated with a Lewis acid such as AlCl<sub>3</sub> and ethanethiol. This protocol causes cyclization to form the naphtho[a]fluorene as well as deprotecting the methyl ether group.

15 Other preferred compounds of formula I are prepared by replacing the 3-position and/or 10-position hydroxy moieties, when present, with a moiety of the formula -O-CO-(C<sub>1</sub>-C<sub>6</sub> alkyl), or -O-SO<sub>2</sub>-(C<sub>2</sub>-C<sub>6</sub> alkyl) via well known procedures. See, for example, U.S. Pat. No. 4,358,593.

20 For example, when an -O-CO(C<sub>1</sub>-C<sub>6</sub> alkyl) group is desired, a mono- or dihydroxy compound of formula I is reacted with an acylating agent such as acyl chloride, bromide, cyanide, or azide, or with an appropriate anhydride or mixed anhydride. The reactions are conveniently carried  
25 out in a basic solvent such as pyridine, lutidine, quinoline or isoquinoline, or in a tertiary amine solvent such as triethylamine, tributylamine, methylpiperidine, and the like. The reaction also may be carried out in an inert solvent such as ethyl acetate, dimethylformamide,  
30 dimethylsulfoxide, dioxane, dimethoxyethane, acetonitrile, acetone, methyl ethyl ketone, and the like, to which at least one equivalent of an acid scavenger (except as noted below), such as a tertiary amine, has been added. If  
35 desired, acylation catalysts such as 4-dimethylaminopyridine or 4-pyrrolidinopyridine may be used. See, for example, Haslam, et al., *Tetrahedron*, 36:2409-2433 (1980).

The present reactions are carried out at moderate temperatures, in the range from about  $-25^{\circ}\text{C}$  to about  $100^{\circ}\text{C}$ , frequently under an inert atmosphere such as nitrogen gas. However, ambient temperature is usually adequate for the reaction to run.

5 Acylation of a 3-position and/or 10-position hydroxy group also may be performed by acid-catalyzed reactions of the appropriate carboxylic acids in inert organic solvents. Acid catalysts such as sulfuric acid, polyphosphoric acid, methanesulfonic acid, and the like are used.

10 The aforementioned  $\text{R}^1$  and/or  $\text{R}^2$  groups of formula I compounds also may be provided by forming an active ester of the appropriate acid, such as the esters formed by such known reagents such as dicyclohexylcarbodiimide, acylimidazoles, nitrophenols, pentachlorophenol, N-hydroxysuccinimide, and 1-hydroxybenzotriazole. See, for example, *Bull. Chem. Soc. Japan*, 38:1979 (1965), and *Chem. Ber.*, 788 and 2024 (1970).

Each of the above techniques which provide  $-\text{O}-\text{CO}-(\text{C}_1-\text{C}_6$  alkyl) moieties are carried out in solvents as discussed above. Those techniques which do not produce an acid product in the course of the reaction, of course, do not call for the use of an acid scavenger in the reaction mixture.

25 When a formula I compound is desired in which the 3-position and/or 10-position hydroxy group of a formula I compound is converted to a group of the formula  $-\text{O}-\text{SO}_2-(\text{C}_2-\text{C}_6$  alkyl), the mono- or dihydroxy compound is reacted with, for example, a sulfonic anhydride or a derivative of the appropriate sulfonic acid such as a sulfonyl chloride, bromide, or sulfonyl ammonium salt, as taught by King and Monoir, *J. Am. Chem. Soc.*, 97:2566-2567 (1975). The dihydroxy compound also can be reacted with the appropriate sulfonic anhydride or mixed sulfonic anhydrides. Such reactions are carried out under conditions such as were explained above in the discussion of reaction with acid halides and the like.

Although the free-base form of formula I compounds can be used in the methods of the instant invention, it is preferred to prepare and use a pharmaceutically acceptable salt form. The term "pharmaceutically acceptable salt" refers to either acid or base addition salts which are known to be non-toxic and are commonly used in the pharmaceutical literature. The pharmaceutically acceptable salts generally have enhanced solubility characteristics compared to the compound from which they are derived, and thus are often more amenable to formulation as liquids or emulsions. The compounds used in the methods of this invention primarily form pharmaceutically acceptable acid addition salts with a wide variety of organic and inorganic acids, and include the physiologically acceptable salts which are often used in pharmaceutical chemistry. Such salts are also part of this invention.

Typical inorganic acids used to form such salts include hydrochloric, hydrobromic, hydroiodic, nitric, sulfuric, phosphoric, hypophosphoric, and the like. Salts derived from organic acids, such as aliphatic mono and dicarboxylic acids, phenyl-substituted alkanolic acids, hydroxyalkanoic and hydroxyalkandioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, may also be used. Such pharmaceutically acceptable salts thus include acetate, phenylacetate, trifluoroacetate, acrylate, ascorbate, benzoate, chlorobenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, methylbenzoate, o-acetoxybenzoate, naphthalene-2-benzoate, bromide, isobutyrate, phenylbutyrate,  $\beta$ -hydroxybutyrate, butyne-1,4-dioate, hexyne-1,4-dioate, caproate, caprylate, chloride, cinnamate, citrate, formate, fumarate, glycolate, heptanoate, hippurate, lactate, malate, maleate, hydroxymaleate, malonate, mandelate, mesylate, nicotinate, isonicotinate, nitrate, oxalate, phthalate, terephthalate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrrophosphate, propiolate, propionate, phenylpropionate, salicylate, sebacate, succinate, suberate, sulfate,

bisulfate, pyrosulfate, sulfite, bisulfite, sulfonate, benzenesulfonate, *p*-bromophenylsulfonate, chlorobenzenesulfonate, ethanesulfonate, 2-hydroxyethanesulfonate, methanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, *p*-toluenesulfonate, xylenesulfonate, tartarate, and the like. A preferred salt is the hydrochloride salt.

The pharmaceutically acceptable acid addition salts are typically formed by reacting a compound of formula I with an equimolar or excess amount of acid. The reactants are generally combined in a mutual solvent such as diethyl ether or ethyl acetate. The salt normally precipitates out of solution within about one hour to 10 days and can be isolated by filtration, or the solvent can be stripped off by conventional means. The instant invention further provides for pharmaceutically acceptable formulations for administering to a mammal, including humans, in need of treatment, which comprises an effective amount of a compound of formula I and a pharmaceutically acceptable diluent or carrier.

As used herein, the term "effective amount" means an amount of compound of the instant invention which is capable of inhibiting bone loss or bone resorption, particularly osteoporosis, and cardiovascular-related pathological conditions including hyperlipidemia, and related cardiovascular pathologies.

In the case of estrogen-dependent cancers, the term "effective amount" means the amount of compound of the instant invention which is capable of alleviating, ameliorating, inhibiting cancer growth, treating, or preventing the cancer and/or its symptoms in mammals, including humans.

By "pharmaceutically acceptable formulation" it is meant that the carrier, diluent, excipients and salt must be compatible with the active ingredient (a compound of formula I) of the formulation, and not be deleterious to the recipient thereof. Pharmaceutical formulations can be

prepared by procedures known in the art. For example, the compounds of this invention can be formulated with common excipients, diluents, or carriers, and formed into tablets, capsules, and the like. Examples of excipients, diluents, and carriers that are suitable for such formulations include the following: fillers and extenders such as starch, sugars, mannitol, and silicic derivatives; binding agents such as carboxymethyl cellulose and other cellulose derivatives, alginates, gelatin, and polyvinyl pyrrolidone; moisturizing agents such as glycerol; disintegrating agents such as agar agar, calcium carbonate, and sodium bicarbonate; agents for retarding dissolution such as paraffin; resorption accelerators such as quaternary ammonium compounds; surface active agents such as cetyl alcohol, glycerol monostearate; adsorptive carriers such as kaolin and bentonite; and lubricants such as talc, calcium and magnesium stearate and solid polyethylene glycols. Final pharmaceutical forms may be: pills, tablets, powders, lozenges, syrups, aerosols, saches, cachets, elixirs, suspensions, emulsions, ointments, suppositories, sterile injectable solutions, or sterile packaged powders, and the like, depending on the type of excipient used.

Additionally, the compounds of this invention are well suited to formulation as sustained release dosage forms. The formulations can also be so constituted that they release the active ingredient only or preferably in a particular part of the intestinal tract, possibly over a period of time. Such formulations would involve coatings, envelopes, or protective matrices which may be made from polymeric substances or waxes.

The particular dosage of a compound of formula I required to inhibit the symptoms and/ or disease of a mammal, including humans, suffering from the above maladies according to this invention will depend upon the particular disease, symptoms, and severity. Dosage, routes of administration, and frequency of dosing is best decided by the attending physician. Generally, accepted and effective

doses will be from 15mg to 1000mg, and more typically from 15mg to 80mg, one to three times per day. Such dosages will be administered to a patient in need thereof usually at least for thirty days, and more typically for six months, or  
5 chronically.

The formulations which follow are given for purposes of illustration and are not intended to be limiting in any way. The total active ingredients in such formulations comprises from 0.1% to 99.9% by weight of the formulation. The term  
10 "active ingredient" means a compound of formula I.

#### Formulation 1: Gelatin Capsules

	Ingredient	Quantity (mg/capsule)
15	Active Ingredient	0.1-1000
	Starch NF	0-500
	Starch flowable powder	0-500
	Silicone fluid 350 centistokes	0-15

20 The ingredients are blended, passed through a No. 45 mesh U.S. sieve, and filled into hard gelatin capsules.

#### Formulation 2: Tablets

	Ingredient	Quantity (mg/tablet)
25	Active Ingredient	2.5-1000
	Starch	10-50
	Cellulose, microcrystalline	10-20
	Polyvinylpyrrolidone	5
30	(as 10% solution in water)	
	Sodium carboxymethylcellulose	5
	Magnesium stearate	1
	Talc	1-5

35 The active ingredient, starch, and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant

powders which are then passed through a No. 14 mesh U.S. sieve. The granules thus produced are dried at 50-60 °C and passed through a No. 18 mesh U.S. sieve. The sodium carboxymethylcellulose, magnesium stearate, and talc, previously passed through a No. 60 mesh U.S. sieve, are added to the above granules and thoroughly mixed. The resultant material is compressed in a tablet forming machine to yield the tablets.

10 Formulation 3: Aerosol

Ingredient	Weight %
Active Ingredient	0.25
15 Ethanol	29.75
Propellant 22 (Chlorodifluoromethane)	70.00
Total	100.00

20

The active ingredient is mixed with ethanol and the mixture added to a portion of the propellant 22, cooled to -30 °C and transferred to a filling device. The required amount is then fed to a stainless steel container and diluted with the remainder of the propellant. The valve units are then fitted to the container.

30 Formulation 4: Suppositories

Ingredient	Weight
Active ingredient	150 mg
Saturated fatty acid glycerides	3000mg

35

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the fatty acid glycerides which had previously heated to their melting point. The mixture is poured into a suppository mold and allowed to cool.

5

#### Formulation 5: Suspension

Suspensions each containing 0.1-1000 mg of a compound of formula I per 5 mL dose.

10

Ingredient	Weight
Active Ingredient	0.1-1000 mg
Sodium carboxymethyl cellulose	50 mg
Syrup	1.25 mL
Benzoic acid solution (0.1M)	0.10 mL
Flavor	q.v.
Color	q.v.
Purified water to total	Total 5 mL

20

A compound of formula I is passed through a No. 45 mesh U.S. sieve and mixed with the sodium carboxymethyl cellulose and syrup to form a smooth paste. The benzoic acid solution, flavor, and color diluted in water are added and mixture stirred thoroughly. Additional water is added to bring the formulation to final volume.

25

The following Examples and Preparations are provided to better elucidate the practice of the instant invention and should not be interpreted in any way as to limit the scope of same. Those skilled in the art will recognize that various modifications may be made while not departing from the spirit and scope of the invention. All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains.

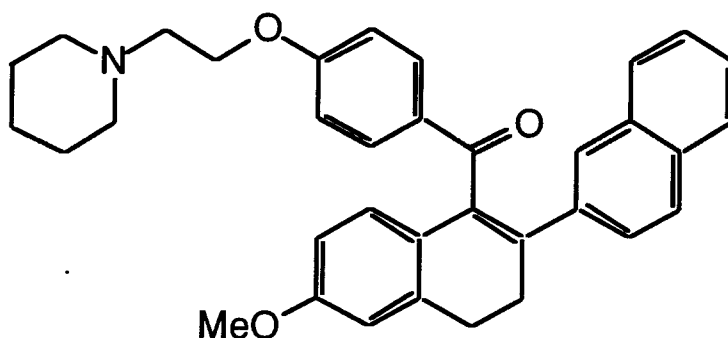
30

35



NMR data for the following Examples were generated on a GE 300 MHz NMR instrument, and anhydrous  $\text{CDCl}_3$  was used as the solvent unless otherwise indicated. Field strength for  $^{13}\text{C}$  NMR spectra was 75.5 MHz, unless otherwise indicated.

## Preparation 1



To a suspension of sodium hydride (0.90 g of a 60% oil dispersion, 22.42 mmol) stirring in THF (100 mL) at 0 °C was added a mixture of diphenylchlorophosphate (3.30 mL, 22.47 mmol) and the compound of formula III (8.3 g, 20.42 mmol) in THF (100 mL). After 2.5 h, the resulting solution was quenched with saturated aqueous ammonium chloride. The reaction mixture was diluted with ethyl acetate and extracted consecutively with saturated aqueous solutions of ammonium chloride, sodium hydroxide, and sodium chloride. The organic extracts were dried (sodium sulfate) and filtered. Concentration afforded a dark oil which was dissolved in THF (150 mL). This solution was cooled to -78 °C and naphthylmagnesium bromide solution (150 ml) (was made using the following procedure: To 3.0 g magnesium turnings in THF (150 ml) under nitrogen was added 2-bromonaphthylene (12.7 g, 61.27 mmol). The solution was allowed to exotherm to reflux, then stirred at room temperature for 4 hours. The resulting solution was decanted away from unreacted magnesium and used as is.) was added. After complete consumption of the enol phosphate intermediate, the reaction

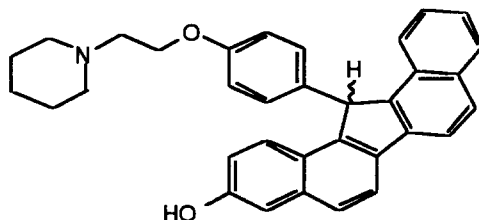
15  
20  
25  
30

was warmed to -30 °C and quenched with saturated aqueous ammonium chloride. The mixture was then extracted with ethyl acetate and the combined organic extracts washed with saturated aqueous ammonium chloride, 1 N aqueous NaOH, and  
5 brine. The resulting dark oil was purified by flash chromatography (silica gel, chloroform to 5% methanol/chloroform gradient) to give 4.9 g (60 %) of the crude desired product as a dark yellow oil: <sup>1</sup>H NMR is consistent with structure. MS/FD - 517.

10

## Example 1

3-Hydroxy-13-[4-(2-piperidin-1-ylethoxy)phenyl]-13H-naphtho[a]fluorene



15

To a solution of a compound of Preparation 1 (4.5 g, 8.70 mmol) stirring in CH<sub>2</sub>Cl<sub>2</sub> (200 mL) at room temperature was added ethanethiol (3.2 mL, 43.5 mmol) followed by AlCl<sub>3</sub>  
20 (5.80 g, 43.5 mmol). After stirring vigorously for 0.5 h, the dark red solution was quenched with saturated aqueous sodium bicarbonate. The resulting mixture was extracted with saturated aqueous sodium bicarbonate and brine. The organic extract was dried (sodium sulfate), filtered, and  
25 concentrated. The resulting dark oil was purified by flash chromatography (silica gel, 2% to 5% MeOH/CHCl<sub>3</sub> gradient) to give 1.6 g (40 %) of the desired product as an orange foam: <sup>1</sup>H NMR is consistent with structure. MS/FD - 485.

30

In the examples illustrating the methods, a postmenopausal model was used in which effects of different treatments upon circulating lipids were determined.

Seventy-five day old female Sprague Dawley rats (weight  
5 range of 200 to 225g) were obtained from Charles River  
Laboratories (Portage, MI). The animals were either  
bilaterally ovariectomized (OVX) or exposed to a Sham  
surgical procedure at Charles River Laboratories, and then  
shipped after one week. Upon arrival, they were housed in  
10 metal hanging cages in groups of 3 or 4 per cage and had *ad  
libitum* access to food (calcium content approximately 0.5%)  
and water for one week. Room temperature was maintained at  
22.2° ± 1.7° C with a minimum relative humidity of 40%. The  
photoperiod in the room was 12 hours light and 12 hours  
15 dark.

Dosing Regimen Tissue Collection. After a one week  
acclimation period (therefore, two weeks post-OVX) daily  
dosing with test compound was initiated. 17 $\alpha$ -ethynyl  
20 estradiol or the test compound were given orally, unless  
otherwise stated, as a suspension in 1%  
carboxymethylcellulose or dissolved in 20% cyclodextrin.  
Animals were dosed daily for 4 days. Following the dosing  
regimen, animals were weighed and anesthetized with a  
25 ketamine: xylazine (2:1, V:V) mixture and a blood sample was  
collected by cardiac puncture. The animals were then  
sacrificed by asphyxiation with CO<sub>2</sub>, the uterus was removed  
through a midline incision, and a wet uterine weight was  
determined.

30 Cholesterol Analysis. Blood samples were allowed to clot at  
room temperature for 2 hours, and serum was obtained  
following centrifugation for 10 minutes at 3000 rpm. Serum  
cholesterol was determined using a Boehringer Mannheim  
35 Diagnostics high performance cholesterol assay. Briefly the  
cholesterol was oxidized to cholest-4-en-3-one and hydrogen  
peroxide. The hydrogen peroxide was then reacted with

phenol and 4-aminophenazone in the presence of peroxidase to produce a p-quinone imine dye, which was read spectrophotometrically at 500 nm. Cholesterol concentration was then calculated against a standard curve. The entire  
5 assay was automated using a Biomek Automated Workstation.

Uterine Eosinophil Peroxidase (EPO) Assay. Uteri were kept at 4° C until time of enzymatic analysis. The uteri were then homogenized in 50 volumes of 50 mM Tris buffer (pH -  
10 8.0) containing 0.005% Triton X-100. Upon addition of 0.01% hydrogen peroxide and 10 mM O-phenylenediamine (final concentrations) in Tris buffer, increase in absorbance was monitored for one minute at 450 nm. The presence of  
15 eosinophils in the uterus is an indication of estrogenic activity of a compound. The maximal velocity of a 15 second interval was determined over the initial, linear portion of the reaction curve.

Source of Compound: 17 $\alpha$ -ethynyl estradiol was obtained  
20 from Sigma Chemical Co., St. Louis, MO.

#### Influence of Formula I Compounds on Serum Cholesterol and Determination of Agonist/Non-Agonist Activity

25 Data presented in Table 1 below show comparative results among ovariectomized rats, rats treated with 17 $\alpha$ -ethynyl estradiol (EE<sub>2</sub>; an orally available form of estrogen), and rats treated with certain compounds of the instant invention. Although EE<sub>2</sub> caused a decrease in serum  
30 cholesterol when orally administered at 0.1 mg/kg/day, it also exerted a stimulatory action on the uterus so that EE<sub>2</sub> uterine weight was substantially greater than the uterine weight of ovariectomized test animals. This uterine response to estrogen is well recognized in the art.

35 Not only did the compounds of the instant invention generally reduce serum cholesterol compared to the ovariectomized control animals, but uterine weight was only

minimally increased to slightly decreased with the majority of the formula compounds tested. Compared to estrogenic compounds known in the art, the benefit of serum cholesterol reduction without adversely affecting uterine weight is quite rare and desirable.

As is expressed in the data below, estrogenicity also was assessed by evaluating the adverse response of eosinophil infiltration into the uterus. The compounds of the instant invention did not cause any increase in the number of eosinophils observed in the stromal layer of ovariectomized rats, while estradiol cause a substantial, expected increase in eosinophil infiltration.

The data presented in Table 1 below reflects the response of 5 to 6 rats per treatment.

Table 1

<u>Compound</u>	<u>Dose mg/kg<sup>a</sup></u>	<u>Uterine Weight<sup>b</sup> (% increase vs. OVX)</u>	<u>Uterine EPO (V<sub>max</sub>)<sup>c</sup></u>	<u>Serum Cholesterol<sup>d</sup> (% decrease vs. OVX)</u>
EE <sub>2</sub> <sup>e</sup>	0.1	139.2	139.8	90.2
Example 1	0.01	71.3	17.4	5
	0.1	81.6	74.1	46.9
	1.0	97.2	113.7	65.2
	10.0	99.4	138.3	66.1

<sup>a</sup> mg/kg PO

<sup>b</sup> Uterine Weight % increase versus the ovariectomized controls

<sup>c</sup> Eosinophil peroxidase, V<sub>max</sub>

<sup>d</sup> Serum cholesterol decrease versus ovariectomized controls

<sup>e</sup> 17- $\alpha$ -Ethinyl-estradiol

\* p<.05

In addition to the demonstrated benefits of the compounds of the instant invention, the above data clearly demonstrate that compounds of formula I are not estrogen

mimetics. Furthermore, no deleterious toxicological effects (for example, survival numbers) were observed with any treatment.

#### 5 Osteoporosis Test Procedure

Following the General Preparation Procedure, *infra*, the rats are treated daily for 35 days (6 rats per treatment group) and sacrificed by carbon dioxide asphyxiation on the 36th day. The 35 day time period is sufficient to allow  
10 maximal reduction in bone density, measured as described herein. At the time of sacrifice, the uteri are removed, dissected free of extraneous tissue, and the fluid contents expelled before determination of wet weight in order to confirm estrogen deficiency associated with complete  
15 ovariectomy. Uterine weight is routinely reduced about 75% in response to ovariectomy. The uteri are then placed in 10% neutral buffered formalin to allow for subsequent histological analysis.

The right femurs are excised and digitized x-rays  
20 generated and analyzed by an image analysis program (NIH image) at the distal metaphysis. The proximal aspect of the tibiae from these animals is also scanned by quantitative computed tomography.

In accordance with the above procedures, compounds of  
25 the instant invention and ethynyl estradiol (EE<sub>2</sub>) in 20% hydroxypropyl  $\beta$ -cyclodextrin are orally administered to test animals. Results are reported as percent protection relative to ovariectomy.

In summary, ovariectomy of the test animals will cause  
30 a significant reduction in femur density compared to intact, vehicle treated controls. Orally administered ethynyl estradiol (EE<sub>2</sub>) prevented this loss, but the risk of uterine stimulation with this treatment is ever-present.

#### 35 MCF-7 Proliferation Assay

MCF-7 breast adenocarcinoma cells (ATCC HTB 22) were maintained in MEM (minimal essential medium, phenol red-

free, Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS) (V/V), L-glutamine (2 mM), sodium pyruvate (1 mM), HEPES {(N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]10 mM)}, non-essential amino acids and  
5 bovine insulin (1 ug/mL) (maintenance medium). Ten days prior to assay, MCF-7 cells were switched to maintenance medium supplemented with 10% dextran coated charcoal stripped fetal bovine serum (DCC-FBS) assay medium) in place of 10% FBS to deplete internal stores of steroids. MCF-7  
10 cells were removed from maintenance flasks using cell dissociation medium (Ca<sup>++</sup>/Mg<sup>++</sup> free HBSS (phenol red-free) supplemented with 10 mM HEPES and 2 mM EDTA). Cells were washed twice with assay medium and adjusted to 80,000 cells/mL. Approximately 100 mL (8,000 cells) were added to  
15 flat-bottom microculture wells (Costar 3596) and incubated at 37° C in a 5% CO<sub>2</sub> humidified incubator for 48 hours to allow for cell adherence and equilibration after transfer. Serial dilutions of drugs or DMSO as a diluent control were prepared in assay medium and 50 mL transferred to triplicate  
20 microcultures followed by 50 mL assay medium for a final volume of 200 mL. After an additional 48 hours at 37° C in a 5% CO<sub>2</sub> humidified incubator, microcultures were pulsed with tritiated thymidine (1 μCi/well) for 4 hours. Cultures were terminated by freezing at -70° C for 24 hours followed  
25 by thawing and harvesting of microcultures using a Skatron Semiautomatic Cell Harvester. Samples were counted by liquid scintillation using a Wallace BetaPlace β counter. Results obtained from this assay indicated a compound of Example 1 was a 4.5 nM inhibitor of MCF-7 breast cancer  
30 proliferation..

#### DMBA-Induced Mammary Tumor Inhibition

Estrogen-dependent mammary tumors are produced in female Sprague-Dawley rats which are purchased from Harlan  
35 Industries, Indianapolis, Indiana. At about 55 days of age, the rats receive a single oral feeding of 20 mg of 7,12-dimethylbenzo[a]anthracene (DMBA). About 6 weeks after DMBA

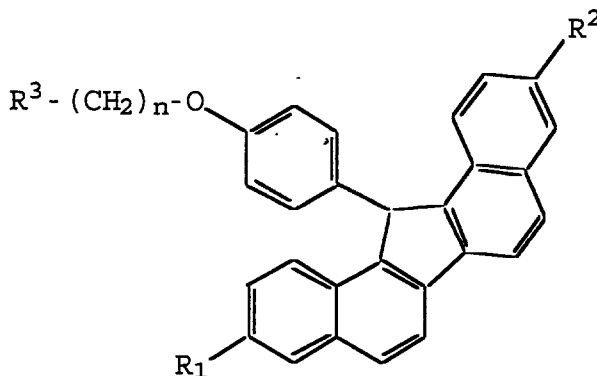
administration, the mammary glands are palpated at weekly intervals for the appearance of tumors. Whenever one or more tumors appear, the longest and shortest diameters of each tumor are measured with a metric caliper, the  
5 measurements are recorded, and that animal is selected for experimentation. An attempt is made to uniformly distribute the various sizes of tumors in the treated and control groups such that average-sized tumors are equivalently distributed between test groups. Control groups and test  
10 groups for each experiment contain 5 to 9 animals.

Compounds of Formula I are administered either through intraperitoneal injections in 2% acacia, or orally. Orally administered compounds are either dissolved or suspended in 0.2 mL corn oil. Each treatment, including acacia and corn  
15 oil control treatments, is administered once daily to each test animal. Following the initial tumor measurement and selection of test animals, tumors are measured each week by the above-mentioned method. The treatment and measurements of animals continue for 3 to 5 weeks at which time the final  
20 areas of the tumors are determined. For each compound and control treatment, the change in the mean tumor area is determined.



The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

- 5 1. A compound of formula I:



10

I

wherein:

$R^1$  is -H, -OH, -O(C<sub>1</sub>-C<sub>4</sub> alkyl),  
 -OCO(C<sub>1</sub>-C<sub>6</sub> alkyl), -O(CO)O(C<sub>1</sub>-C<sub>6</sub> alkyl), -OCOAr, -O(CO)OAr,  
 15 where Ar is phenyl or optionally substituted phenyl, or -  
 OSO<sub>2</sub>(C<sub>2</sub>-C<sub>6</sub> alkyl);

$R^2$  is -H, -OH, -O(C<sub>1</sub>-C<sub>4</sub> alkyl),  
 -OCO(C<sub>1</sub>-C<sub>6</sub> alkyl), -O(CO)O(C<sub>1</sub>-C<sub>6</sub> alkyl), -OCOAr, -O(CO)OAr,  
 where Ar is phenyl or optionally substituted phenyl, or -  
 20 OSO<sub>2</sub>(C<sub>2</sub>-C<sub>6</sub> alkyl);

$R^3$  is 1-piperidinyl, 1-pyrrolidinyl, methyl-1-pyrrolidinyl, dimethyl-1-pyrrolidinyl, 4-morpholino, dimethylamino, diethylamino, or 1-hexamethyleneimino; and  
 n is 2 or 3;

25 or a pharmaceutically acceptable salt or solvate thereof.

2. A compound according to Claim 1 wherein n is two.

3. A compound according to Claim 1 wherein  $R^3$  is  
 30 1-piperidinyl.

4. A pharmaceutical composition comprising a compound according to Claims 1-3, or a pharmaceutically acceptable salt thereof, and optionally an effective amount of estrogen or progestin, in combination with a pharmaceutically acceptable carrier, diluent, or excipient.

5. A compound of formula I as claimed in any of Claims 1-3 for use in alleviating the symptoms of postmenopausal syndrome.

6. A compound of formula I as claimed in any of Claims 1-3 for use in alleviating the symptoms of the postmenopausal syndrome pathological condition of osteoporosis.

7. A compound of formula I as claimed in any of Claims 1-3 for use in alleviating the symptoms of the postmenopausal syndrome pathological condition related to a cardiovascular disease.

8. A compound of formula I as claimed in any of Claims 1-3 for use in alleviating the symptoms of a cardiovascular disease related to hyperlipidemia.

9. A compound of formula I as claimed in any of Claims 1-3 for use in alleviating the symptoms of the postmenopausal syndrome pathological condition of estrogen-dependent cancer.

10. A compound of formula I as claimed in any of Claims 1-3 for use in alleviating the symptoms of breast or uterine cancer.

11. A compound of formula I as claimed in any of Claims 1-3 for use in alleviating the symptoms of uterine fibroid disease.

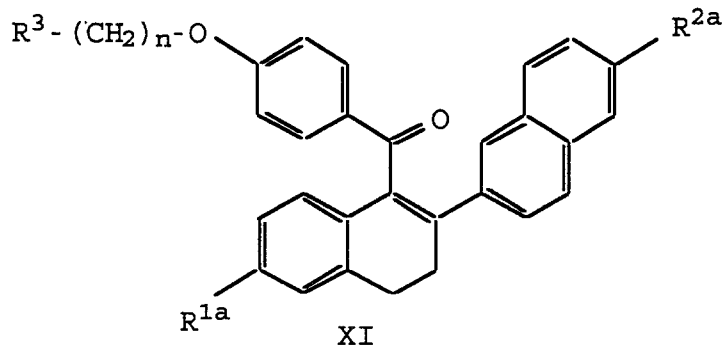
12. A compound of formula I as claimed in any of Claims 1-3 for use in alleviating the symptoms of endometriosis.

5 13. A compound of formula I as claimed in any of Claims 1-3 for use in alleviating the symptoms of aortal smooth muscle cell prolifiration.

10 14. A compound of formula I as claimed in any of Claims 1-3 for use in alleviating the symptoms of restenosis.

15. A compound of formula XI:

15



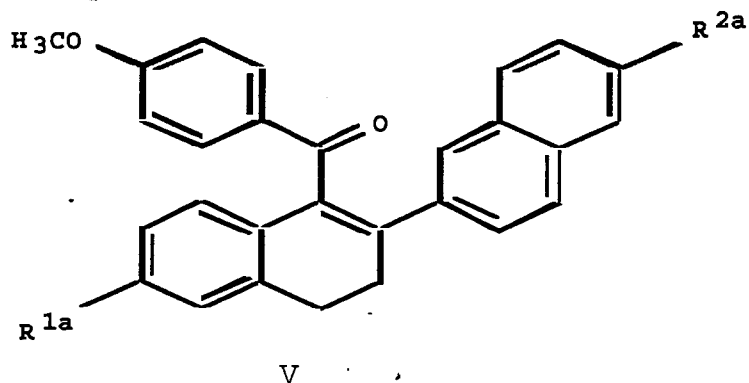
wherein:

20 R<sup>1a</sup> is -H or -OR<sup>5</sup>, wherein R<sup>5</sup> is a hydroxy protecting group;

R<sup>2a</sup> is -H, -OR<sup>6</sup>, wherein R<sup>6</sup> is a hydroxy protecting group, or halo;

25 R<sup>3</sup> is 1-piperidinyl, 1-pyrrolidinyl, methyl-1-pyrrolidinyl, dimethyl-1-pyrrolidinyl, 4-morpholino, dimethylamino, diethylamino, or 1-hexamethyleneimino; and n is 2 or 3.

16. A compound of formula V:



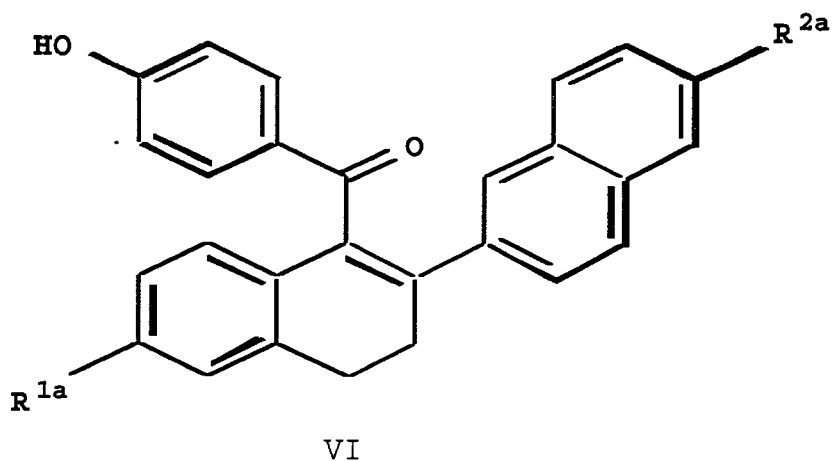
5 wherein:

R<sup>1a</sup> is -H or -OR<sup>5</sup>, wherein R<sup>5</sup> is a hydroxy protecting group; and

R<sup>2a</sup> is -H, -OR<sup>6</sup>, wherein R<sup>6</sup> is a hydroxy protecting group, or halo.

10

17. A compound of formula VI:

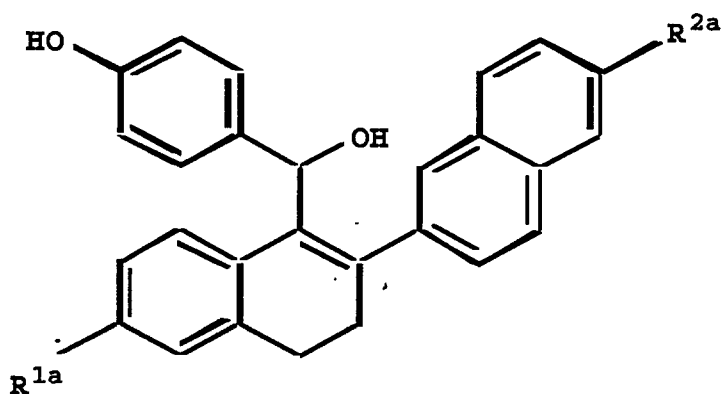


15 wherein:

R<sup>1a</sup> is -H or -OR<sup>5</sup>, wherein R<sup>5</sup> is a hydroxy protecting group; and

R<sup>2a</sup> is -H, -OR<sup>6</sup>, wherein R<sup>6</sup> is a hydroxy protecting group, or halo.

18. A compound of formula VII:



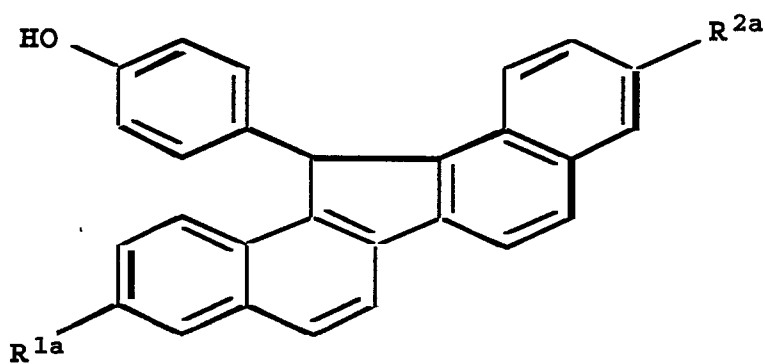
VII

wherein:

R<sup>1a</sup> is -H or -OR<sup>5</sup>, wherein R<sup>5</sup> is a hydroxy protecting group; and

10 R<sup>2a</sup> is -H, -OR<sup>6</sup>, wherein R<sup>6</sup> is a hydroxy protecting group, or halo.

19. A compound of formula II:



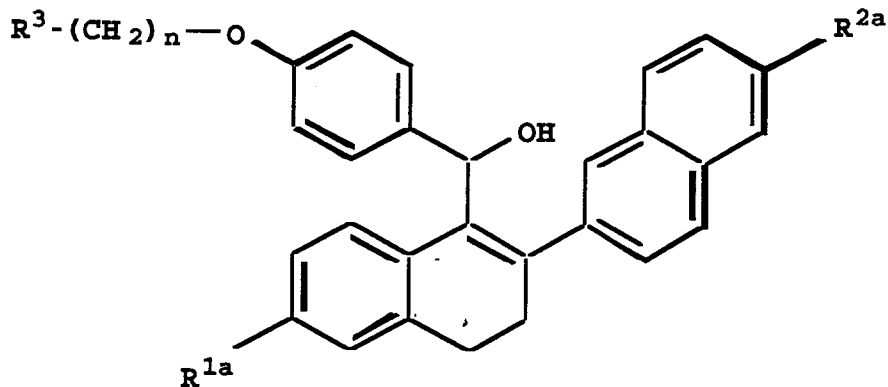
II

wherein:

R<sup>1a</sup> is -H or -OR<sup>5</sup>, wherein R<sup>5</sup> is a hydroxy protecting group; and

20 R<sup>2a</sup> is -H, -OR<sup>6</sup>, wherein R<sup>6</sup> is a hydroxy protecting group, or halo.

20. A compound of formula XII:



XII

wherein:

R<sup>1a</sup> is -H or -OR<sup>5</sup>, wherein R<sup>5</sup> is a hydroxy protecting group;

R<sup>2a</sup> is -H, -OR<sup>6</sup>, wherein R<sup>6</sup> is a hydroxy protecting group, or halo.

R<sup>3</sup> is 1-piperidinyl, 1-pyrrolidinyl, methyl-1-pyrrolidinyl, dimethyl-1-pyrrolidinyl, 4-morpholino, dimethylamino, diethylamino, or 1-hexamethyleneimino; and n is 2 or 3.