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(54) **TETRAHYDROBENZO-A-FLUORENES ET METHODE
D'UTILISATION**

(54) **TETRAHYDROBENZO-A-FLUORENE COMPOUNDS AND
METHOD OF USE**

(57) Tétrahydrobenzo[a]fluorènes, formulations 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.

(57) The invention provides tetrahydrobenzo[a]fluorene compounds, formulations, and methods of inhibiting bone loss or bone resorption, particularly osteoporosis, and cardiovascular-related pathological conditions, including hyperlipidemia.

Abstract

The invention provides tetrahydrobenzo[a]fluorene compounds, formulations, and methods of inhibiting bone loss or bone resorption, particularly osteoporosis, and cardiovascular-related pathological conditions, including hyperlipidemia.

TETRAHYDROBENZO[A]FLUORENE COMPOUNDS AND METHODS OF USE

Osteoporosis describes a group of diseases which arises from diverse etiologies, but which are characterized by the 5 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 associated with menopause. Most women lose from about 20% 10 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 resorptive cycle is more dominant and the result is a net 15 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 results of osteoporosis are personally harmful, and also 20 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. Additionally, although osteoporosis is generally 25 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.

The most vulnerable tissue in the bone to the effects 30 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 trabecular tissue is characterized by small osteoid 35 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 5 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 10 example, the vertebrae, the neck of the weight-bearing bones such as the femur and the forearm. Indeed, hip fracture, collar fractures, and vertebral crush fractures are hallmarks of postmenopausal osteoporosis.

The most generally accepted method for the treatment 15 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 20 administration of a bisphosphonate compound, such as, for example, Fosamax[®] (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 25 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 30 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 35 beneficial effects on cardiovascular health.

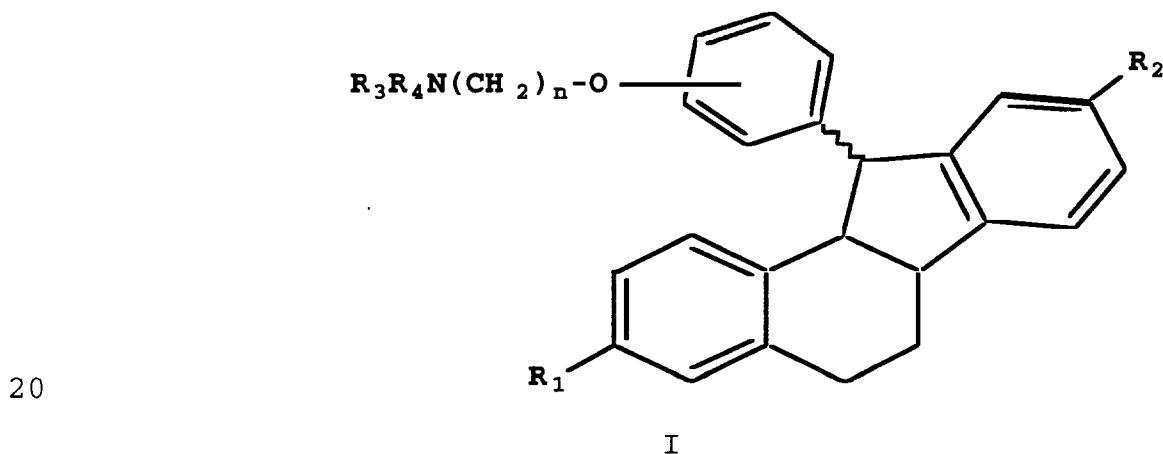
It has been reported in the literature that serum lipid levels in postmenopausal women having estrogen replacement

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therapy return to concentrations found in the premenopausal state. Thus, estrogen would appear to be a reasonable 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 which is devoid of the side effects and risks associated with estrogen therapy.

The instant invention provides tetrahydrobenzo[a]fluorene compounds, pharmaceutical formulations thereof, and methods of using such compounds for the treatment of postmenopausal syndrome and other estrogen-related pathological conditions such as those mentioned below.

The instant invention relates to compounds of formula I:



wherein:

R₁ is -H, -OH, -O(C₁-C₄ alkyl), -OCO(C₁-C₆ alkyl), -O(CO)O(C₁-C₆ alkyl), -COAr, -OCOAr, -O(CO)OAr, where Ar is phenyl or optionally substituted phenyl, or -OSO₂-(C₄-C₆ alkyl);

R₂ is -OH, -O(C₁-C₄ alkyl), -OCO(C₁-C₆ alkyl), -O(CO)O(C₁-C₆ alkyl), -COAr, -OCOAr, -O(CO)OAr, where Ar is phenyl or optionally substituted phenyl, or -OSO₂-(C₄-C₆ alkyl);

5 R₃ and R₄ are each independently -C₁-C₄ alkyl, or combine to form 1-piperidinyl, 1-pyrrolidinyl, methyl-1-pyrrolidinyl, dimethyl-1-pyrrolidinyl, 4-morpholino, dimethylamino, diethylamino, or 1-hexamethyleneimino;

n is 2 or 3;

10 or a pharmaceutically acceptable salt or solvate thereof.

The instant invention further provides pharmaceutical formulations containing compounds of formula I, and the use of said compounds at least for the inhibition of bone loss
15 or bone resorption, particularly osteoporosis and cardiovascular-related pathological conditions, including hyperlipidemia, and other cardiovascular pathologies.

20 General terms used in the description of compounds herein described bear their usual meanings. For example, "C₁-C₆ 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, 25 isohexyl, and the like. Similarly, the term "-OC₁-C₄ alkyl" represents a C₁-C₄ alkyl group attached through an oxygen such as, for example, methoxy, ethoxy, n-propoxy, isopropoxy, and the like. Of these C₁-C₄ alkoxy groups, methoxy is preferred.

30 The term "substituted phenyl" refers to a phenyl group having one or more substituents selected from the group consisting of C₁-C₄ alkyl, -OC₁-C₄ alkyl, hydroxy, nitro, chloro, fluoro, or tri(chloro or fluoro)methyl.

The term "hydroxy protecting group" contemplates 35 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, alkylsilyloxys, -OC₁-C₄ alkyls, and the like. Numerous reactions for the formation and removal of such protecting groups are described in a number of standard works

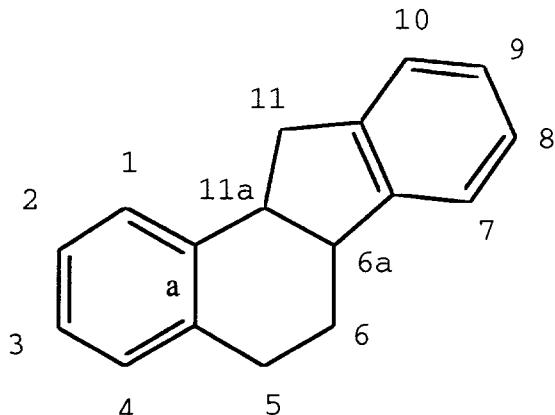
5 including, for example, *Protective Groups in Organic Chemistry*, Plenum Press (London and New York, 1973); Green, T.W., *Protective Groups in Organic Synthesis*, Wiley, (New York, 1981); and *The Peptides*, Vol. I, Schroeder and Lubke, Academic Press (London and New York, 1965).

10 The term "substituted phenyl" refers to a phenyl group having one or more substituents selected from the group consisting of C₁-C₄ alkyl, C₁-C₃ alkoxy, hydroxy, nitro, chloro, fluoro, or tri(chloro or fluoro)methyl. "C₁-C₃ alkoxy" refers a C₁-C₃ alkyl group attached through an 15 oxygen bridge such as, methoxy, ethoxy, n-propoxy, iso-propoxy.

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.

20 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.

25 Compounds of the present invention are named as derivatives of benzo[a]fluorene in accordance to the Ring Index, The American Chemical Society, as follows:

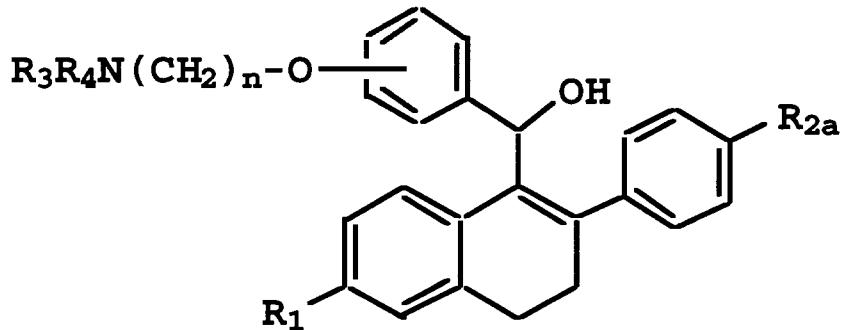


Thus, a compound of formula I, wherein R₁ and R₂ are methoxy, R₃ and R₄ combine to form 1-piperidinyl, and n is two, is named as follows:

1-[2-[4-(6,6a,11,11a-tetrahydro-3,9-dimethoxy-5H-5
benzo[a]fluoren-11-yl)phenoxy]ethyl]piperidine. Additional
representative compounds of the instant invention include 1-[2-[4-(6,6A,11,11A-tetrahydro-9-methoxy-5H-benzo[a]fluoren-11-yl)phenoxy]ethyl]pyrrolidine,
1-[2-[4-(6,6A,11,11A-tetrahydro-9-methoxy-5H-5
benzo[a]fluoren-11-yl)phenoxy]ethyl]piperidine, and
1-[2-[4-(6,6A,11,11A-tetrahydro-3,9-dimethoxy-5H-5
benzo[a]fluoren-11-yl)phenoxy]ethyl]pyrrolidine.

Included within the scope of the invention are the various stereochemical isomers at the 6A, 11, and 11A carbon atoms.

Compounds of formula I may be prepared from their corresponding 3,4-dihydroronaphthylmethanol precursors, such as, for example, a compound of formula II, by a chemically unique re-arrangement and reduction.



wherein:

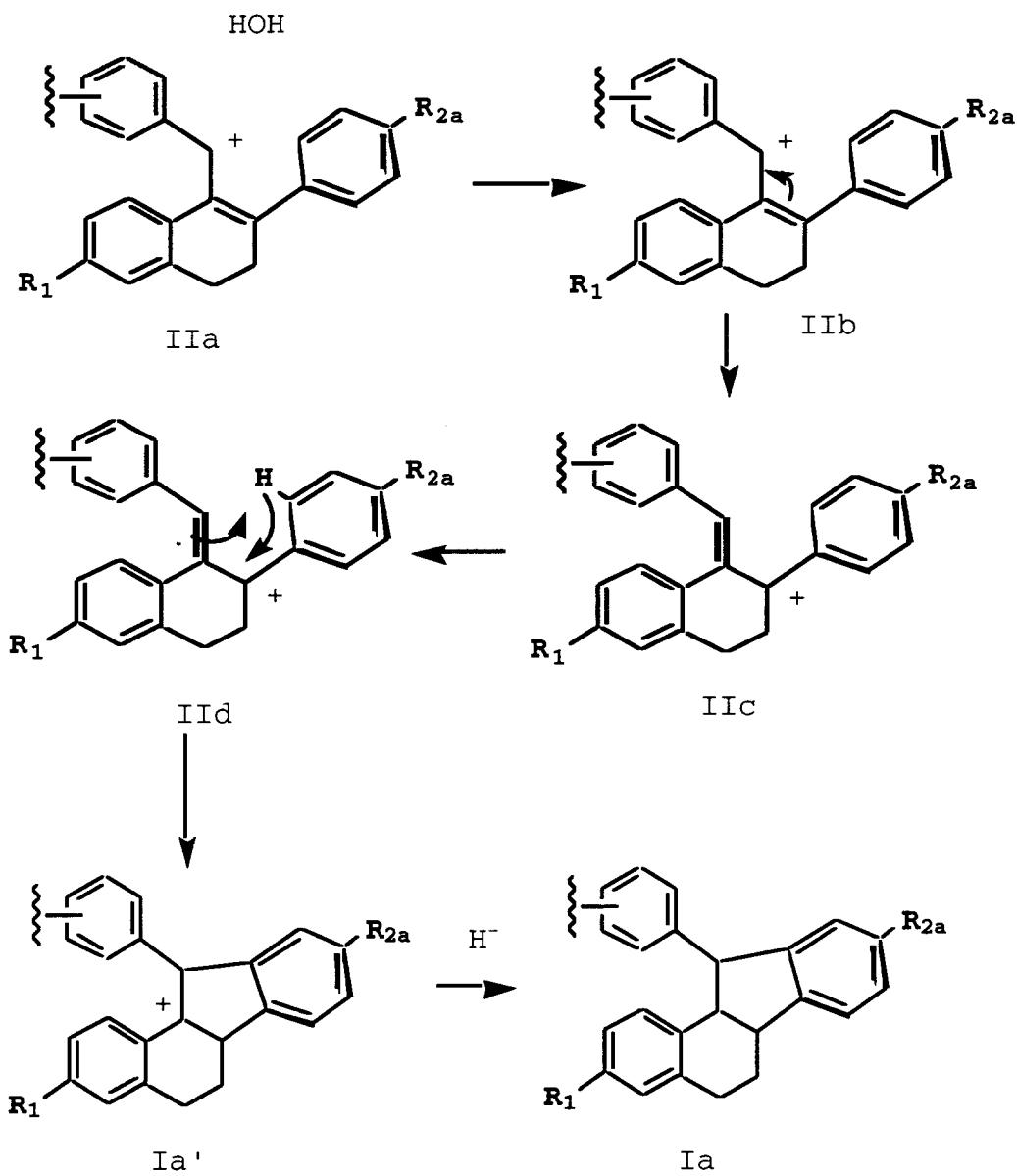
R_{2a} is hydroxy or -O(C₁-C₄ alkyl); and

R₁, R₃, R₄, and n have their previous meanings.

25 A compound of formula II is treated with a trialkylsilane reducing agent, such as triethylsilane, in the presence of a strong acid, such as trifluoroacetic acid, in THF at ambient temperature. The strong acid protonates the secondary alcohol, which then undergoes the elimination of water, thus generating a carbonium ion. The 1,2 double
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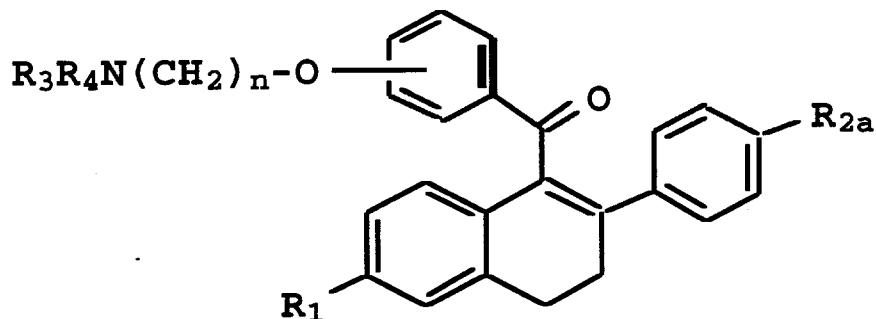
bond of the dihydronaphthyl system migrates to the exo-cyclic position, regenerating the carbonium ion at the 2-position of the naphthyl system. The system then undergoes a rearrangement, whereby the exo-cyclic double bond migrates 5 to form a bridge between the carbinol carbon and the 2-position of the phenyl ring, thus forming the benzo[a]fluorenyl system. At the same time, the hydrogen on the 2-position of the phenyl ring undergoes a 1,3 shift to the 2-position of the naphthalene. This re-arrangement 10 generates the benzo[a]fluorenyl system with the carbonium ion at the 11a-position. The final ion is then reduced by the silane to yield the final product, as illustrated in Scheme I below. As a result of the mechanism of this reaction, the stereochemical centers are not 15 stereochemically controlled, thus resulting in a mixture of isomers (for example, the 6a, 11, and 11a-positions). R_{2a} in formula II must be hydroxy or -O(C₁-C₄ alkyl), with methoxy being preferred. If other substituents are used, the above-described reaction does not occur, but instead, 20 the compounds of formula II form naphthalenes via the elimination of water and aromatization.

Scheme I



The compounds of formula II are obtained by a reduction of a compound of formula III with LiAlH₄, NaBH₄, or the like, in an appropriate solvent at temperatures of 0-30°C. The methods for this reduction are disclosed in U.S. Pat. No. 5,484,797, the disclosure of which is herein incorporated by reference.

The compounds of formula III are synthesized by methods known in the art.



III

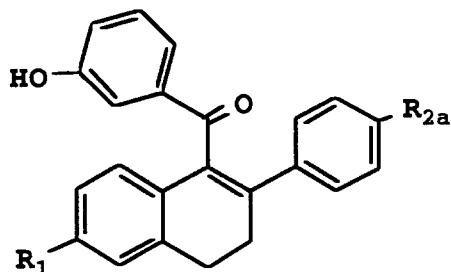
5 wherein R_1 , R_{2a} , R_3 , R_4 , and n have their previous meanings.

Specifically, the compounds of formula III, wherein R_{2a} is $-\text{O}(\text{C}_1\text{-C}_4 \text{ alkyl})$ or $-\text{OH}$ and the basic side chain is in the 4(*para*)-position of the benzoyl moiety, are synthesized by methods as provided in U.S. Pat. Nos. 4,230,862 and

10 5,147,880 and also in Jones, C.D., et al., *J. Med. Chem.*, 22, 962-966 (1979) and Jones C.D., et al., *J. Med. Chem.*

35(5), 931-938 (1992), the disclosures of each of which are herein incorporated by reference. The compounds of formula III, wherein R_{2a} is $-\text{O}(\text{C}_1\text{-C}_4 \text{ alkyl})$ or $-\text{OH}$ and the basic

15 side chain is in the 3(*meta*)-position of the benzoyl moiety, are synthesized from a compound of formula IV via the methods of Jones, *ibid.*



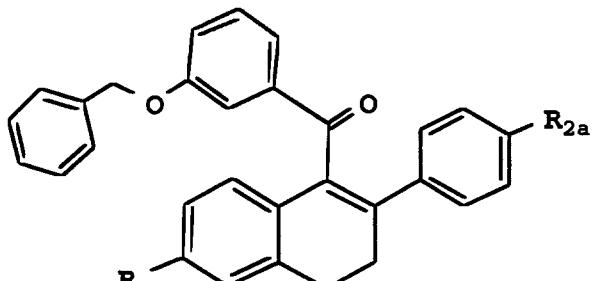
IV

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wherein R_1 and R_{2a} have their previous meanings.

The compounds of formula IV may be prepared by the methods of Jones, *ibid.* starting with the reaction of the 25 appropriate beta-tetralone with sodium hydride and 3-

benzyloxybenzoyl phenylester to form a compound of formula V.



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V

wherein R1 and R2a have their previous meanings.

A compound of formula V is then converted to a compound of formula IV by removal of the benzyl protecting group with catalytic hydrogenation, typically by refluxing in 10% EtOH in toluene with tetrakis(triphenylphosphine) palladium (0) for several hours. (See: Barton, J.W., "Protective Groups in Organic Chemistry", McOhmie, W. Ed., Plenum Press, NYC, 1973, Chapter 2 or Green, T.W., "Protective Groups in Organic Synthesis", John Wiley and Sons, NYC, 1981, Chapter 7.) Other derivatives of formula I wherein R1 and R2 are esters of sulfonates are prepared from the phenolic starting material by methods known in the art, such as, for example U.S. Pat. No. 5,393,763, the disclosure of which is herein incorporated by reference. The phenols are prepared from the methoxy precursors by de-alkylation with AlCl₃ and EtSH in CH₂Cl₂ via methods as described in Jones *supra*.

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 5 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, 10 phosphoric, hypophosphoric, and the like. Salts derived from organic acids, such as aliphatic mono and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxyalkanoic and hydroxyalkadioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, may also be used. Such 15 pharmaceutically acceptable salts thus include acetate, phenylacetate, trifluoroacetate, acrylate, ascorbate, benzoate, chlorobenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, methylbenzoate, o-acetoxybenzoate, naphthalene-2-benzoate, bromide, isobutyrate, 20 phenylbutyrate, β -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, 25 nitrate, oxalate, phthalate, terephthalate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, propiolate, propionate, phenylpropionate, salicylate, sebacate, succinate, suberate, sulfate, bisulfate, pyrosulfate, sulfite, bisulfite, sulfonate, 30 benzenesulfonate, *p*-bromophenylsulfonate, chlorobenzenesulfonate, ethanesulfonate, 2-hydroxyethanesulfonate, methanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, *p*-toluenesulfonate, xylenesulfonate, tartarate, and the like. A preferred salt 35 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 5 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 10 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, alleviating, ameliorating, treating, or 15 preventing further symptoms in mammals, including humans, suffering from bone loss or bone resorption, particularly osteoporosis, and cardiovascular-related pathological conditions including hyperlipidemia and related cardiovascular pathologies.

20 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 25 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 30 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 35 such as agar agar, calcium carbonate, and sodium bicarbonate; agents for retarding dissolution such as paraffin; resorption accelerators such as quaternary

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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.

10 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 15 period of time. Such formulations would involve coatings, envelopes, or protective matrices which may be made from polymeric substances or waxes.

10 The particular dosage of a compound of formula I required to treat, inhibit, or prevent 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 25 effective doses will be from 15mg to 1000mg, and more typically from 15mg to 80mg, from 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 chronically.

30 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 "active ingredient" means a compound of formula I.

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Formulation 1: Gelatin Capsules

Ingredient	Quantity (mg/capsule)
Active Ingredient	0.1-1000
5 Starch NF	0-500
Starch flowable powder	0-500
Silicone fluid 350 centistokes	0-15
The ingredients are blended, passed through a No. 45 mesh U.S. sieve, and filled into hard gelatin capsules.	

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Formulation 2: Tablets

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

The active ingredient, starch, and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The 25 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, 30 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.

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Formulation 3: Aerosol

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

10 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
15 units are then fitted to the container.

Formulation 4: Suppositories

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

25 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.

30 Formulation 5: Suspension

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

35 Ingredient	Weight
Active Ingredient	0.1-1000 mg

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

10 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.

15 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 20 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.

25 NMR data for the following Examples were generated on a GE 300 MHz NMR instrument, and anhydrous CDCl_3 was used as the solvent unless otherwise indicated. Field strength for ^{13}C NMR spectra was 75.5 MHz, unless otherwise indicated.

Preparation 1

30 [2-(4-Methoxyphenyl)-3,4-dihydronaphth-1-yl][4-(1-pyrrolidinyl-2-ethoxy)phenyl]carbinol

35 850mg (1.9 mmol) of [2-(4-methoxyphenyl)-3,4-dihydronaphth-1-yl][4-(1-pyrrolidinyl-2-ethoxy)phenyl]methanone was dissolved in 100mL of THF and 160mg (4 mmol) of LiAlH_4 was added. The reaction mixture was stirred at

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room temperature and under a N₂ atmosphere for eighteen hours. The reaction mixture was evaporated to dryness *in vacuo*. Fifty mL of water was carefully added to the residue and the reaction mixture was extracted twice with 100 mL of EtOAc. The EtOAc extracts were combined and washed with water, then the EtOAc layer was removed and dried by filtration through anhydrous Na₂SO₄ and evaporated to dryness *in vacuo*. This yielded 670 mg of the title compound as a white amorphous powder.

10 PMR: consistent with the proposed structure

MS: m/e=455 (M⁺) FD

EA: Calc: C, 79.2; H, 7.26; N, 3.08 Found: C, 79.11; H, 7.47; N, 2.93

C₃₀H₃₃NO₃

15 R_f=0.46 silica gel eluted with CHCl₃-MeOH (19:1)

Example 1

1-[2-[4-(6,6A,11,11A-Tetrahydro-9-methoxy-5H-benzo[a]fluoren-11-yl)phenoxy]ethyl]pyrrolidine

20 1.8g (0.004 mol) of [2-(4-methoxyphenyl)-3,4-dihydronaphth-1-yl][4-(1-pyrrolidinyl-2-ethoxy)phenyl] carbinol was dissolved in 100mL of CH₂Cl₂ and 0.6g (0.005 mol) of Et₃SiH was added. The reaction mixture was stirred 25 for several minutes under a N₂ atmosphere. 25 mL of CF₃COOH was slowly added and the reaction was allowed proceed for eighteen hours at room temperature. The reaction mixture was evaporated to dryness *in vacuo*. The reaction residue was dissolved in 100mL of hot water and 25 mL of MeOH. This solution was made basic with the addition 30 of saturated aqueous NaHCO₃ solution and extracted twice with 100 ml portions of EtOAc. The EtOAc extracts were combined and dried by filtration through anhydrous Na₂SO₄ and evaporated to dryness *in vacuo*. This resulted in 1.23g 35 of the title compound as a tan, amorphous powder.
R_f= 0.62 silica gel eluted with CHCl₃-MeOH (19:1)

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Example 2

1-[2-[4-(6,6A,11,11A-Tetrahydro-9-methoxy-5H-benzo[a]fluoren-11-yl)phenoxy]ethyl]pyrrolidine hydrochloride

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1.23g of 1-[2-[4-(6,6A,11,11A-tetrahydro-9-methoxy-5H-benzo[a]fluoren-11-yl)phenoxy]ethyl]pyrrolidine was dissolved in 10 mL of EtOAc and a saturated solution of HCl-10 Et₂O was added until the product stopped precipitating out.

The liquid was decanted off and the remaining solid was triturated (2x) with Et₂O. The solid was dried *in vacuo* at room temperature for several hours. This resulted in 960 mg of the title compound as a pink, amorphous powder.

15 PMR: consistent with the proposed structure

EA: Calc: C, 75.69; H, 7.20; N, 2.94 Found: C, 75.42; H, 7.43; N, 2.85

C₃₀H₃₄ClNO₂

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Example 3

[2-(4-Methoxyphenyl)-3,4-dihydro-6-methoxynaphth-1-yl][4-(1-piperidinyl-2-ethoxy)phenyl]carbinol

4.45g (0.009 mol) of [2-(4-methoxyphenyl)-3,4-dihydro-25 6-methoxynaphth-1-yl][4-(1-piperidinyl-2-ethoxy) phenyl] methanone was dissolved in 100 mL of THF and 1g (0.026 mol) of LiAlH₄ was carefully added. The reaction mixture was stirred at room temperature and under N₂ for eighteen hours. The reaction mixture was evaporated to dryness and 100 mL of 30 water was carefully added. The reaction mixture was extracted twice with 100 mL portions of EtOAc. The combined EtOAc extracts were dried by filtration through anhydrous Na₂SO₄. The EtOAc extract was evaporated to dryness *in vacuo*. This yielded 3.38g of the title compound as a white, 35 amorphous powder.

MS: m/e=500 (M+1) FD

R_f = 0.55 silica gel eluted with CHCl₃-MeOH (19:1)

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Example 4

1-[2-[4-(6,6A,11,11A-Tetrahydro-3,9-dimethoxy-5H-benzo[a]fluoren-11-yl)phenoxy]ethyl]piperidine

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2.5g (0.005 mol) of [2-(4-Methoxyphenyl)-3,4-dihydro-6-methoxynaphth-1-yl][4-(1-piperidinyl-2-ethoxy)phenyl] carbinol was dissolved in 100 mL of CH₂Cl₂ and 810 mg (0.007 mol) of Et₃SiH was added. The reaction mixture was stirred at room temperature and under N₂ for several minutes. 12 mL of CF₃COOH was slowly added. The reaction was allowed to proceed for thirty-six hours. The reaction mixture was evaporated to dryness. The reaction mixture was dissolved in 100 mL of CH₂Cl₂ and was washed with 100 mL of 1N NaOH and the CH₂Cl₂ layer was removed. The CH₂Cl₂ solution was dried by filtration through anhydrous Na₂SO₄ and evaporated to dryness. The residue was crystallized from EtOAc and Hexane. This resulted in 240 mg of the title compound as a white crystalline powder.

10 20 PMR: consistent with the proposed structure

MS: m/e=484 (M+) FD

EA: Calc: C, 79.47; H, 7.71; N, 2.90 Found: C, 75.14; H, 7.28; N, 2.94
C₃₂H₃₇NO₃

25

Example 5

[2-(4-Methoxyphenyl)-3,4-dihydronaphth-1-yl][4-(1-piperidinyl-2-ethoxy)phenyl]carbinol

30 2g (0.00335 mol) of [2-(4-methoxyphenyl)-3,4-dihydronaphth-1-yl][4-(1-piperidinyl-2-ethoxy)phenyl] methanone mesylate was suspended in 100 mL of THF. 1g (0.026 mol) of LiAlH₄ was slowly added and the reaction was stirred and allowed to proceed at room temperature and under N₂ for eighteen hours. The reaction mixture was evaporated to dryness *in vacuo*. 100 mL of EtOAc was added and 50 mL of water was slowly added. The EtOAc layer was removed and

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the cloudy aqueous layer was extracted twice more with 100 mL portions of EtOAc. All of the EtOAc extracts were combined and dried by filtration through anhydrous Na₂SO₄ and evaporated to dryness. The resulting, glassy residue
5 was chromatographed (HPLC) on a silica gel column eluted with a linear gradient starting with CHCl₃ and ending with CHCl₃ - MeOH (3:1, v/v). The fractions containing the desired product were determined by tlc and combined and evaporated to dryness. This resulted in 1g of the title
10 compound as a white, amorphous powder.

PMR: consistent with the proposed structure

MS: m/e= 469 (M+) FD

R_f= 0.62 silica gel eluted with CHCl₃-MeOH (19:1)

15

Example 6

1-[2-[4-(6,6A,11,11A-Tetrahydro-9-methoxy-5H-benzo[a]fluoren-11-yl)phenoxy]ethyl]piperidine

1g (0.002 mol) of [2-(4-methoxyphenyl)-3,4-
20 dihydronaphth-1-yl][4-(1-piperidinyl-2-ethoxy)phenyl] carbinol was dissolved in 50 mL of CH₂Cl₂ and 460 mg (0.004 mol) of Et₃SiH was added. The reaction mixture was stirred for several minutes at room temperature and under N₂. 12 mL of CF₃COOH was slowly added. The reaction was allowed to
25 proceed for seventy-two hours. The reaction mixture was evaporated to dryness. 50 mL of CH₂Cl₂ was added and the solution was extracted with 50 mL of 1 N NaOH. The organic layer was dried with Na₂SO₄ and evaporated to dryness. The residue was crystallized from Et₂O-Hexane. This resulted in
30 270 mg of the title compound as a tan, crystalline powder.

PMR: consistent with the proposed structure

MS: 454 (M+) FD

EA: Calc: C, 82.08; H, 7.78; N, 3.09 Found: C, 81.95; H,
7.87; N, 3.10

35 C₃₁H₃₅NO₂

Test Procedures

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

5 Seventy-five day old female Sprague Dawley rats (weight 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 10 surgical procedure at Charles River Laboratories, and then shipped after one week. Upon arrival, they were housed in 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 15 22.2° ± 1.7° C with a minimum relative humidity of 40%. The photoperiod in the room was 12 hours light and 12 hours dark.

Dosing Regimen Tissue Collection. After a one week 20 acclimation period (therefore, two weeks post-OVX) daily dosing with test compound was initiated. 17 α -ethynodiol or the test compound were given orally, unless otherwise stated, as a suspension in 1% carboxymethylcellulose or dissolved in 20% cyclodextrin. 25 Animals were dosed daily for 4 days. Following the dosing regimen, animals were weighed and anesthetized with a ketamine: Xylazine (2:1, V:V) mixture and a blood sample was collected by cardiac puncture. The animals were then sacrificed by asphyxiation with CO₂, the uterus was removed 30 through a midline incision, and a wet uterine weight was determined.

Cholesterol Analysis. Blood samples were allowed to clot at room temperature for 2 hours, and serum was obtained 35 following centrifugation for 10 minutes at 3000 rpm. Serum cholesterol was determined using a Boehringer Mannheim 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

5 spectrophotometrically at 500 nm. Cholesterol concentration was then calculated against a standard curve.

Uterine Eosinophil Peroxidase (EPO) Assay. Uteri were kept at 4° C until time of enzymatic analysis. The uteri were

10 then homogenized in 50 volumes of 50 mM Tris buffer (pH - 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.

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

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

Data presented in Table 1 below show comparative results among ovariectomized rats, rats treated with 17 α -ethynyl estradiol (EE₂; an orally available form of estrogen), and rats treated with certain compounds of the instant invention. Although EE₂ caused a decrease in serum cholesterol when orally administered at 0.1 mg/kg/day, it also exerted a stimulatory action on the uterus so that EE₂ uterine weight was substantially greater than the uterine weight of ovariectomized test animals. This uterine response to estrogen is well recognized in the art.

30 Not only did the compounds of the instant invention generally reduce serum cholesterol compared to the

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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

Compound No.	Dose mg/kg ^a	Uterine Weight % Inc ^b	Uterine Eosinophil (Vmax) ^c	Serum Cholest. % Dec. ^d
EE2 ^e	0.1	206.1*	155.2*	97.9*
Compound 3	0.1	47.9*	28.1*	58.6*
	1.0	53.3*	92.8*	65.9*
25	10.0	97.6*	64.0*	52.4*
Compound 5	0.1	59.4*	15.7	41.8*
	1.0	43.3*	55.4*	55.2*
	10.0	109.2*	50.9*	51.8*
Compound 7	0.1	18	4.3	40.5*
30	1.0	67.5*	46.3*	69.6*
	10.0	77.7*	43.2*	47.4*

^a mg/kg PO

^b Uterine Weight % increase versus the ovariectomized controls

^c Eosinophil peroxidase Vmax

5 ^d Serum cholesterol decrease versus ovariectomized controls

^e 17- α -Ethynyl estradiol

* p<.05

In addition to the demonstrated benefits of the
10 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.

15

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
20 36th day. The 35 day time period is sufficient to allow 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 are expelled before determination of wet weight in order to
25 confirm estrogen deficiency associated with complete 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.

30

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

35

In accordance with the above procedures, compounds of the instant invention and ethynyl estradiol (EE₂) in 20% hydroxypropyl β -cyclodextrin are orally administered to test animals. Distal femur metaphysis and proximal tibiae data

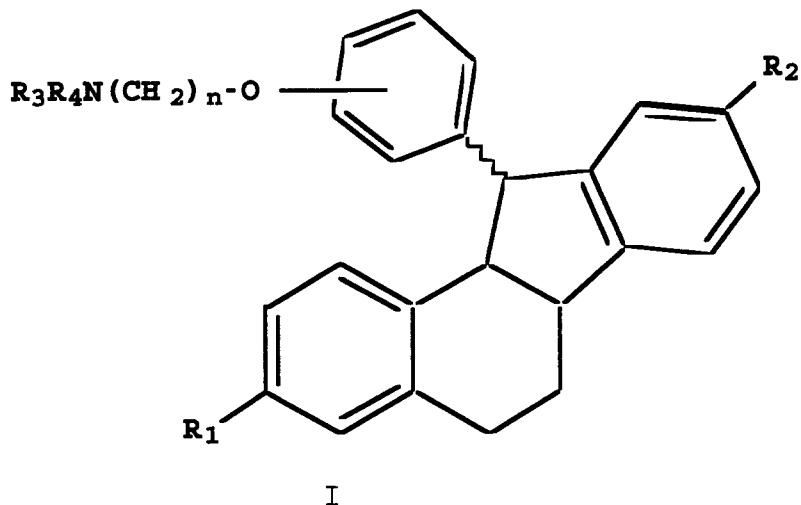
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are the results of formula I compound treatments compared to intact and ovariectomized test animals. Results are reported as percent protection relative to ovariectomy.

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

1. A compound of formula I:



wherein:

10 R₁ is -H, -OH, -O(C₁-C₄ alkyl), -OCO(C₁-C₆ alkyl), -O(CO)O(C₁-C₆ alkyl), -COAr, -OCOAr, -O(CO)OAr, where Ar is phenyl or optionally substituted phenyl, or -OSO₂-(C₄-C₆ alkyl);

15 R₂ is -OH, -O(C₁-C₄ alkyl), -OCO(C₁-C₆ alkyl), -O(CO)O(C₁-C₆ alkyl), -COAr, -OCOAr, -O(CO)OAr, where Ar is phenyl or optionally substituted phenyl, or -OSO₂-(C₄-C₆ alkyl);

R₃ and R₄ are each independently -C₁-C₄ alkyl, or combine to form 1-piperidinyl, 1-pyrrolidinyl, methyl-1-pyrrolidinyl, dimethyl-1-pyrrolidinyl, 4-morpholino, dimethylamino, diethylamino, or 1-hexamethyleneimino;

20 n is 2 or 3;

or a pharmaceutically acceptable salt or solvate thereof.

25 2. A compound according to Claim 1 wherein R₃ and R₄ combine to form 1-piperidinyl.

3. A compound according to Claim 1 wherein R₃ and R₄ combine to form 1-pyrrolidinyl.

4. A pharmaceutical composition comprising a compound according to Claim 1, or a pharmaceutically acceptable salt thereof, and optionally an effective amount of estrogen or 5 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 post-10 menopausal syndrome.

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

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

20 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.

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

30 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.

35 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 proliferation.

10

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