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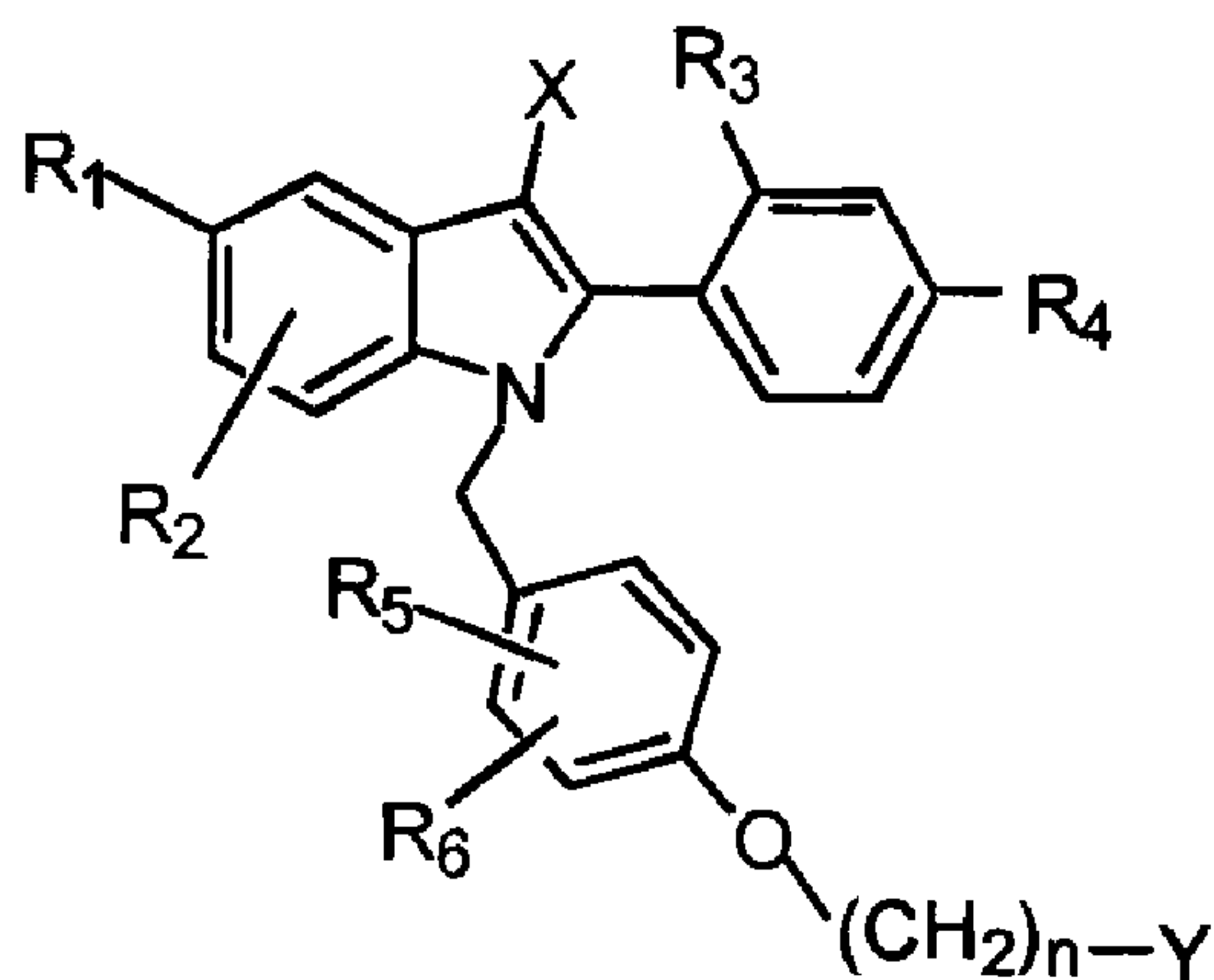
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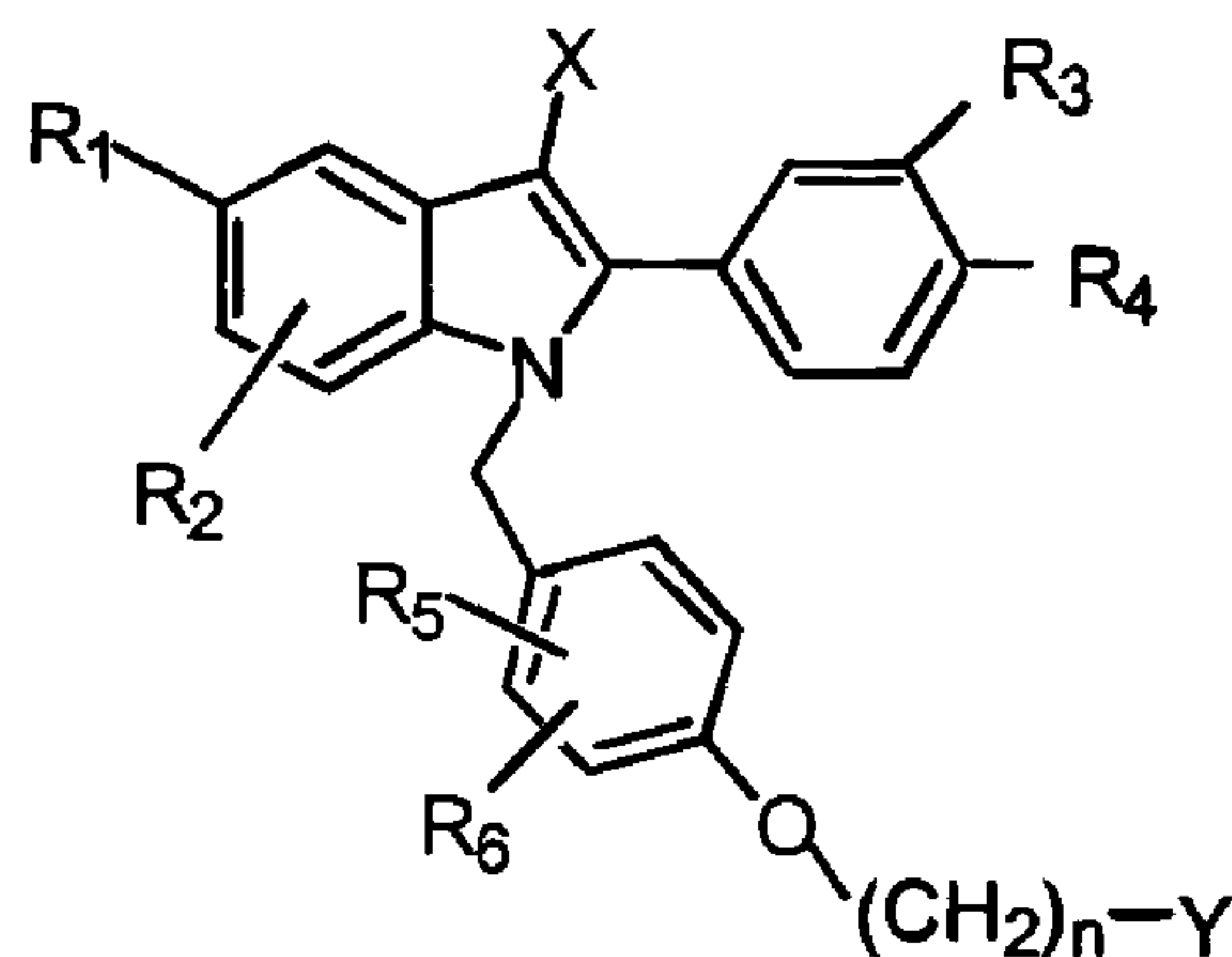
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(54) Titre : 2-PHENYL-1-[4-(2-AMINOETHOXY)-BENZYL]INDOLES EN TANT QU'AGENTS ESTROGENIQUES

(54) Title: 2-PHENYL-1-[4-(2-AMINOETHOXY)-BENZYL]INDOLES AS ESTROGENIC AGENTS



(I)



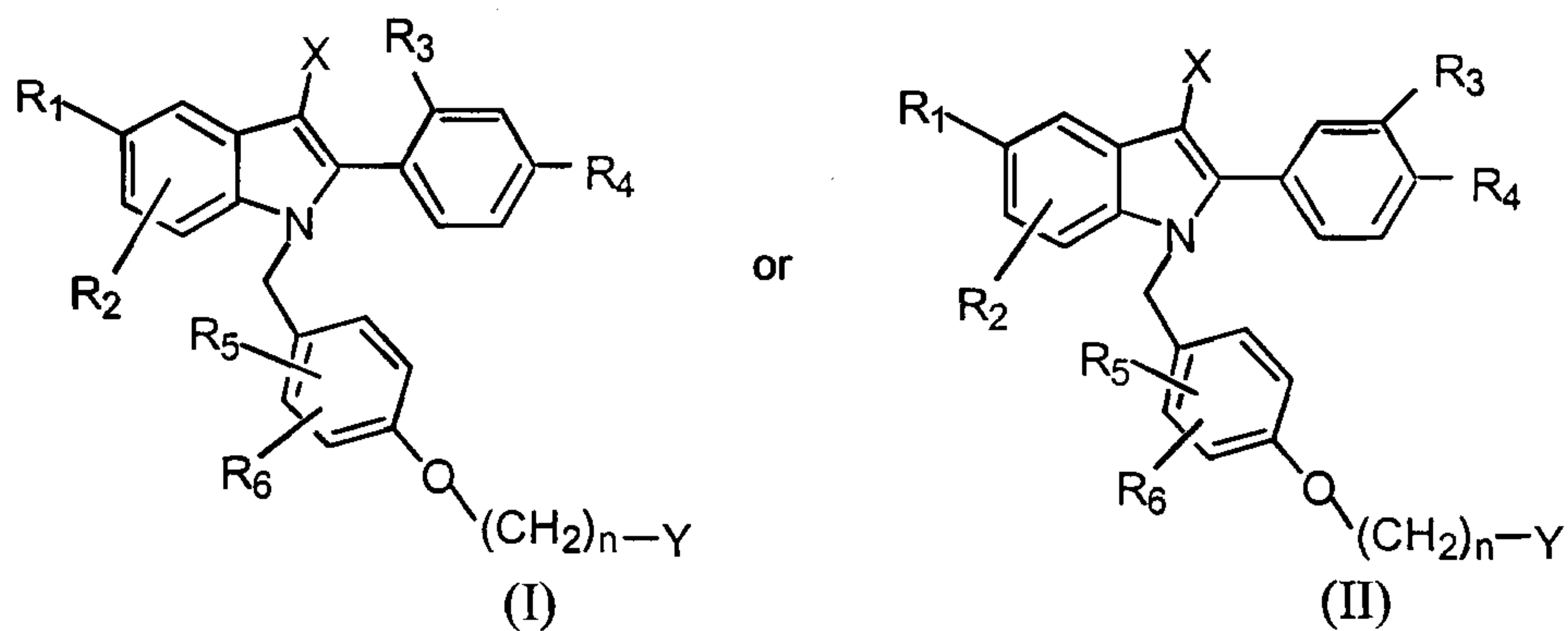
(II)

(57) Abrégé/Abstract:

The present invention relates to new 2-Phenyl-1-[4-(2-Aminoethoxy)-Benzyl]-Indole compounds which are useful as estrogenic agents, as well as pharmaceutical compositions and methods of treatment utilizing these compounds. These compounds have a structure selected from the formulas I or II: (see formula I or II) wherein the variables R₁, R₂, R₃, R₄, R₅, R₆, X, n, and Y are defined in the claims.

ABSTRACT OF THE DISCLOSURE

The present invention relates to new 2-Phenyl-1-[4-(2-Aminoethoxy)-Benzyl]-Indole compounds which are useful as estrogenic agents, as well as pharmaceutical compositions and methods of treatment utilizing these compounds. These compounds have a structure selected from the formulas I or II:



wherein the variables R₁, R₂, R₃, R₄, R₅, R₆, X, n, and Y are defined in the claims.

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2-PHENYL-1-[4-(2-AMINOETHOXY)-BENZYL]INDOLES AS ESTROGENIC AGENTS

The present invention relates to new 2-Phenyl-1-[4-(2-Aminoethoxy)-Benzyl]-
5 Indole compounds which are useful as estrogenic agents, as well as pharmaceutical compositions and methods of treatment utilizing these compounds.

Background of the Invention

10 The use of hormone replacement therapy for bone loss prevention in post-menopausal women is well preceded. The normal protocol calls for estrogen supplementation using such formulations containing estrone, estriol, ethynyl estradiol or conjugated estrogens isolated from natural sources (i.e. Premarin® conjugated
15 estrogens from Wyeth-Ayerst). In some patients, therapy may be contraindicated due to the proliferative effects unopposed estrogens (estrogens not given in combination with progestins) have on uterine tissue. This proliferation is associated with increased risk for endometriosis and/or endometrial cancer. The effects of unopposed estrogens on breast tissue is less clear, but is of some concern. The need for estrogens which can maintain the bone sparing effect while minimizing the proliferative effects in the uterus
20 and breast is evident. Certain nonsteroidal antiestrogens have been shown to maintain bone mass in the ovariectomized rat model as well as in human clinical trials. Tamoxifen (sold as Novadex® brand tamoxifen citrate by Zeneca Pharmaceuticals, Wilmington, Delaware), for example, is a useful palliative for the treatment of breast cancer and has been demonstrated to exert an estrogen agonist-like effect on the bone,
25 in humans. However, it is also a partial agonist in the uterus and this is cause for some concern. Raloxifene, a benzothiophene antiestrogen, has been shown to stimulate uterine growth in the ovariectomized rat to a lesser extent than Tamoxifen while maintaining the ability to spare bone. A suitable review of tissue selective estrogens is seen in the article "Tissue-Selective Actions Of Estrogen Analogs", *Bone* Vol. 17, No.
30 4, October 1995, 181S-190S.

The use of indoles as estrogen antagonists has been reported by Von Angerer, Chemical Abstracts, Vol. 99, No. 7 (1983), Abstract No. 53886u. Also, see,

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J.Med.Chem. 1990, 33, 2635-2640; J.Med.Chem. 1987, 30, 131-136. Also see Ger. Offen., DE 3821148 A1 891228 and WO 96/03375. These prior art compounds share structural similarities with the present compounds, but are functionally different. For compounds containing a basic amine, there is no phenyl group to rigidify the side chain. The reported data for these compounds indicates that they have a considerably weaker binding to estrogen receptor than the compounds of the present invention and they possess some uterotrophic effect in the rat uterus. One compound from the listed family of compounds in WO 96/03375 possesses a benzyl group, but does not have a basic side chain. This compound is considerably weaker in binding to the estrogen receptor.

WO A 95 17383 (Kar Bio AB) describes indole antiestrogens with long straight chains. Another related patent WO A 93 10741 describes 5-Hydroxyindoles with a broad range of side chains. WO 93/23374 (Otsuka Pharmaceuticals, Japan) describes compounds sharing structural similarities with those of the present invention, except with the structure referred to as R₃ in the present formulas I and II, below, is defined as thioalkyl and the reference discloses no such compounds having chains from the indole nitrogen having the same structure as the ones provided by the present invention.

Description of the Invention

2-Phenylindoles of the general structure type shown in formulas (I) and (II) are estrogen agonists/antagonists useful for the treatment of diseases associated with estrogen deficiency. The compounds of the present invention show strong binding to the estrogen receptor. *In vitro* assays, including an Ishikawa alkaline phosphatase assay and an ERE transfection assay, show these compounds are antiestrogens with little to no intrinsic estrogenicity and they have proven capable of completely antagonizing the effects of 17 β - estradiol while showing little or no uterine stimulation in a rat uterine assay when dosed alone.

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The present invention includes compounds of the formulas I or II, below:



R₂, R₃, R₄, R₅, and R₆ are independently selected from H, OH or the C₁-C₄ esters or alkyl ethers thereof, halogen, cyano, C₁-C₆ alkyl, or trifluoromethyl, with the proviso that, when R₁ is H, R₂ is not OH;

n is 2 or 3;

Y is selected from:

$$\begin{array}{c} \text{R}_7 \\ | \\ \text{N} \\ | \\ \text{R}_8 \end{array}$$

wherein R₇ and R₈ are independently selected from the group of H, C₁-phenyl.

b) a five-membered saturated, unsaturated or partially unsaturated heterocycle containing up to two heteroatoms selected from the group consisting of -O-, -NH-, -N(C₁C₄ alkyl)-, -N=, and -S(O)_m-, wherein m is an integer of from 0-2, optionally substituted with 1-3 substituents independently selected from the group consisting of hydrogen, hydroxyl, halo, C₁-C₄ alkyl, trihalomethyl, C₁-C₄ alkoxy, trihalomethoxy, C₁-C₄ acyloxy, C₁-C₄ alkylthio, C₁-C₄ alkylsulfinyl, C₁-C₄ alkylsulfonyl, hydroxy (C₁-C₄)alkyl, phenyl optionally substituted with 1-3 (C₁-

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C₄)alkyl, -CO₂H-, -CN-, -CONHR¹-, -NH₂-, C₁-C₄ alkylamino, C₁-C₄ dialkylamino, -NHSO₂R¹-, -NHCOR¹-, -NO₂;

c) a six-membered saturated, unsaturated or partially unsaturated
 5 heterocycle containing up to two heteroatoms selected from the group consisting of -O-,
 -NH-, -N(C₁C₄ alkyl)-, -N=, and -S(O)_m-, wherein m is an integer of from 0-2,
 optionally substituted with 1-3 substituents independently selected from the group
 consisting of hydrogen, hydroxyl, halo, C₁-C₄ alkyl, trihalomethyl, C₁-C₄ alkoxy,
 trihalomethoxy, C₁-C₄ acyloxy, C₁-C₄ alkylthio, C₁-C₄ alkylsulfinyl, C₁-C₄
 10 alkylsulfonyl, hydroxy (C₁-C₄)alkyl, phenyl optionally substituted with 1-3 (C₁-
 C₄)alkyl, -CO₂H-, -CN-, -CONHR¹-, -NH₂-, C₁-C₄ alkylamino, C₁-C₄ dialkylamino,
 -NHSO₂R¹-, -NHCOR¹-, -NO₂;

d) a seven-membered saturated, unsaturated or partially
 15 unsaturated heterocycle containing up to two heteroatoms selected from the group
 consisting of -O-, -NH-, -N(C₁C₄ alkyl)-, -N=, and -S(O)_m-, wherein m is an integer
 of from 0-2, optionally substituted with 1-3 substituents independently selected from
 the group consisting of hydrogen, hydroxyl, halo, C₁-C₄ alkyl, trihalomethyl, C₁-C₄
 alkoxy, trihalomethoxy, C₁-C₄ acyloxy, C₁-C₄ alkylthio, C₁-C₄ alkylsulfinyl, C₁-C₄
 20 alkylsulfonyl, hydroxy (C₁-C₄)alkyl, phenyl optionally substituted with 1-3 (C₁-
 C₄)alkyl, -CO₂H-, -CN-, -CONHR¹-, -NH₂-, C₁-C₄ alkylamino, C₁-C₄ dialkylamino,
 -NHSO₂R¹-, -NHCOR¹-, -NO₂- ; or

e) a bicyclic heterocycle containing from 6-12 carbon atoms either
 25 bridged or fused and containing up to two heteroatoms selected from the group
 consisting of -O-, -NH-, -N(C₁C₄ alkyl)-, and -S(O)_m-, wherein m is an integer of
 from 0-2, optionally substituted with 1-3 substituents independently selected from the
 group consisting of hydrogen, hydroxyl, halo, C₁-C₄ alkyl, trihalomethyl, C₁-C₄
 alkoxy, trihalomethoxy, C₁-C₄ acyloxy, C₁-C₄ alkylthio, C₁-C₄ alkylsulfinyl, C₁-C₄
 30 alkylsulfonyl, hydroxy (C₁-C₄)alkyl, phenyl optionally substituted with 1-3 (C₁-
 C₄)alkyl, -CO₂H-, -CN-, -CONHR¹-, -NH₂-, C₁-C₄ alkylamino, C₁-C₄ dialkylamino,
 -NHSO₂R¹-, -NHCOR¹-, -NO₂;

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and the pharmaceutically acceptable salts thereof.

The more preferred compounds of this invention are those having the general structures I or II, above, wherein:

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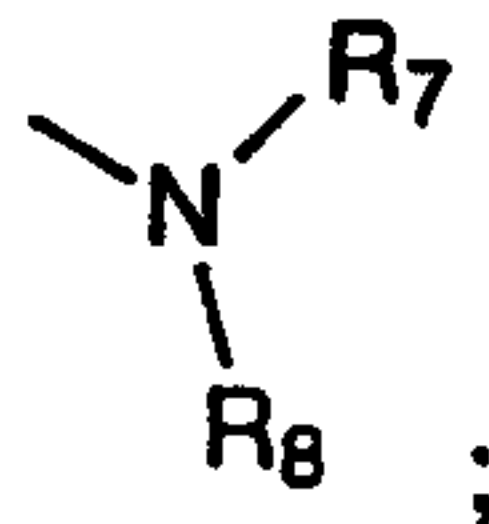
R_1 is selected from H, OH or the C_1 - C_4 esters or alkyl ethers thereof, halogen;

R_2 , R_3 , R_4 , R_5 , and R_6 are independently selected from H, OH or the C_1 - C_4 esters or alkyl ethers thereof, halogen, cyano, C_1 - C_6 alkyl, or trifluoromethyl, with the proviso that, when R_1 is H, R_2 is not OH;

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X is selected from H, C_1 - C_6 alkyl, cyano, nitro, trifluoromethyl, halogen;

Y is the moiety



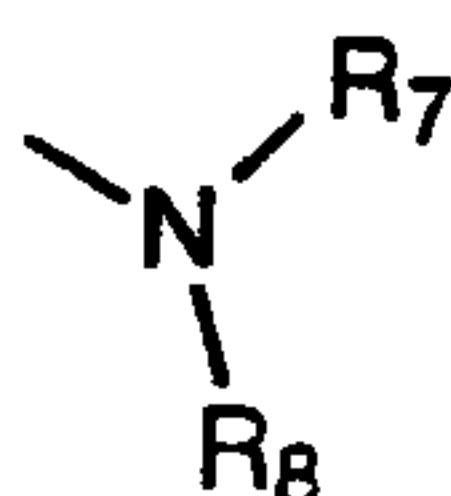
R_7 and R_8 are selected independently from H, C_1 - C_6 alkyl, or combined by -
(CH_2) p -, wherein p is an integer of from 2 to 6, so as to form a ring, the ring being
optionally substituted by up to three substituents selected from the group of hydrogen,
hydroxyl, halo, C_1 - C_4 alkyl, trihalomethyl, C_1 - C_4 alkoxy, trihalomethoxy, C_1 - C_4
alkylthio, C_1 - C_4 alkylsulfinyl, C_1 - C_4 alkylsulfonyl, hydroxy (C_1 - C_4)alkyl, - CO_2H , -
CN, - $\text{CONH}(C_1-C_4)$, - NH_3 , C_1 - C_4 alkylamino, C_1 - C_4 dialkylamino, - $\text{NHSO}_2(C_1-$
 $C_4)$, - $\text{NHCO}(C_1-C_4)$, and - NO_2 ;

and the pharmaceutically acceptable salts thereof.

The rings formed by a concatenated R_7 and R_8 , mentioned above, may include, but are not limited to, aziridine, azetidine, pyrrolidine, piperidine, or hexamethylenamine rings.

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The most preferred compounds of the present invention are those having the structural formulas I or II, above, wherein R_1 is OH; R_2 - R_6 are as defined above; X is selected from the group of Cl, NO_2 , CN, CF_3 , or CH_3 ; and Y is the moiety



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and R₇ and R₈ are concatenated together as -(CH₂)_r-, wherein r is an integer of from 4 to 6, to form a ring optionally substituted by up to three substituents selected from the group of hydrogen, hydroxyl, halo, C₁-C₄ alkyl, trihalomethyl, C₁-C₄ alkoxy, trihalomethoxy, C₁-C₄ alkylthio, C₁-C₄ alkylsulfinyl, C₁-C₄ alkylsulfonyl, hydroxy (C₁-C₄)alkyl, -CO₂H, -CN, -CONH(C₁-C₄), -NH₃, C₁-C₄ alkylamino, C₁-C₄ dialkylamino, -NHSO₂(C₁-C₄), -NHCO(C₁-C₄), and -NO₂; and the pharmaceutically acceptable salts thereof.

It is further preferred that, when R₇ and R₈ are concatenated together as -(CH₂)_q-, the ring so formed is optionally substituted with 1-3 substituents selected from a group containing C₁-C₃ alkyl, trifluoromethyl, halogen, hydrogen, phenyl, nitro, -CN.

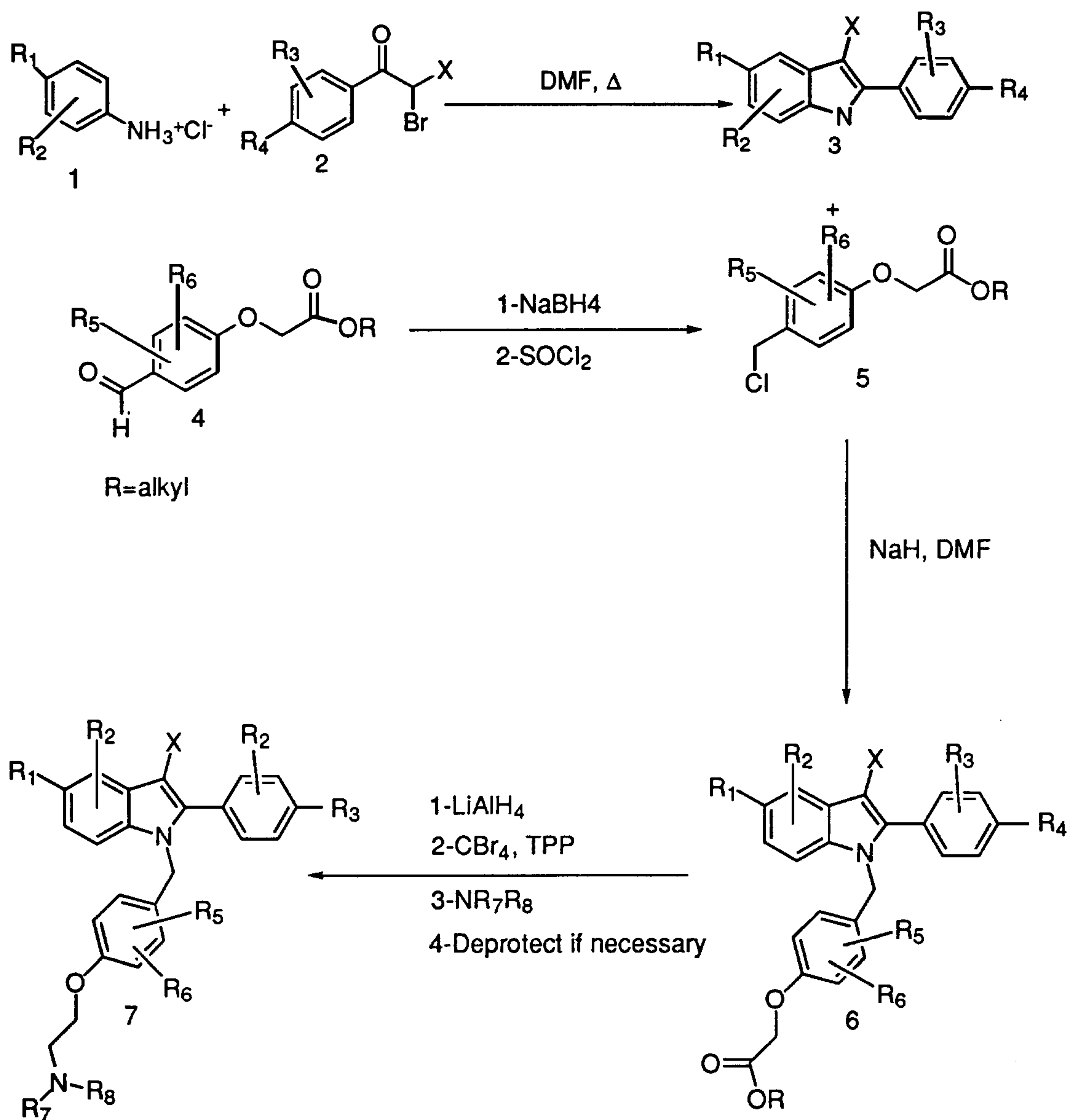
The invention includes acceptable salt forms formed from the addition reaction with either inorganic or organic acids. Inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, phosphoric acid, nitric acid useful as well as organic acids such as acetic acid, propionic acid, citric acid, maleic acid, malic acid, tartaric acid, phthalic acid, succinic acid, methanesulfonic acid, toluenesulfonic acid, naphthalenesulfonic acid, camphorsulfonic acid, benzenesulfonic acid are useful. It is known that compounds possessing a basic nitrogen can be complexed with many different acids (both protic and not protic) and usually it is preferred to administer a compound of this invention in the form of an acid addition salt.

Methods

Compounds of this invention can be synthesized in a general sense according to Scheme 1, below. It is understood in the following reaction schemes that the substituents symbolized by R₂ may be in either the 2 or 3 position.

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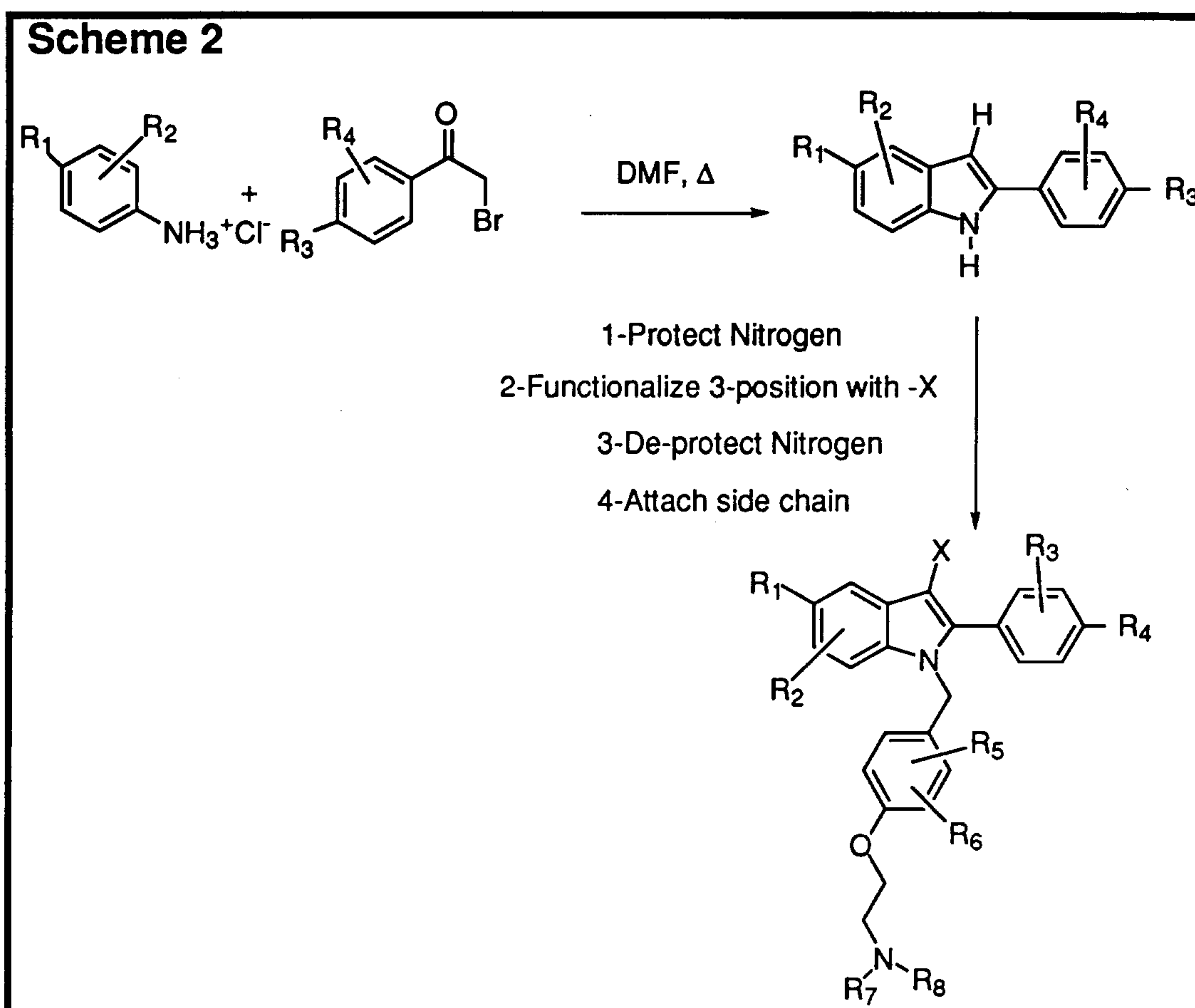
Scheme 1

- 5 The initial indole synthesis is accomplished by heating an appropriately substituted aniline (1) with an appropriately substituted α -bromophenylalkylphenone (2) in a suitably high boiling solvent such as DMF. The product is then alkylated with a benzyl chloride (5) to give the substituted indole (6). The benzyl chloride (5) can be readily prepared from the aldehyde (4) in 2 steps as

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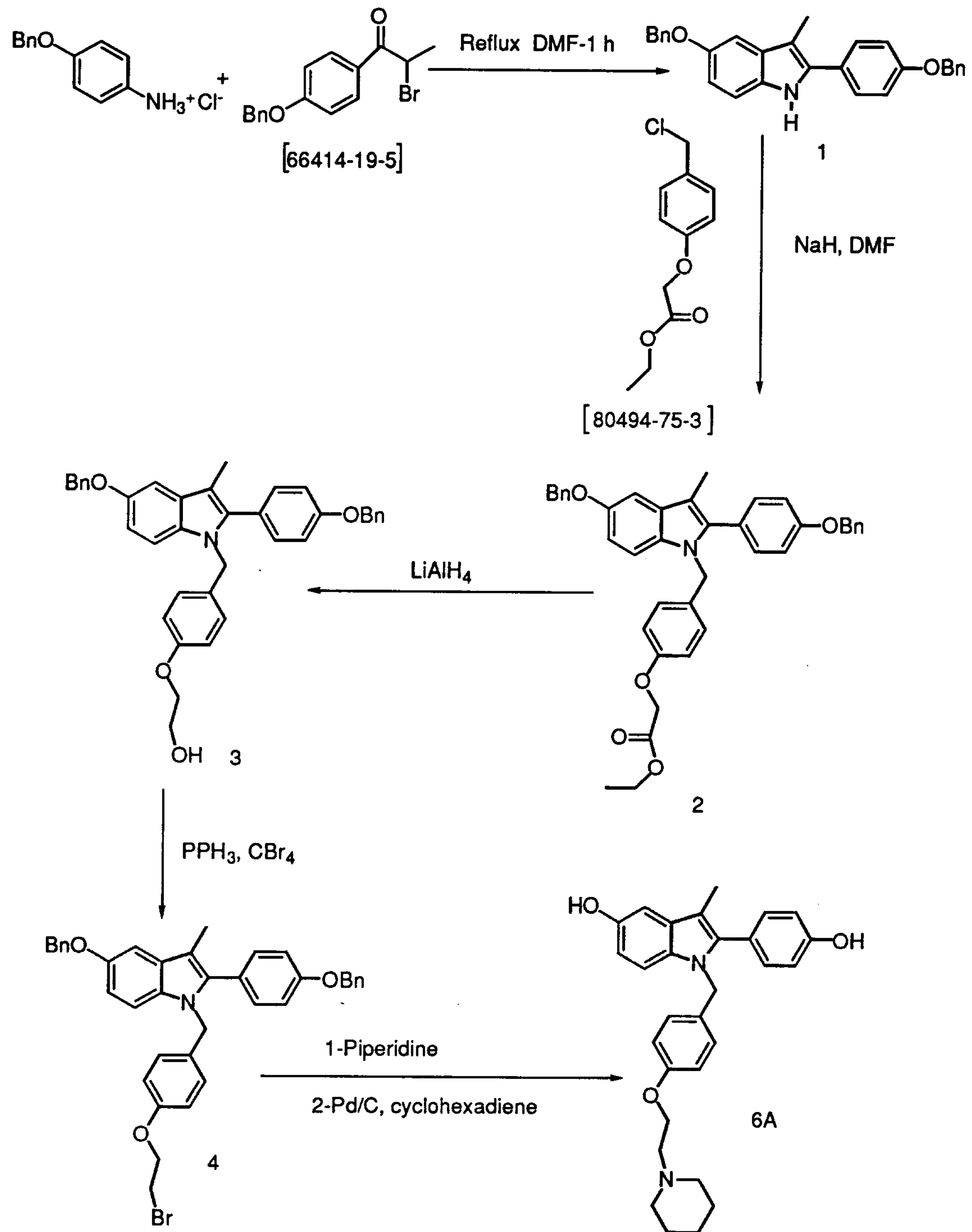
given. Product (7) can be prepared from (6) by reduction of the ester, conversion of the alcohol to a bromide, displacement of the bromide in a suitable solvent such as THF or DMF, and finally, deprotection if necessary. Deprotection is necessary when either R_1 or R_2 or both is a protected phenol. The preferred protecting group is a benzyl group which can be conveniently removed by several conventional methods, including hydrogenolysis. For the synthesis of compounds with $X=H$, halogen, trifluoromethyl, cyano, nitro, an alternative synthesis shown in scheme 2 may be preferable. The formation of halogens at the 3-position can be easily performed with such reagents as bromine or iodine or alternatively N-chlorosuccinamide, N-bromosuccinamide, or N-iodosuccinamide. The Iodo compound obtained can be used as a precursor to the 3-trifluoromethyl compound by a coupling reaction utilizing a palladium catalyst and bistrifluoromethyl mercury (II). A compound with a cyano group in the 3-position can be prepared by electrophilic cyanation or alternatively the 3-position can be formylated (with a formyl iminium salt, for example) then the formyl group converted to an oxime and subsequently dehydrated to a nitrile. A compound with the nitro group in the 3-position can be prepared by treating the indole with sodium nitrite and acetic acid. One skilled in the art recognizes these routes are not limiting and other routes are also available.

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- 5 The synthesis of the final product of Example 1 (step g), below, can be carried out via scheme 3:

- 10 -

Scheme 3

The compounds of the invention are partial estrogen agonists and display high affinity for the estrogen receptor. Unlike many estrogens, however, these compounds

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do not cause increases in uterine wet weight. These compounds are antiestrogenic in the uterus and can completely antagonize the trophic effects of estrogen agonists in uterine tissue. These compounds are useful in treating or preventing in a mammal disease states or syndromes which are caused or associated with an estrogen deficiency.

The present compounds have the ability to behave like estrogen agonists by lowering cholesterol and preventing bone loss. These compounds are useful for treating many maladies including osteoporosis, prostatic hypertrophy, infertility, breast cancer, endometrial cancer, cardiovascular disease, contraception, Alzheimer's disease and other CNS disorders, as well as certain cancers including melanoma. Additionally, these compounds can be used for hormone replacement therapy in post-menopausal women or in other estrogen deficiency states where estrogen supplementation would be beneficial.

The compounds of this invention may also be used in methods of treatment for bone loss, which may result from an imbalance in an individual's formation of new bone tissues and the resorption of older tissues, leading to a net loss of bone. Such bone depletion results in a range of individuals, particularly in post-menopausal women, women who have undergone hysterectomy, those receiving or who have received extended corticosteroid therapies, those experiencing gonadal dysgenesis, and those suffering from Cushing's syndrome. Special needs for bone replacement can also be addressed using these compounds in individuals with bone fractures, defective bone structures, and those receiving bone-related surgeries and/or the implantation of prosthesis. In addition to those problems described above, these compounds can be used in treatments for osteoarthritis, Paget's disease, osteomalacia, osteomalacia, multiple myeloma and other forms of cancer having deleterious effects on bone tissues. Methods of treating the maladies listed herein are understood to comprise administering to an individual in need of such treatment a pharmaceutically effective amount of one or more of the compounds of this invention or a pharmaceutically acceptable salt thereof. This invention also includes pharmaceutical compositions utilizing one or more of the present compounds, and/or the pharmaceutically acceptable salts thereof, along with one or more pharmaceutically acceptable carriers, excipients, etc.

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It is understood that the dosage, regimen and mode of administration of these compounds will vary according to the malady and the individual being treated and will be subject to the judgement of the medical practitioner involved. It is preferred that the administration of one or more of the compounds herein begin at a low dose and be increased until the desired effects are achieved.

Effective administration of these compounds may be given at a dose of from about 0.1 mg/day to about 1,000 mg/day. Preferably, administration will be from about 10 mg/day to about 600 mg/day in a single dose or in two or more divided doses. Such doses may be administered in any manner useful in directing the active compounds herein to the recipient's bloodstream, including orally, parenterally (including intravenous, intraperitoneal and subcutaneous injections), and transdermally. For the purposes of this disclosure, transdermal administrations are understood to include all administrations across the surface of the body and the inner linings of bodily passages including epithelial and mucosal tissues. Such administrations may be carried out using the present compounds, or pharmaceutically acceptable salts thereof, in lotions, creams, foams, patches, suspensions, solutions, and suppositories (rectal and vaginal).

Oral formulations containing the active compounds of this invention may comprise any conventionally used oral forms, including tablets, capsules, buccal forms, troches, lozenges and oral liquids, suspensions or solutions. Capsules may contain mixtures of the active compound(s) with inert fillers and/or diluents such as the pharmaceutically acceptable starches (e.g. corn, potato or tapioca starch), sugars, artificial sweetening agents, powdered celluloses, such as crystalline and microcrystalline celluloses, flours, gelatins, gums, etc. Useful tablet formulations may be made by conventional compression, wet granulation or dry granulation methods and utilize pharmaceutically acceptable diluents, binding agents, lubricants, disintegrants, suspending or stabilizing agents, including, but not limited to, magnesium stearate, stearic acid, talc, sodium lauryl sulfate, microcrystalline cellulose, carboxymethylcellulose calcium, polyvinylpyrrolidone, gelatin, alginic acid, acacia gum, , xanthan gum, sodium citrate, complex silicates, calcium carbonate, glycine,

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dextrin, sucrose, sorbitol, dicalcium phosphate, calcium sulfate, lactose, kaolin, mannitol, sodium chloride, talc, dry starches and powdered sugar. Oral formulations herein may utilize standard delay or time release formulations to alter the absorption of the active compound(s). Suppository formulations may be made from traditional materials, including cocoa butter, with or without the addition of waxes to alter the suppository's melting point, and glycerin. Water soluble suppository bases, such as polyethylene glycols of various molecular weights, may also be used.

Aldrich Sure SealTM Solvents, anhydrous without further purification, may be used for the reactions described herein and may be obtained from Aldrich Chemical Company. All reactions were carried out under a nitrogen atmosphere. Chromatography was performed using 230-400 mesh silica gel (Merck^{*} Grade 60, Aldrich Chemical Company). Thin layer chromatography was performed with Silica Gel 60 F254 plates from EM Science. ¹H NMR spectra were obtained on a Bruker^{*} AM-400 instrument in DMSO and chemical shifts reported in ppm. Melting points were determined on a Thomas-Hoover^{*} apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer diffraction grating or Perkin-Elmer^{*} 784 spectrophotometers. Mass spectra were recorded on a Kratos MS 50 or Finnigan^{*} 8230 mass spectrometers. Elemental analyses were obtained with a Perkin-Elmer^{*} 2400 elemental analyzer. Compounds for which CHN are reported are within 0.4% of the theoretical value for the formula given.

The present invention is further illustrated by the following non-limiting examples.

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EXAMPLE 1**2-(4-Hydroxy-phenyl)-3-methyl-1-[4-(2-piperidin-1-yl-ethoxy)-benzyl]-1H-indol-5-ol**

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Step a**5-Benzyloxy-2-(4-benzyloxy-phenyl)-3-methyl-1H-indole**

A flask was charged with 4-benzyloxyaniline (45 g, 0.23 mol), 4'-benzyloxy-2-bromophenylpropiophenone (66414-19-5) (21g, 0.066 mol), and 50 mL DMF. The reaction was heated at reflux for 30 minutes and then cooled to rt and then partitioned between 250 mL EtOAc and 100 mL 1N HCl (aq). The EtOAc was washed with NaHCO₃ (aq) and brine, dried over MgSO₄. The solution was concentrated and the residue taken up in CH₂Cl₂ and hexanes added to precipitate out 25g of a crude solid. The solid was dissolved in CH₂Cl₂ and evaporated onto silica gel and chromatographed using CH₂Cl₂/Hexane (1:5) to yield 9.2 g of a tan solid (33%): Mpt = 150-152°C; ¹H NMR (DMSO) 10.88 (s, 1 H), 7.56 (d, 2 H, J = 8.8 Hz), 7.48 (d, 4 H, J = 7.9 Hz), 7.42-7.29 (m, 6 H), 7.21 (d, 1 H, J = 7.0 Hz), 7.13 (d, 2 H, J = 8.8 Hz), 7.08 (d, 1 H, J = 2.2 Hz), 6.94 (dd, 1 H, J = 8.8, 2.4 Hz), 5.16 (s, 2 H), 5.11 (s, 2 H), 2.33 (s, 3 H); IR (KBr) 3470, 2880, 2820, 1620 cm⁻¹; MS eI m/z 419.

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Step b**{4-[5-Benzyloxy-2-(4-benzyloxy-phenyl)-3-methyl-indol-1-ylmethyl]-phenoxy}-acetic acid ethyl ester**

25

A solution of 5-Benzyloxy-2-(4-benzyloxy-phenyl)-3-methyl-1H-indole (Step a) (32g, 77 mmol) in DMF (0.15 L) was cooled to 0°C and treated with sodium hydride (2.2 g, 89 mmol). The reaction was stirred for 20 minutes and then the benzyl chloride [80494-75-3] (29g, 127 mmol) was added and the reaction stirred for 18 hours at room temperature. The reaction mixture was poured into water and extracted with ethyl acetate. The ethyl acetate was washed with brine and dried over magnesium sulfate. The ethyl acetate was concentrated and triturated with ether to obtain 21 g of a white solid. The filtrate was concentrated and triturated with ether to give an additional 7 g of

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white solid for a total yield of 31 g: Mpt=129-131°C; ¹H NMR (DMSO) 7.47 (d, 4 H, J = 7.2 Hz), 7.39 (q, 4 H, J = 7.9 Hz), 7.36-7.32 (m, 1 H), 7.29 (d, 2 H, J = 8.8 Hz), 7.19 (d, 1 H, J = 9.0 Hz), 7.13-7.09 (m, 4 H), 6.80 (dd, 1 H, J = 8.8, 2.4 Hz), 6.73 (s, 4 H), 5.16 (s, 2 H), 5.13 (s, 2 H), 5.11 (s, 2 H), 4.66 (s, 2 H), 4.11 (q, 2 H, J = 7.2 Hz), 2.15 (s, 3 H), 1.16 (t, 3 H, J = 7.2 Hz); MS eI m/z 612.

Step c**2-{4-[5-Benzyloxy-2-(4-benzyloxy-phenyl)-3-methyl-indol-1-ylmethyl]-phenoxy}-ethanol**

10

A solution of the compound of Step b (5.5 g, 8.8 mmol) in THF (50 mL) was cooled to 0°C and a solution of LiAlH₄ (10mL, 1 M) in THF was added dropwise. After 30 minutes at 0°C the reaction was carefully quenched with water, and partitioned between EtOAc and 1 N HCl. The EtOAc was dried with MgSO₄, concentrated, and chromatographed on silica gel EtOAc/hexane (2:3) to yield 4.0 g of a white foam: ¹H NMR (DMSO) 7.48-7.46 (m, 4 H), 7.42-7.27 (m, 8 H), 7.20 (d, 1 H, J = 8.8 Hz), 7.12-7.10 (m, 3 H), 6.80 (dd, 1 H, J = 8.8, 2.4 Hz), 6.73 (s, 4 H), 5.15 (s, 2 H), 5.13 (s, 2 H), 5.11 (s, 2 H), 4.80 (t, 1 H, J = 5.5 Hz), 3.86 (t, 2 H, J = 4.8 Hz), 3.63 (q, 2 H, J = 5.3 Hz), 2.15 (s, 3 H).

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Step d**5-Benzyloxy-2-(4-benzyloxy-phenyl)-1-[4-(2-bromo-ethoxy)-benzyl]-3-methyl-1H-indole**

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A solution of the compound of Step c (3.3 g, 5.8 mmol) in THF (50 mL) was added CBr₄ (2.9 g, 8.7 mmol) and PPH₃ (2.3 g, 8.7 mmol). The reaction was stirred at rt for 3 h and then concentrated and chromatographed on silica gel using a gradient elution from EtOAc/hexane (1:4) to EtOAc to give 3.2 g of a white solid: Mpt=131-134°C; ¹H NMR (DMSO) 7.64-7.30 (m, 10 H), 7.29 (d, 2 H, J = 8.8 Hz), 7.20 (d, 1 H, J = 8.8 Hz), 7.12-7.09 (m, 3 H), 6.80 (dd, 1 H, J = 8.8, 2.4 Hz), 6.77-6.73 (m, 4 H), 5.16 (s, 2 H), 5.13 (s, 2 H), 5.11 (s, 2 H), 4.20 (t, 2 H, J = 5.3 Hz), 3.73 (t, 2 H, J = 5.5 Hz), 2.15 (s, 3 H); MS FAB 631/633 (M+H⁺, Br present).

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Step e5-Benzylloxy-2-(4-benzylloxy-phenyl)-3-1-[4-(2-piperidin-1-yl-ethoxy)-benzyl]-1H-indole

5 A solution of the compound of Step d (3.2 g, 5.0 mmol) in THF (50 mL) was treated with piperidine (5.0 mL, 50 mmol) and heated to reflux. After 5 hours, the reaction mixture was concentrated and taken up in EtOAc, washed with saturated NaHCO₃, dried over MgSO₄ and column chromatographed on silica gel using a gradient elution of EtOAc/Hexane to EtOAc. The product (2.7 g) was a white solid
 10 with a Mpt=93-95°C; ¹H NMR (DMSO) 7.48-7.46 (m, 4 H), 7.42-7.38 (m, 4 H), 7.38-7.32 (m, 2 H), 7.29 (d, 2 H, J = 8.8 Hz), 7.19 (d, 1 H, J = 9.0 Hz), 7.12-7.10 (m, 3 H), 6.80 (dd, 1 H, J = 8.8, 2.4 Hz), 6.73 (s, 4 H), 5.15 (s, 2 H), 5.13 (s, 2 H), 5.11 (s, 2 H), 3.93 (t, 2 H, J = 5.7 Hz), 2.60-2.50 (m, 2 H), 2.41-2.30 (m, 4 H), 2.15 (s, 3 H), 1.47-1.42 (m, 4 H), 1.36-1.32 (m, 2 H); MS FAB 637 (M+H⁺).

15

Step f2-(4-Hydroxy-phenyl)-3-methyl-1-[4-(2-piperidin-1-yl-ethoxy)-benzyl]-1H-indol-5-ol

20 A suspension of 10% Pd/C (1.1 g) in EtOH was treated with a solution of Step e (2.2 g, 3.4 mmol) in THF/EtOH. Cyclohexadiene (6.0 mL, 63 mmol) was added and the reaction was stirred for 48 hours. The catalyst was filtered through Celite* and the reaction mixture was concentrated and chromatographed on silica gel using a gradient elution of MeOH/CH₂Cl₂ (1:19 to 1:10) to yield 0.8 g of the product as a white
 25 solid. Mpt=109-113°C; CHN calc'd for C₂₉H₃₂N₂O₃ + 0.5 H₂O; ¹H NMR 9.64 (s, 1 H), 8.67 (s, 1 H), 7.14 (d, 2 H, J = 8.6 Hz), 7.05 (d, 1 H, J = 8.6 Hz), 6.84 (d, 2 H, J = 8.8 Hz), 6.79 (d, 1 H, J = 2.2 Hz), 6.74 (s, 4 H), 6.56 (dd, 1 H, J = 8.8, 2.4 Hz), 5.09 (s, 2 H), 3.95-3.93 (m, 2 H), 2.60-2.51 (m, 2 H), 2.39-2.38 (m, 4 H), 2.09 (s, 3 H), 1.46-1.45 (m, 4 H), 1.35-1.34 (m, 2 H); IR (KBr) 3350 (br), 2920,
 30 1620, 1510 cm⁻¹; MS (EI) m/z 456.

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5-Benzoyloxy-2-(4-benzoyloxy-phenyl)-3-1-[4-(2-pyrrolidin-1-yl-ethoxy)-benzyl]-1H-indole

This compound was made analogously to the compound of Step e. Product
 5 was isolated as a white solid with a Mpt=132-134°C; ¹H NMR (DMSO) 7.48-7.46 (m, 4 H), 7.42-7.35 (m, 4 H), 7.35-7.32 (m, 1 H), 7.29 (d, 2 H, J = 8.8 Hz), 7.20 (d, 1 H, J = 8.8 Hz), 7.12-7.10 (m, 3 H), 6.80 (dd, 1 H, J = 8.8, 2.4 Hz), 6.73 (s, 4 H), 5.15 (s, 2 H), 5.13 (s, 2 H), 5.11 (s, 2 H), 3.94 (t, 2 H, J = 5.9 Hz), 2.71 (t, 2 H, J = 5.7 Hz), 2.45-2.41 (m, 4 H), 2.15 (s, 3 H), 1.65-1.62 (m, 4 H); MS (EI) m/z 622.

10

EXAMPLE 2

2-(4-Hydroxy-phenyl)-3-methyl-1-[4-(2-pyrrolidin-1-yl-ethoxy)-benzyl]-1H-indol-5-ol

15

Compound was prepared from 6 in a similar fashion to the compound of Example 1. Compound was isolated as a foam. Mpt=105-110°C; CHN calc'd for C₂₈H₃₀N₂O₃ + 0.4 H₂O; ¹H NMR (DMSO) 9.65 (s, 1 H), 8.67 (s, 1 H), 7.15 (d, 2 H, J = 8.6 Hz), 7.05 (d, 1 H, J = 8.6 Hz), 6.84 (d, 2 H, J = 2 H), 6.79 (d, 1 H, J = 2.4 Hz), 6.56 (dd, 1 H, J = 8.6, 2.2 Hz), 6.74 (s, 4 H), 5.09 (s, 2 H), 3.95 (t, 2 H, J = 5.7 Hz), 3.39-3.23 (m, 4 H), 2.80-2.75 (m, 2 H), 2.09 (s, 3 H), 1.67-1.64 (m, 4 H); IR (KBr) 3410 (br), 1620, 1510 cm⁻¹; MS (EI) m/z 442.

20

EXAMPLE 3

25

2-(4-Hydroxy-phenyl)-3-methyl-1-[4-(2-N,N'-diethyl-1-yl-ethoxy)-benzyl]-1H-indol-5-ol

Compound was prepared by hydrogenation of the benzyl ether precursor (data
 30 not given) in a similar fashion to the compound of Example 1. Compound was isolated as a foam. Mpt=100-107°C; CHN calc'd for C₂₈ H₃₂ N₂O₃ + 0.25 H₂O; ¹H NMR (DMSO) 9.64 (s, 1 H), 8.67 (s, 1 H), 7.14 (d, 2 H, J = 8.6 Hz), 7.05 (d, 1 H, J = 8.8 Hz), 6.84 (d, 2 H, J = 8.6 Hz), 6.79 (d, 1 H, J = 2.2 Hz), 6.74 (s, 4 H), 6.56 (dd, 1 H, J

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= 8.8, 2.4 Hz), 5.09 (s, 2 H), 3.95-3.85 (m, 2 H), 2.80-2.60 (m, 2 H), 2.58-2.40 (m, 4 H), 2.49 (t, 6 H, J = 1.76 Hz), 2.09 (s, 3 H), 0.93 (t, 3 H, J = 7.0 Hz); IR (KBr) 3410 (br), 2950, 1610, 1510 cm^{-1} ; MS FAB 445 ($\text{M}+\text{H}^+$).

5

EXAMPLE 4**2-(4-Hydroxy-phenyl)-3-methyl-1-[4-(2-piperidin-1-yl-ethoxy)-benzyl]-1H-indol-5-ol-hydrochloride**

10 1.0 g of the compound of Example 1 in a large test tube was dissolved in 20 mL of MeOH. This was treated with slow addition of 2.6 mL 1.0 N HCl and then 4.0 mL deionized water. The tube was partially opened to the atmosphere to encourage slow evaporation of the solvents. After about ten minutes, crystals began to appear and after 4 hours the solution was filtered and the solid crystals washed with water. The product
15 was present as 0.42 g of white crystalline plates with a melting point of 184-185°C. The mother liquor yielded an additional crop of 0.30 g of white solid with a melting point of 177-182°C. CHN calc'd for $\text{C}_{29}\text{H}_{32}\text{N}_2\text{O}_3 + \text{HCL} + 1 \text{ H}_2\text{O}$.

EXAMPLE 5

20

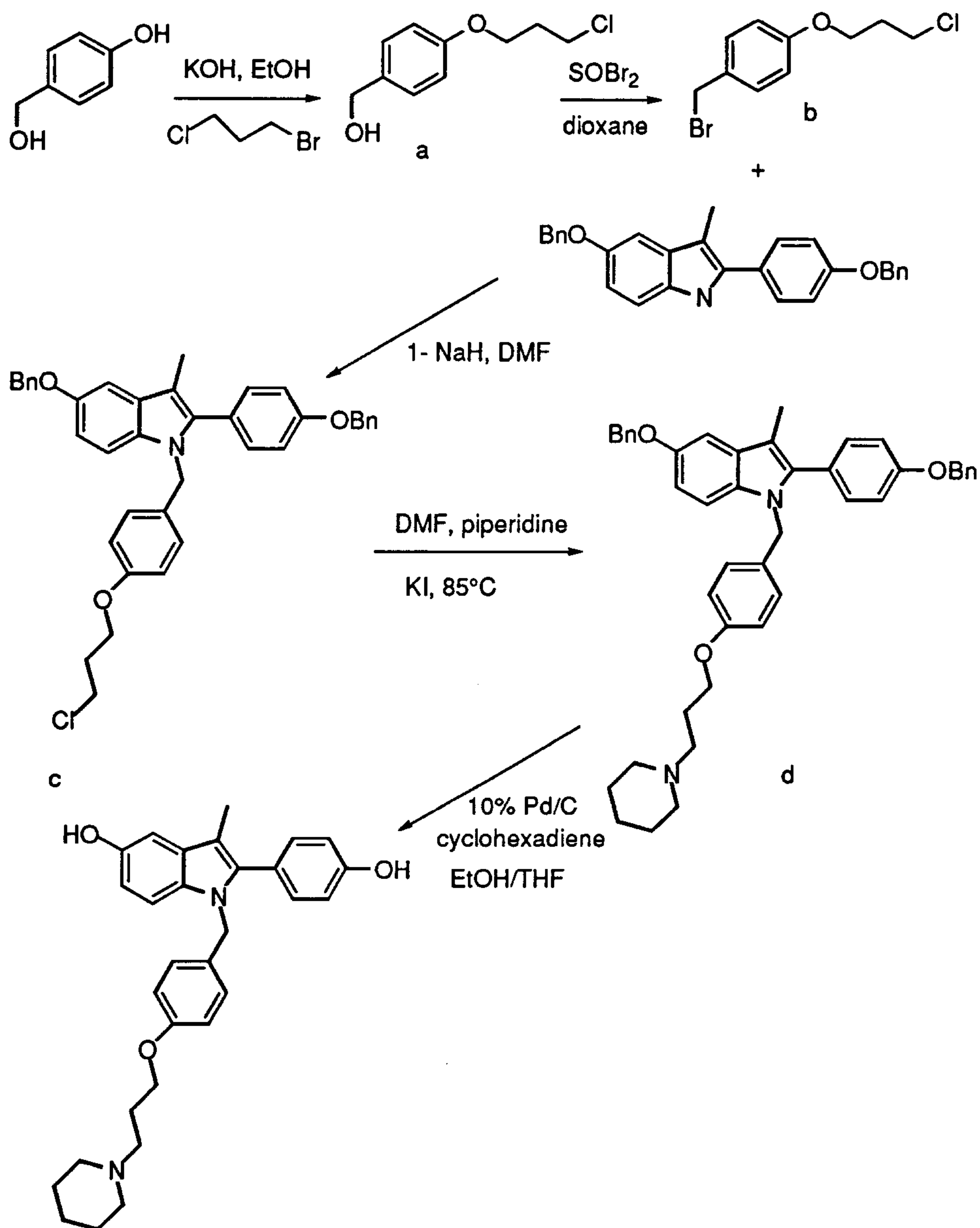
1-{4-[2-(3,3-Dimethyl-piperidin-1-yl)-ethoxyl-benzyl]}-2-(4-hydroxy-phenyl)-3-methyl-1H-indol-5-ol

 The title compound was prepared analogously to that of Example 1:
25 Mpt=100°C; ^1H NMR (DMSO) 9.65 (s, 1 H), 8.67 (s, 1 H), 7.15 (d, 2 H, J = 8.6 Hz), 7.05 (d, 1 H, J = 8.8 Hz), 6.84 (d, 2 H, J = 8.6 Hz), 6.79 (d, 1 H, J = 2.4 Hz), 6.74 (s, 4 H), 6.56 (dd, 1 H, J = 8.8, 2.4 Hz), 5.09 (s, 2 H), 3.93 (t, 2 H, J = 5.7 Hz), 2.60-2.50 (m, 2 H), 2.37-2.25 (m, 2 H), 2.09 (s, 3 H), 2.10-1.99 (m, 2 H), 1.46 (t, 2 H, J = 5.9 Hz), 1.13 (t, 2 H, J = 6.4 Hz), 0.86 (s, 6 H); MS eI m/z 484.

30

EXAMPLE 6**2-(4-Hydroxy-phenyl)-3-methyl-1-{4-[3-(piperidin-1-yl)-propoxy]-benzyl}-1H-indol-5-ol**

5 The title compound was prepared according to Scheme 4 and the steps provided below:

Scheme 4

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Synthesis of 4-(3-chloropropoxy)-benzyl alcohol (a)

A solution of 4-hydroxy benzyl alcohol (10g, 80.5 mmol) in ethanol (70 mL) was treated with 1, 3 bromochloro propane (16.0 g, 100 mmol) and potassium hydroxide (5.0 g, 89 mmol) was refluxed for 2 hours. The solution was cooled and filtered and then the filtrate concentrated. The concentrate was taken up in ether and washed with water, brine and dried over magnesium sulfate. The material was chromatographed on silica gel using ethyl acetate/hexanes (3:7) to yield 11.6 g of the product as a white solid: Mpt=65°C; ¹H NMR (DMSO) 7.21 (d, 2 H, J = 8.8 Hz), 6.88 (d, 2 H, J = 8.8 Hz), 5.03 (t, 1 H, J = 5.7 Hz), 4.40 (d, 2H, J = 5.5 Hz), 4.05 (t, 2 H, J = 6.1 Hz), 3.77 (t, 2 H, J = 6.4 Hz), 2.17-2.13 (m, 2 H); MS eI m/z 200.

Synthesis of 4-(3-chloropropoxy)-benzyl bromide (b)

A solution consisting of 4-(3-chloropropoxy)-benzyl alcohol (a) (10.6 g, 52.8 mmol) in dioxane (0.125 l) was cooled to 0° C and treated with a dropwise addition of thionyl bromide (12.0 g, 58.0 mmol). After 10 minutes the reaction was complete. The dioxane was diluted with ethyl ether and washed with water, brine, and then dried over MgSO₄. The material was concentrated down to yield 15 g of an oil: ¹H NMR (DMSO) 7.36 (d, 2 H, J = 8.8 Hz), 6.92 (d, 2 H, J = 8.6 Hz), 4.68 (s, 2 H), 4.08 (t, 2 H, J = 5.9 Hz), 3.77 (t, 2 H, J = 6.4 Hz), 2.20-2.10 (m, 2 H); MS (FAB) 266 (M+H⁺).

5-Benzyloxy-2-(4-benzyloxy-phenyl)-1-[4-(3-chloro-propoxy)-benzyl]-3-methyl-1H-indole (c)

A solution consisting of 5-Benzyloxy-2-(4-benzyloxy-phenyl)-3-methyl-1H-indole (1) (6.5 g, 15.5 mmol) in DMF (60 mL) was cooled to 0°C and treated with addition of sodium hydride (0.68 g, 17.0 mmol) and stirred for 20 minutes. A solution of 4-(3-chloropropoxy)-benzyl bromide (b) in DMF (10 mL) was then added slowly. The reaction was allowed to come to rt and stirred for 2 hours. The reaction was poured into water and extracted with ethyl acetate. The ethyl acetate was washed with water, brine and dried over magnesium sulfate and concentrated. The concentrate was

treated with methanol and 5 g of the desired product precipitated as a white solid with a melting point of 130-132°C.

5-Benzyloxy-2-(4-benzyloxy-phenyl)-1-[4-(3-piperidin-1-yl-propoxy)-benzyl]-3-methyl-1H-indole (d)

A solution of 5-Benzyloxy-2-(4-benzyloxy-phenyl)-1-[4-(3-chloro-propoxy)-benzyl]-3-methyl-1H-indole (c) (3g, 5.1 mmol), potassium iodide (2.5 g, 15.3 mmol) and piperidine (3.0 mL, 30.6 mmol) were heated in DMF (30 mL) at 100°C for 18 hours. The reaction was worked up by pouring into water and extracting with ethyl acetate. The organic layer was washed with water, brine and dried over magnesium sulfate. The solution was concentrated to an oil and the product precipitated out by adding methanol. The product was obtained as a white solid: Mpt=104-106°C; ¹H NMR (DMSO) 7.47 (d, 4 H, J = 7.5 Hz), 7.38 (q, 4 H, J = 7.9 Hz), 7.36-7.30 (m, 1 H), 7.28 (d, 2 H, J = 8.3 Hz), 7.19 (d, 1 H, J = 8.8 Hz), 7.12-7.10 (m, 4 H), 6.80 (dd, 1 H, J = 8.8, 2.0 Hz), 6.72 (s, 4 H), 5.14 (s, 2 H), 5.13 (s, 2 H), 5.11 (s, 2 H), 3.86 (t, 2 H, J = 6.4 Hz), 2.35-2.20 (m, 6 H), 2.14 (s, 3 H), 1.78-1.75 (m, 2 H), 1.47-1.42 (m, 4 H), 1.40-1.31 (m, 2 H); MS EI m/z 650.

2-(4-Hydroxy-phenyl)-3-methyl-1-[4-[3-(piperidin-1-yl)-propoxy]-benzyl]-1H-indol-5-ol

A solution of 5-Benzyloxy-2-(4-benzyloxy-phenyl)-1-[4-(3-piperidin-1-yl-propoxy)-benzyl]-3-methyl-1H-indole (d) in tetrahydrofuran (25 mL) and ethanol (25 mL) was added to 2.3 g of 10% palladium on carbon. Cyclohexadiene (10 mL) was added and the reaction allowed to stir at room temperature for 18 hours. The catalyst was filtered through celite and the reaction mixture was concentrated and chromatographed on silica gel using dichloromethane/methanol (4:1) to elute the product (0.8 g) as a white foam: Mpt=125-130°C; ¹H NMR 9.68 (s, 1 H), 8.70 (s, 1 H), 7.15 (d, 2 H, J = 8.6 Hz), 7.05 (d, 1 H, J = 8.8 Hz), 6.85 (d, 2 H, J = 8.6 Hz), 6.80 (d, 1 H, J = 2.4 Hz), 6.74 (d, 4 H, J = 2.6 Hz), 6.57 (dd, 1 H, J = 8.6, 2.2 Hz), 5.09 (s, 2 H), 3.88 (t, 2 H, J = 6.4 Hz), 3.60-3.15 (m, 2 H), 2.62-2.38 (m, 4 H),

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2.09 (s, 3 H), 1.92-1.78 (m, 2 H), 1.55-1.43 (m, 4 H), 1.42-1.30 (m, 2 H); IR (Kbr) 3400 (br), 2900, 1620, 1515 cm^{-1} ; MS eI m/z 470.

EXAMPLE 7

5

2-(4-methoxy-phenyl)-3-methyl-1-{4-[2-(piperidin-1-yl)-ethoxy]-benzyl}-1H-indol-5-ol

The title compound was synthesized in a similar manner to the compound of Example 1, except the methyl ether of phenylpropiophenone was used instead of the benzyl ether in the indole synthesis: Mpt = 188-189°C; CHN calcd for $\text{C}_{30}\text{H}_{34}\text{N}_2\text{O}_3 + 0.1 \text{ H}_2\text{O}$; ^1H NMR (DMSO) 8.70 (s, 1 H), 7.27 (d, 2 H, J = 8.6 Hz), 7.06 (d, 1 H, J = 8.6 Hz), 7.02 (d, 2 H, J = 8.8 Hz), 6.81 (d, 1 H, J = 2.2 Hz), 6.73 (s, 4 H), 6.58 (dd, 1 H, J = 8.8, 2.4 Hz), 5.10 (s, 2 H), 3.93 (t, 2 H, J = 5.9 Hz), 3.79 (s, 3 H), 2.56 (t, 2 H, J = 5.9 Hz), 2.41-2.32 (m, 4 H), 2.10 (s, 3 H), 1.47-1.41 (m, 4 H), 1.34-1.31 (m, 2 H); MS eI m/z 470.

EXAMPLE 8

4-{5-Methoxy-3-methyl-1-{4-[2-(piperidin-1-yl)-ethoxy]-benzyl}-1H-indol-2-yl}-phenol

The title compound was prepared analogously to the compound of Example 1, except 4-methoxy aniline was used in the indole synthesis: Mpt = 87-90°C; CHN calcd for $\text{C}_{30}\text{H}_{34}\text{N}_2\text{O}_3 + 0.1 \text{ H}_2\text{O}$; ^1H NMR (DMSO) 9.67 (s, 1 H), 7.16 (d, 2 H, J = 8.6 Hz), 7.16 (1 H buried), 6.98 (d, 1 H, J = 2.4 Hz), 6.85 (d, 2 H, J = 8.6 Hz), 6.73 (s, 4 H), 6.69 (dd, 1 H, J = 8.8, 2.4 Hz), 5.13 (s, 2 H), 3.94 (t, 2 H, J = 5.7 Hz), 3.76 (s, 3 H), 2.63-2.50 (m, 2 H), 2.43-2.31 (m, 4 H), 2.15 (s, 3 H), 1.49-1.40 (m, 4 H), 1.39-1.25 (m, 2 H); IR (Kbr) 3400 (br), 2920, 1610, 1520 cm^{-1} ; MS eI m/z 470.

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EXAMPLE 9**2-(4-methoxy-phenyl)-3-methyl-1-[4-[2-(piperidin-1-yl)-ethoxy]-benzyl]-1H-indol-5-ol**

5

The title compound was prepared analogously to the compound of example 1, except the methyl ether of phenylpropiophenone was used instead of the benzyl ether in the indole synthesis: Mpt = 188-189°C; CHN calcd for C₃₀H₃₄N₂O₃ + 0.1 H₂O; ¹H NMR (DMSO) 8.70 (s, 1 H), 7.27 (d, 2 H, J = 8.6 Hz), 7.06 (d, 1 H, J = 8.6 Hz), 7.02 (d, 2 H, J = 8.8 Hz), 6.81 (d, 1 H, J = 2.2 Hz), 6.73 (s, 4 H), 6.58 (dd, 1 H, J = 8.8, 2.4 Hz), 5.10 (s, 2 H), 3.93 (t, 2 H, J = 5.9 Hz), 3.79 (s, 3 H), 2.56 (t, 2 H, J = 5.9 Hz), 2.41-2.32 (m, 4 H), 2.10 (s, 3 H), 1.47-1.41 (m, 4 H), 1.34-1.31 (m, 2 H); MS eI m/z 470.

15 **In vitro estrogen receptor binding assay****Receptor preparation**

CHO cells overexpressing the estrogen receptor were grown in 150 mm² dishes in DMEM + 10% dextran coated charcoal, stripped fetal bovine serum. The plates were washed twice with PBS and once with 10mM Tris-HCl, pH 7.4, 1mM EDTA. Cells were harvested by scraping the surface and then the cell suspension was placed on ice. Cells were disrupted with a hand-held motorized tissue grinder using two, 10-second bursts. The crude preparation was centrifuged at 12,000g for 20 minutes followed by a 60 minute spin at 100,000g to produce a ribosome free cytosol. The cytosol was then frozen and stored at -80°C. Protein concentration of the cytosol was estimated using the BCA assay with reference standard protein.

Binding assay conditions

30

The competition assay was performed in a 96-well plate (polystyrene*) which binds <2.0% of the total input [³H]-17β-estradiol and each data point was gathered in triplicate. 100uG/100uL of the receptor preparation was aliquoted per well. A

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saturating dose of 2.5 nM [³H]17β-estradiol + competitor (or buffer) in a 50 uL volume was added in the preliminary competition when 100x and 500x competitor were evaluated, only 0.8 nM [³H] 17β-estradiol was used. The plate was incubated at room temperature for 2.5 h. At the end of this incubation period 150 uL of ice-cold dextran coated charcoal (5% activated charcoal coated with 0.05% 69K dextran) was added to each well and the plate was immediately centrifuged at 99g for 5 minutes at 4°C. 200 uL of the supernatant solution was then removed for scintillation counting. Samples were counted to 2% or 10 minutes, whichever occurs first. Because polystyrene absorbs a small amount of [³H]17β-estradiol, wells containing radioactivity and cytosol, but not processed with charcoal were included to quantitate amounts of available isotope. Also, wells containing radioactivity but no cytosol were processed with charcoal to estimate unremovable DPM of [³H] 17β-estradiol. Corning* #25880-96, 96-well plates were used because they have proven to bind the least amount of estradiol.

15

Analysis of results

Counts per minute (CPM) of radioactivity were automatically converted to disintegrated per minute (DPM) by the Beckman* LS 7500 Scintillation Counter using a set of quenched standards to generate a H# for each sample. To calculate the % of estradiol binding in the presence of 100 or fold 500 fold competitor the following formula was applied:

$$\frac{((\text{DPM sample} - \text{DPM not removed by charcoal}) / (\text{DPM estradiol} - \text{DPM not removed by charcoal})) \times 100\%}{1} = \% \text{ of estradiol binding}$$

25

For the generation of IC₅₀ curves, % binding is plotted vs compound. IC₅₀'s are generated for compounds that show >30% competition at 500x competitor concentration. For a description of these methods, see Hulme, E.C., ed. 1992. Receptor-Ligand Interactions: A Practical Approach. IRL Press, New York.(see especially chapter 8). Reference in the tables below to the compound of Example 1 refer to the final product, 2-(4-Hydroxy-phenyl)-3-methyl-1-[4-(2-piperidin-1-yl-

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ethoxy)-benzyl]-1H-indol-5-ol. Example 2 refers to (4-Hydroxy-phenyl)-3-methyl-1-[4-(2-pyrrolidin-1-yl-ethoxy)-benzyl]-1H-indol-5-ol.

Estrogen Receptor Affinity (reported as RBA: 17 β -estradiol=100)

5

<u>Compound</u>	<u>RBA</u>
Raloxifene	200
Tamoxifen	1.8
Equilin	5.3
Example 1	50-400
Example 2	100

EXAMPLE 10

Ishikawa Cell Alkaline Phosphatase Assay

10

Cell Maintenance and Treatment:

Ishikawa cells were maintained in DMEM/F12 (50%:50%) containing phenol red + 10% fetal bovine serum and the medium was supplemented with 2 mM Glutamax, 1% Pen/Strap and 1 mM sodium pyruvate. Five days prior to the beginning of each experiment (treatment of cells) the medium was changed to phenol red-free DMEM/F12 + 10% dextran coated charcoal stripped serum. On the day before treatment, cells were harvested using 0.5% trypsin/EDTA and plated at a density of 5×10^4 cells/well in 96-well tissue culture plates. Test compounds were dosed at 10^{-6} , 10^{-7} and 10^{-8} M in addition to 10^{-6} M (compound) + 10^{-9} M 17 β -estradiol to evaluate the ability of the compounds to function as antiestrogens. Cells were treated for 48 h prior to assay. Each 96-well plate contained a 17 β -estradiol control. Sample population for at each dose was n=8.

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Alkaline Phosphatase Assay:

At the end of 48h the media is aspirated and cells are washed three times with phosphate buffered saline (PBS). 50µL of lysis buffer (0.1 M Tris-HCl, pH 9.8, 0.2% Triton X-100) is added to each well. Plates are placed at -80°C for a minimum of 15 minutes. Plates are thawed at 37°C followed by the addition of 150µL of 0.1 M Tris-HCl, pH 9.8, containing 4 mM para-nitrophenylphosphate (pNPP) to each well (final concentration, 3 mM pNPP).

Absorbance and slope calculations were made using the KineticCalc^{*} Application program (Bio-Tek Instruments, Inc., Winooski, VT). Results are expressed as the mean +/- S.D. of the rate of enzyme reaction (slope) averaged over the linear portion of the kinetic reaction curve (optical density readings every 5 minutes for 30 minutes absorbance reading). Results for compounds are summarized as percent of response related to 1 nM 17β-estradiol.

Various compounds were assayed for estrogenic activity by the alkaline phosphatase method and corresponding ED50 values (95% C.I.) were calculated. The four listed in the following were used as as reference standards:

20

17β-estradiol	0.03 nM
17α-estradiol	1.42 nM
estriol	0.13 nM
estrone	0.36 nM

25

A description of these methods is described by Holinka, C.F., Hata, H., Kuramoto, H. and Gurpide, E. (1986) Effects of steroid hormones and antisteroids on alkaline phosphatase activity in human endometrial cancer cells (Ishikawa Line). Cancer Research, 46:2771-2774, and by Littlefield, B.A., Gurpide, E., Markiewicz, L., McKinley, B. and Hochberg, R.B. (1990) A simple and sensitive microtiter plate estrogen bioassay based on stimulation alkaline phosphatase in Ishikawa cells; Estrogen action of D5 adrenal steroids. Endocrinology, 6:2757-2762.

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Ishikawa Alkaline Phosphatase Assay

	<u>Compound</u>	<u>% Activation</u>
	17 β -estradiol	100% activity
5	tamoxifen	0% activity (45% with 1 nM 17 β -estradiol)
	raloxifene	5% activity (5% with 1 nM 17 β -estradiol)
	Example 1	1% activity (1% with 1 nM 17 β -estradiol)

EXAMPLE 11

10

2X VIT ERE Transfection Assay**Cell Maintenance and Treatment**

15 Chinese Hamster Ovary cells (CHO) which had been stably transfected with the human estrogen receptor were maintained in DMEM + 10% fetal bovine serum (FBS). 48h prior to treatment the growth medium was replaced with DMEM lacking phenol red + 10% dextran coated charcoal stripped FBS (treatment medium). Cells were plated at a density of 5000 cells/well in 96-well plates containing 200 μ L of medium/well.

20

Calcium Phosphate Transfection

Reporter DNA (Promega plasmid pGL2 containing two tandem copies of the vitellogenin ERE in front of the minimal thymidine kinase promoter driving the luciferase gene) was combined with the B-galactosidase expression plasmid pCH110 (Pharmacia) and carrier DNA (pTZ18U) in the following ratio:

25

30

10uG of reporter DNA
5uG of pCH110DNA
5 uG of pTZ18U
20 uG of DNA/1 mL of transfection solution

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The DNA (20uG) was dissolved in 500 uL of 250 mM sterile CaCl₂ and added dropwise to 500 uL of 2 X HeBS (0.28 M NaCl, 50 mM HEPES, 1.5 mM Na₂HPO₄, pH 7.05) and incubated at room temperature for 20 minutes. 20 uL of this mixture was added to each well of cells and remained on the cells for 16 h. At the end of this incubation the precipitate was removed, the cells were washed with media, fresh treatment media was replaced and the cells were treated with either vehicle, 1 nM 17β-estradiol, 1uM compound or 1 uM compound + 1 nM 17β-estradiol (tests for estrogen antagonism). Each treatment condition was performed on 8 wells (n=8) which were incubated for 24 h prior to the luciferase assay.

Luciferase Assay

After 24h exposure to compounds, the media was removed and each well washed with 2 X with 125 uL of PBS lacking Mg⁺⁺ and Ca⁺⁺. After removing the PBS, 25 uL of Promega^{*} lysis buffer was added to each well and allowed to stand at room temperature for 15 min, followed by 15 min at -80°C and 15 min at 37°C. 20 uL of lysate was transferred to an opaque 96 well plate for luciferase activity evaluation and the remaining lysate (5 uL) was used for the B-galactosidase activity evaluation (normalize transfection). The luciferan substrate (Promega^{*}) was added in 100 uL aliquots to each well automatically by the luminometer and the light produced (relative light units) was read 10 seconds after addition.

Infection Luciferase Assay

<u>Compound</u>	<u>% Activation</u>
17β-estradiol	100% activity
estriol	38% activity
tamoxifen	0% activity (10% with 1 nM 17β-estradiol)
raloxifene	0% activity (0% with 1 nM 17β-estradiol)
Example 1	0% activity (0% with 1 nM 17β-estradiol)

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B-Galactosidase Assay

To the remaining 5 uL of lysate 45 uL of PBS was added. Then 50 uL of Promega B-galactosidase 2X assay buffer was added, mixed well and incubated at 37°C for 1 hour. A plate containing a standard curve (0.1 to 1.5 milliunits in triplicate) was set up for each experimental run. The plates were analyzed on a Molecular Devices* spectrophotometric plate reader at 410 nm. The optical densities for the unknown were converted to milliunits of activity by mathematical extrapolation from the standard curve.

10

Analysis of Results

The luciferase data was generated as relative light units (RLUs) accumulated during a 10 second measurement and automatically transferred to a JMP (SAS Inc) file where background RLUs were subtracted. The B-galactosidase values were automatically imported into the file and these values were divided into the RLUs to normalize the data. The mean and standard deviations were determined from a n=8 for each treatment. Compounds activity was compared to 17β-estradiol for each plate. Percentage of activity as compared to 17β-estradiol was calculated using the formula $\% = ((\text{Estradiol-control}) / (\text{compound value})) \times 100$. These techniques are described by Tzukerman, M.T., Esty, A., Santiso-Mere, D., Danielian, P., Parker, M.G., Stein, R.B., Pike, J.W. and McDonnell, D.P. (1994). Human estrogen receptor transactivational capacity was determined by both cellular and promoter context and mediated by two functionally distinct intramolecular regions (see Molecular Endocrinology, 8:21-30).

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Rat Uterotrophic/Antiuterotrophic Bioassay

The estrogenic and antiestrogenic properties of the compounds were determined in an immature rat uterotrophic assay (4 day) that (as described previously by L.J.Black and R.L.Goode, Life Sciences, 26, 1453 (1980)). Immature Sprague-Dawley rats (female, 18 days old) were tested in groups of six. The animals were treated by daily ip injection with 10 uG compound, 100 uG compound, (100 uG compound + 1 uG

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17 β -estradiol) to check antiestrogenicity, and 1 uG 17 β -estradiol, with 50% DMSO/50% saline as the injection vehicle. On day 4 the animals were sacrificed by CO₂ asphyxiation and their uteri were removed and stripped of excess lipid, any fluid removed and the wet weight determined. A small section of one horn was submitted
 5 for histology and the remainder used to isolate total RNA in order to evaluate complement component 3 gene expression.

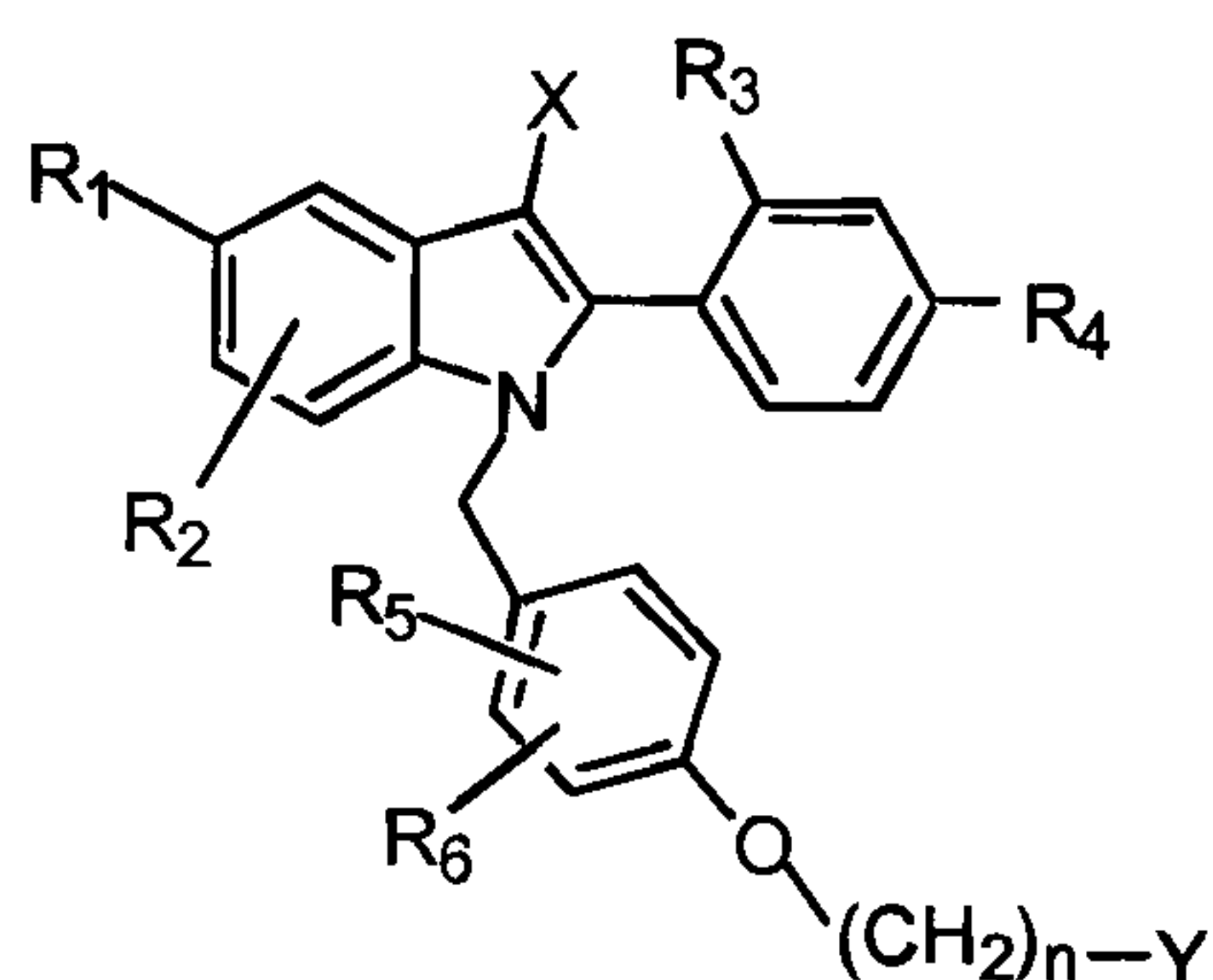
3 day Ovariectomized Rat Model

10	<u>Compound</u>	<u>10uG</u>	<u>100uG</u>	
	Tamoxifen	69.6 mg	71.4 mg	
	Raloxifen	47.5	43.2	
	control = 42.7 mg		1 uG 17 β -estradiol = 98.2	
15	<u>Compound</u>	<u>10uG</u>	<u>100uG</u>	<u>100uG + 1uG_</u> <u>17β-estradiol</u>
	Example 1	39.9 mg	27.4 mg	24.3 mg
20	control = 30.7 mg		1 uG 17 β -estradiol = 63.2	

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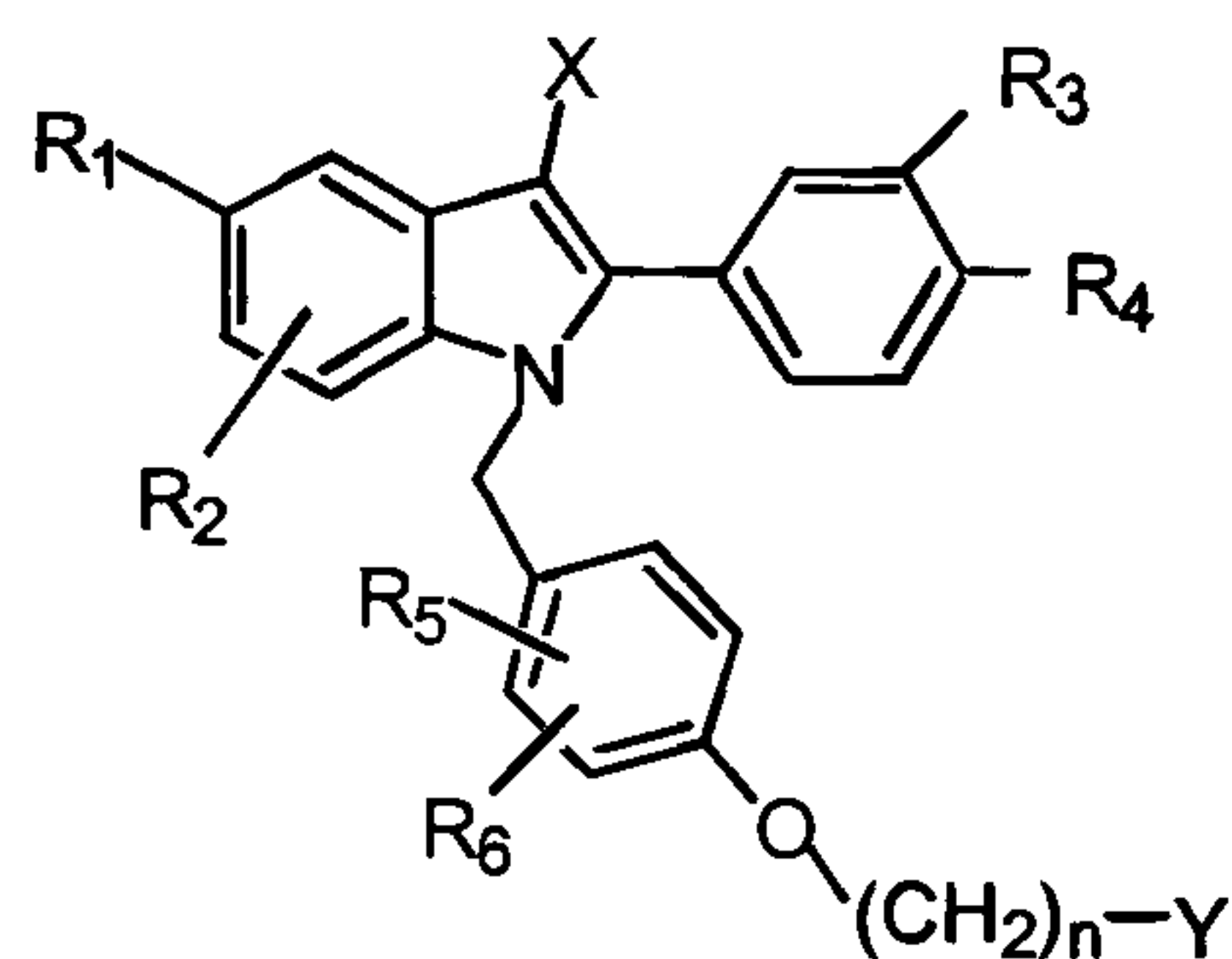
What is Claimed:

1. A compound selected from the formulas I or II:



(I)

or



(II)

wherein:

R_1 is selected from the group consisting of H, OH, a C_1 - C_4 ester, a C_1 - C_4 alkyl ether, and a halogen;

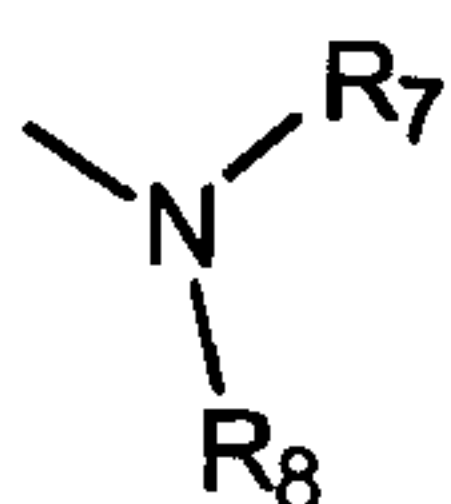
R_2 , R_3 , R_4 , R_5 , and R_6 are independently selected from the group consisting of H, OH, a C_1 - C_4 ester, a C_1 - C_4 alkyl ether, a halogen, cyano, C_1 - C_6 alkyl, and trifluoromethyl, with the proviso that, when R_1 is H, R_2 is not OH;

X is selected from the group consisting of H, C_1 - C_6 alkyl, cyano, nitro, trifluoromethyl, and halogen;

n is 2 or 3;

Y is selected from:

- a) the moiety:



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wherein R_7 and R_8 are independently selected from the group of H, C_1 - C_6 alkyl, and phenyl or

R_7 and R_8 are concatenated by $-(CH_2)_p-$, wherein p is an integer of from 2 to 6, so as to form a ring, the ring being optionally substituted by up to three substituents selected from the group of hydrogen, hydroxyl, halo, C_1 - C_4 alkyl, trihalomethyl, C_1 - C_4 alkoxy, trihalomethoxy, C_1 - C_4 alkylthio, C_1 - C_4 alkylsulfinyl, C_1 - C_4 alkylsulfonyl, hydroxy (C_1 - C_4)alkyl, $-CO_2H$, $-CN$, $-CONH(C_1-C_4 \text{ alkyl})$, $-NH_2$, C_1 - C_4 alkylamino, (C_1 - C_4)dialkylamino, $-NHSO_2(C_1-C_4 \text{ alkyl})$, $-NHCO(C_1-C_4 \text{ alkyl})$, and $-NO_2$;

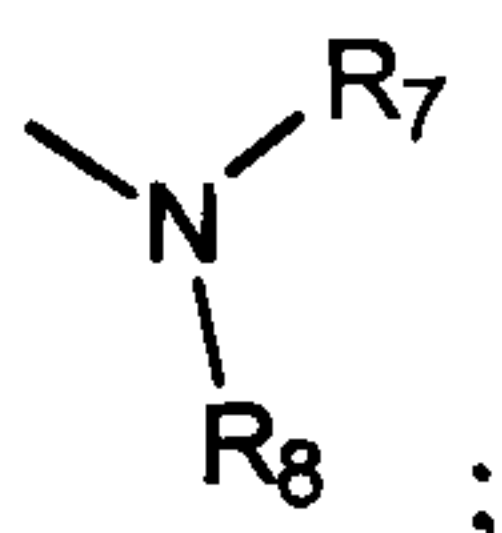
and

b) a six-membered, unsaturated heterocycle containing two $-NH-$, optionally substituted with 1-3 substituents independently selected from the group consisting of hydrogen, hydroxyl, halo, C_1 - C_4 alkyl, trihalomethyl, C_1 - C_4 alkoxy, trihalomethoxy, C_1 - C_4 acyloxy, C_1 - C_4 alkylthio, C_1 - C_4 alkylsulfinyl, C_1 - C_4 alkylsulfonyl, hydroxy (C_1 - C_4)alkyl, and phenyl, wherein said phenyl may be substituted with 1-3 (C_1 - C_4)alkyl, $-CO_2H$, $-CN$, $-CONHR_1$, $-NH_2$, C_1 - C_4 alkylamino, di(C_1 - C_4)alkylamino, $-NHSO_2R_1$, $-NHCOR_1$, and $-NO_2$,

or a pharmaceutically acceptable salt thereof.

2. A compound of Claim 1 wherein:

Y is the moiety



and

R_7 and R_8 are selected independently from H, C_1 - C_6 alkyl, or combined by $-(CH_2)_p-$, wherein p is an integer of from 2 to 6, so as to form a ring, the ring being

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optionally substituted by up to three substituents selected from the group of hydrogen, hydroxyl, halo, C₁-C₄ alkyl, trihalomethyl, C₁-C₄ alkoxy, trihalomethoxy, C₁-C₄ alkylthio, C₁-C₄ alkylsulfinyl, C₁-C₄ alkylsulfonyl, hydroxy (C₁-C₄)alkyl, -CO₂H, -CN, -CONH(C₁-C₄ alkyl), -NH₂, C₁-C₄ alkylamino, (C₁-C₄)dialkylamino, -NHSO₂(C₁-C₄ alkyl), -NHCO(C₁-C₄ alkyl), and -NO₂;

or a pharmaceutically acceptable salt thereof.

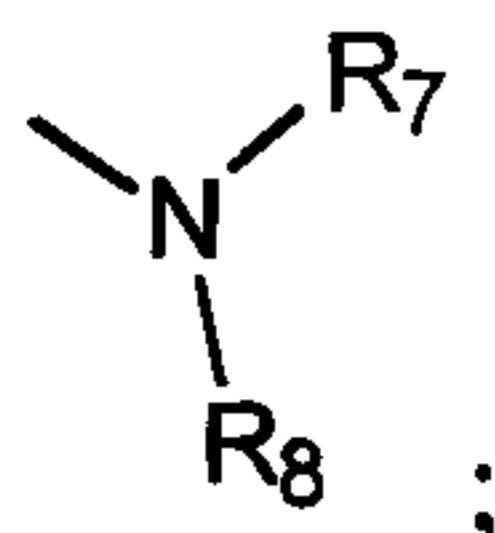
3. A compound of Claim 1 wherein:

R₁ is OH;

R₂, R₃, R₄, R₅, and R₆ are independently selected from H, OH, a C₁-C₄ ester, a C₁-C₄ alkyl ether, halogen, cyano, C₁-C₆ alkyl, and trifluoromethyl, with the proviso that, when R₁ is H, R₂ is not OH;

X is selected from the group consisting of Cl, NO₂, CN, CF₃, and CH₃;

Y is the moiety



and

R₇ and R₈ are concatenated by (CH₂)_p and p is an integer of from 4 to 6, to form a ring optionally substituted by up to three substituents selected from the group of hydrogen, hydroxyl, halo, C₁-C₄ alkyl, trihalomethyl, C₁-C₄ alkoxy, trihalomethoxy, C₁-C₄ alkylthio, C₁-C₄ alkylsulfinyl, C₁-C₄ alkylsulfonyl, hydroxy (C₁-C₄)alkyl, -CO₂H, -CN, -CONH(C₁-C₄ alkyl), -NH₂, C₁-C₄ alkylamino, (C₁-C₄)dialkylamino, -NHSO₂(C₁-C₄ alkyl), -NHCO(C₁-C₄ alkyl), and -NO₂;

or a pharmaceutically acceptable salt thereof.

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4. A compound of Claim 1 which is 2-(4-Hydroxy-phenyl)-3-methyl-1-[4-(2-pyrrolidine-1-yl-ethoxy)-benzyl]-1H-indol-5-ol or a pharmaceutically acceptable salt thereof.
5. A compound of Claim 1 which is 2-(4-Hydroxy-phenyl)-3-methyl-1-[4-(2-N,N'-diethyl-1-yl-ethoxy)-benzyl]-1H-indol-5-ol or a pharmaceutically acceptable salt thereof.
6. A compound of Claim 1 which is 2-(4-Hydroxy-phenyl)-3-methyl-1-[4-(2-piperidin-1-yl-ethoxy)-benzyl]-1H-indol-5-ol hydrochloride.
7. A compound of Claim 1 which is 1-{4-[2-(3,3-Dimethyl-piperidin-1-yl)-ethoxy]-benzyl}-2-(4-hydroxy-phenyl)-3-methyl-1H-indol-5-ol or a pharmaceutically acceptable salt thereof.
8. A compound of Claim 1 which is 2-(4-Hydroxy-phenyl)-3-methyl-1-{4-[3-(piperidin-1-yl)-propoxy]-benzyl}-1H-indol-5-ol or a pharmaceutically acceptable salt thereof.
9. A compound of claim 1 which is 2-(4-methoxy-phenyl)-3-methyl-1-{4-[2-(piperidin-1-yl)-ethoxy]-benzyl}-1H-indol-5-ol or a pharmaceutically acceptable salt thereof.
10. A compound of Claim 1 which is 4-{5-methoxy)-3-methyl-1-{4-[2-(piperidin-1-yl)-ethoxy]-benzyl}-1H-indol-2-yl}-phenol or a pharmaceutically acceptable salt thereof.
11. A pharmaceutical composition comprising a compound of Claim 1, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or excipient.
12. A pharmaceutical composition of Claim 11 in which the compound is 2-(4-Hydroxy-phenyl)-3-methyl-1-[4-(2-pyrrolidin-1-yl-ethoxy)-benzyl]-1H-indol-5-ol or a pharmaceutically acceptable salt thereof.

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13. A pharmaceutical composition of Claim 11 in which the compound is 2-(4-Hydroxy-phenyl)-3-methyl-1-[4-(2-N,N'-diethyl-1-yl-ethoxy)-benzyl]-1H-indol-5-ol or a pharmaceutically acceptable salt thereof.

14. A pharmaceutical composition of Claim 11 in which the compound is 2-(4-Hydroxy-phenyl)-3-methyl-1-[4-(2-piperidin-1-yl-ethoxy)-benzyl]-1H-indol-5-ol hydrochloride.

15. A pharmaceutical composition of Claim 11 in which the compound is 1-{4-[2-(3,3-Dimethyl-piperidin-1-yl)-ethoxy]-benzyl}-2-(4-hydroxy-phenyl)-3-methyl-1H-indol-5-ol or a pharmaceutically acceptable salt thereof.

16. A pharmaceutical composition of Claim 11 in which the compound is 2-(4-Hydroxy-phenyl)-3-methyl-1-{4-[3-(piperidin-1-yl)-propoxy]-benzyl}-1H-indol-5-ol or a pharmaceutically acceptable salt thereof.

17. A pharmaceutical composition of Claim 11 in which the compound is 2-(4-methoxy-phenyl)-3-methyl-1-{4-[2-(piperidin-1-yl)-ethoxy]-benzyl}-1H-indol-5-ol or a pharmaceutically acceptable salt thereof.

18. A pharmaceutical composition of Claim 11 in which the compound is 4-{5-methoxy)-3-methyl-1-{4-[2-(piperidin-1-yl)-ethoxy]-benzyl}-1H-indol-2-yl}-phenol or a pharmaceutically acceptable salt thereof.

19. Use of a compound according to any one of Claims 1 to 10, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament for treating or preventing bone loss in a mammal.

20. Use of a compound according to any one of Claims 1 to 10, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament for treating or preventing disease states or syndromes which are caused or associated with an estrogen deficiency in a mammal.

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21. Use of a compound according to any one of Claims 1 to 10, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament for treating or preventing cardiovascular disease in a mammal.

22. Use of a compound according to any one of claims 1 to 10, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament for treating or preventing disease in a mammal which results from proliferation or abnormal development, actions or growth of endometrial or endometrial-like tissue.

23. Use of a compound according to any one of Claims 1 to 10, or a pharmaceutically acceptable salt thereof, to treat bone loss in a mammal.

24. Use of a compound according to any one of Claims 1 to 10, or a pharmaceutically acceptable salt thereof, to prevent bone loss in a mammal.

25. Use of a compound according to any one of Claims 1 to 10, or a pharmaceutically acceptable salt thereof, to treat disease states or syndromes which are caused or associated with an estrogen deficiency in a mammal.

26. Use of a compound according to any one of Claims 1 to 10, or a pharmaceutically acceptable salt thereof, to prevent disease states or syndromes which are caused or associated with an estrogen deficiency in a mammal.

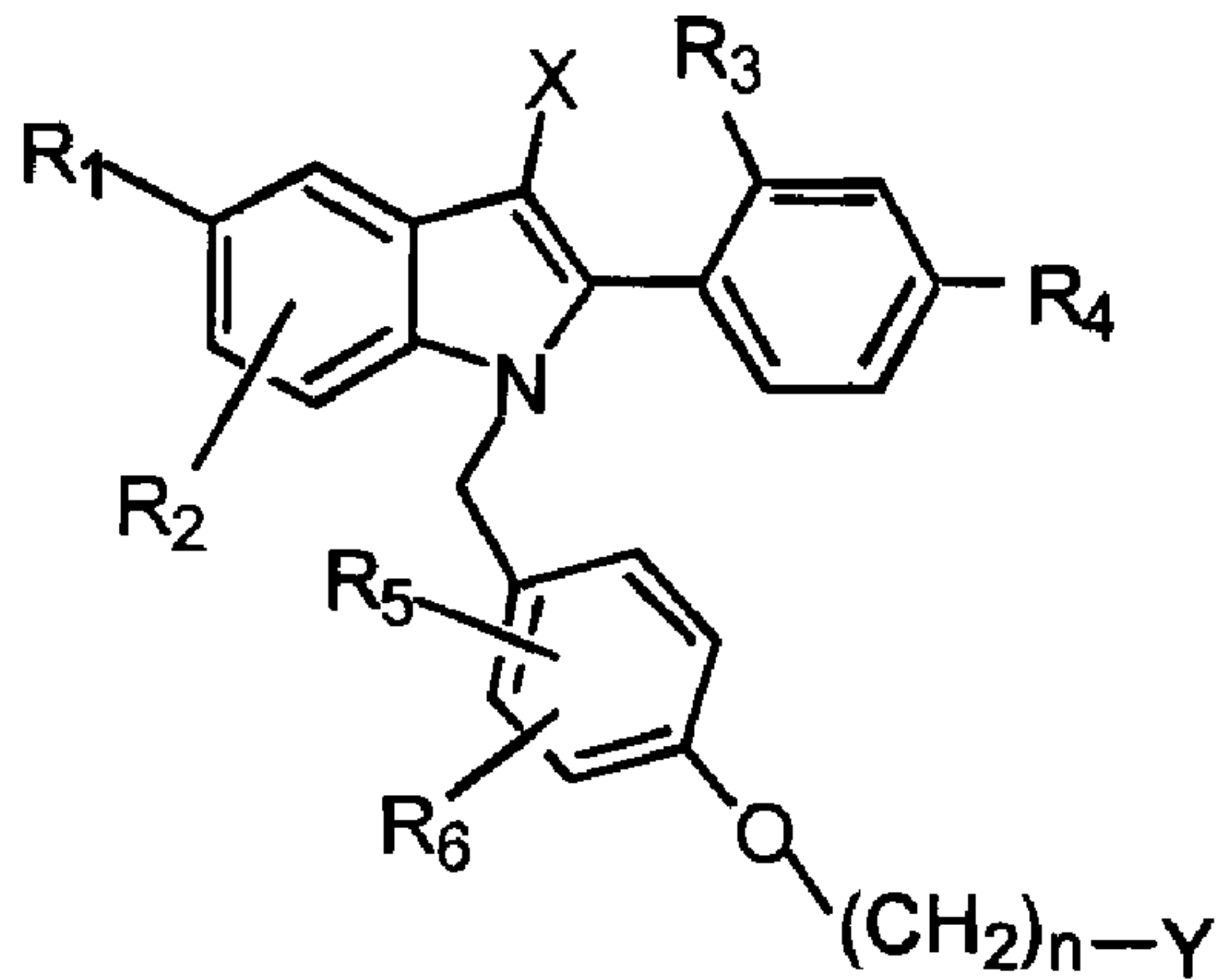
27. Use of a compound according to any one of Claims 1 to 10, or a pharmaceutically acceptable salt thereof, to treat cardiovascular disease in a mammal.

28. Use of a compound according to any one of Claims 1 to 10, or a pharmaceutically acceptable salt thereof, to prevent cardiovascular disease in a mammal.

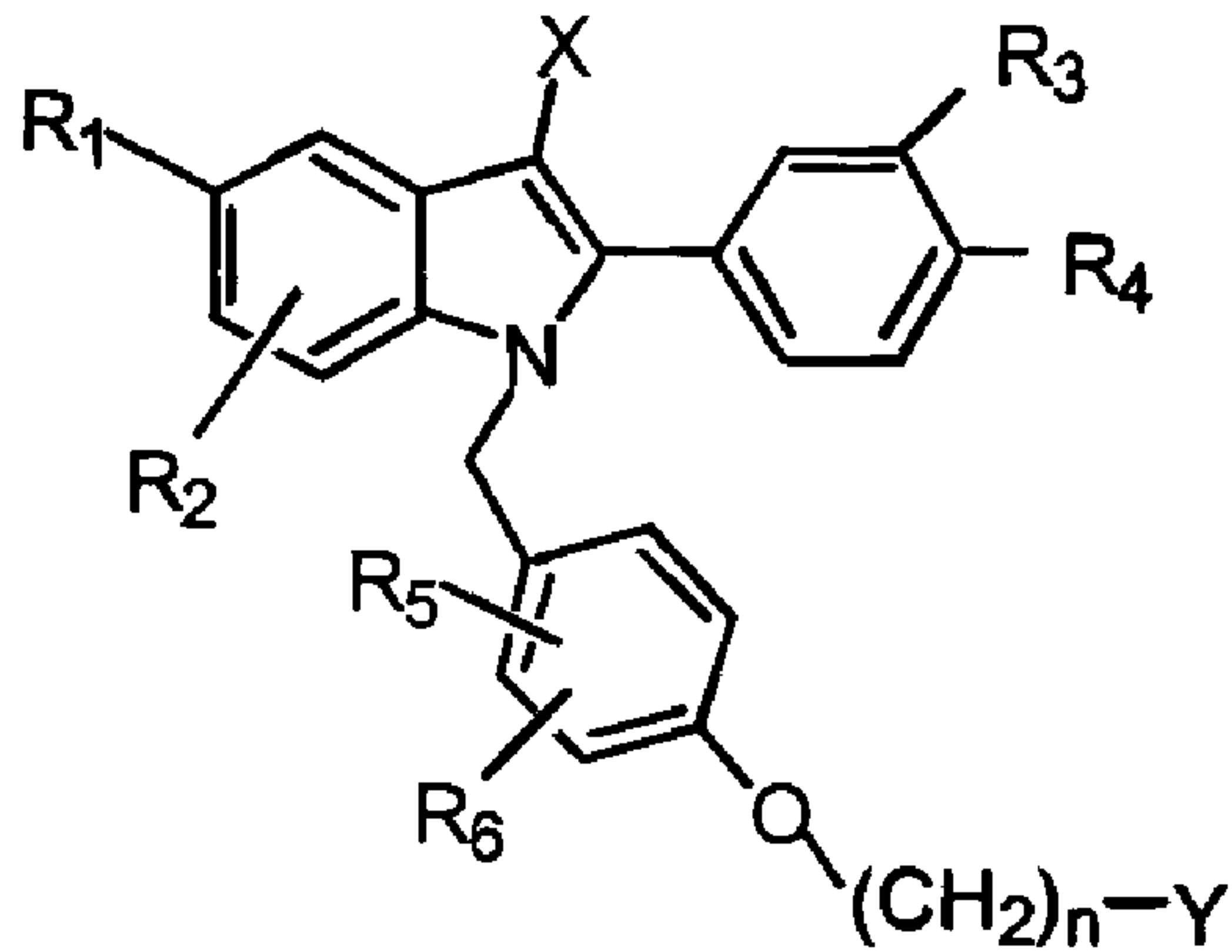
29. Use of a compound according to any one of Claims 1 to 10, or a pharmaceutically acceptable salt thereof, to treat disease in a mammal which results from proliferation or abnormal development, actions or growth of endometrial or endometrial-like tissue.

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30. Use of a compound according to any one of Claims 1 to 10, or a pharmaceutically acceptable salt thereof, to prevent disease in mammal which results from proliferation or abnormal development, actions or growth of endometrial or endometrial-like tissue.



(I)



(II)