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(54) Benævnelse: **6-substituerede estradiolderivater samt anvendelsesmåder**

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DESCRIPTION

FIELD OF THE INVENTION

[0001] The present invention relates to compositions and methods of making and using 6-substituted estradiol compounds and their pharmaceutically acceptable salts as articulated and described herein. The present invention also pertains to pharmaceutical compositions comprising such compounds, present either *in vitro* or *in vivo,* for both diagnostic applications and also treatment of proliferative conditions, such as cancer.

BACKGROUND OF THE INVENTION

[0002] Proliferative cell disorders such as tumors and primary malignant tumors {herein, cancer(s)} in particular are problematic given their tendency to invade surrounding tissues and metastasize to distant organs in the body. To date the most frequently used methods for treating neoplasia, especially solid tumor forms of neoplasia, include surgical procedures, radiation therapy, drug chemotherapies, and combinations of the foregoing.

[0003] With over million cases of cancer being diagnosed annually, and cancer claiming more than half a million lives in the United States each year, there is increased need in new therapeutic modalities against such condition. Prostate, lung and colorectal remain the most common cancers among men; while breast, colorectal and lung cancers are the most common cancers among women.

[0004] In recent years, there have been significant gains in the management of these conditions. At least one of the success stories in the clinical management of a cancer is the early diagnosis and treatment options now available for primary breast cancer. The other is employment of effective and nontoxic anti-estrogen agents that block the actions of estrogen either at its receptor sites or at a point of its synthesis.

[0005] Obviously research on the function and activity of estrogen receptors, the structure and their function has been the subject of many recent investigations. Estrogen receptors belong to a large family of structurally related ligand-inducible transcription factors, including steroid receptors, thyroid/retinoid receptors, vitamin D receptors known as nuclear receptors. While the true ligand for nuclear receptors have not been described, there are distinct small molecules that are able to bind to such receptors and trigger a cellular response.

[0006] Estrogens and estrogen receptor modulators bind to estrogen receptors, classified into two types; α and β, to form discrete molecular complexes that exert pleiotropic tissue-specific effects by modulating the expression of target genes. The ligand-bound estrogen receptor acts as a key transcription factor in various molecular pathways, and modulation of ER expression levels is important in determining cellular growth potential.

[0007] While both these types of receptors bind to estrogen, as well as other agonists and antagonists, the two receptors have distinctly different localization concentration within the body. Aside from some structural differences between the α and β types, when complexes with estrogen, the two were shown to signal in opposite way, with estrogen activating transcription in the presence of Estrogen Receptor α (ERα) and inhibiting transcription in the presence of Estrogen Receptor β (ERβ).

[0008] Tamoxifen is primarily one of the first selective estrogen receptor modulators that have become first-line therapy for hormonal treatment of breast cancer, both for adjuvant treatment and for therapy of metastatic disease. Tamoxifen is a competitive inhibitor of estradiol binding to the estrogen receptor inhibiting its estrogen binding to the estrogen binding element on DNA. It has been suggested that Tamoxifen's binding to the estrogen receptors significantly alters the structural configuration of the estrogen receptors, rendering the binding sites dysfunctional towards any endogenous estrogen. Such structural deformation of the receptor could explain the profound side effect profile associated with the use of Tamoxifen.

[0009] At least another shortcoming of Tamoxifen is its ineffectiveness against non-estrogen-dependent tumors and lower efficacy in pre-menopausal women. Additionally, Tamoxifen undergoes an isomerization under physiological conditions from a therapeutically useful antiestrogenic compound to an estrogenic isomer which can stimulate the growth of estrogen-dependent tumor cells, providing an undesired clinical outcome, particularly among patients suffering from estrogen dependent tumors.

[0010] U.S. Patent No. 4,732,904 discloses other type of estrogen receptor antagonists conventionally known as hydrazone

compounds. It is thought that these antiestrogenic hydrazone compounds do not undergo isomerization to estrogenic compounds under physiological conditions and the estrogenic side effects observed for Tamoxifen are therefore absent. These hydrazone compounds have been proposed as alternative treatments for estrogen-dependent breast cancers. Among these, the substituted benzophenone nitrophenyl hydrazones, such as 4,4'-dihydroxybenzophenone-2,4- dinitrophenylhydrazone are described to be superior.

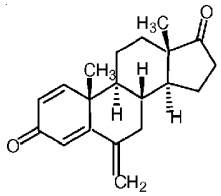
[0011] The complex of the receptor and the antiestrogen such as hydrazone based compounds or Tamoxifen may then bind to nuclear chromatin in an atypical manner for a longer time than the normal hormone receptor complex. Antiestrogens may also be able to deplete the cytoplasm of free receptor. Either or both of these effects could severely impair the continued growth of an estrogen-dependent tumor.

[0012] There has also been an increased interest in the use of aromatase inhibitors to block specifically the local production of estrogens that may contribute substantially to hormone responsive disease such as breast cancer. Aromatase(CYP19) is described as the principal enzyme that converts androgens to estrogens both in pre- and postmenopausal women. Estrogen deprivation through aromatase inhibition is described as an effective and selective treatment for some postmenopausal patients with hormone-dependent breast cancer.

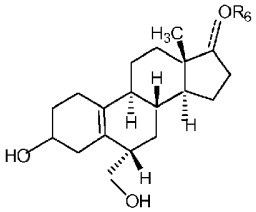
[0013] Exemestane (which is sold as Aromasin, is chemically described as 6-methylenandrosta-1,4-diene-3,17-dione)and acts as an irreversible, steroidal aromatase inactivator. It is believed to act as a false substrate for the aromatase enzyme, and processed to an intermediate that binds irreversibly to the active site of the enzyme causing its inactivation. U.S. Patent Nos. 4,808,616, and 4, 904, 650 disclose 6-alkylidenandrosta-1,4-diene-3,17-dione derivatives, such as exemestane, and methods of making them. U.S. Patent No. 4,876,045 discloses a method of preparing 6-methylene derivatives of androsta-1,4-diene-3,17-diones. U.S. Patent No. 4,990,635 discloses a process for making 6-methylene derivatives of androsta-1,4-diene-3,17-diones.

[0014] The preparation of intermediates that may be useful in preparing exemestane is disclosed in U.S. Patent No. 3,274,176. In German patent DD 258820, 6-hydroxymethyl-androsta-1,4-diene-3,17-dione is prepared from androsta-1,4-diene-3,17-dione via 1,3-dipyrrolidinoandrosta-3,5-dien-17-one.

[0015] Co-pending international application no. PCT/US2005/001248 filed January 14, 2005 (PCT Publication Number WO 2005/070951) also describes the preparation of intermediates that are useful in preparing exemestane. The structure of Exemestane is shown below.



[0016] Schneider et. al, in "Course of the reaction of steroidal 3,5-dienamines with formaldehyde", Helvetica Chimica Acta (1973), 56(7), 2396-2404, discloses the following compounds:



where the ---- symbol represents a double bond, it means a keto group and no R_6 is present; and where thesymbol represents a single bond R_6 is hydrogen (i.e. an alcohol group). Unlike the compounds of the present invention, Schneider's compounds do not embrace estradiol, testostrone or dihydrotestostrone variations.

[0017] A tri-hydroxyl substituted derivative of estranes is disclosed in U.S. Patent No. 3,377,363 to Tadanier et. al, and the 3 hydroxy substituent on the aromatic ring of the present compounds is not disclosed.

[0018] U.S. Patent No. 5,914,324 to De Funari et.al, discloses 6-hydroxy and oxy androstane derivatives for hypertension and heart failure. U.S. Patent 6,384,250 to Gobbini, et al., discloses the hydroxyl and ketone substituents at the 6 position in the preparation of (E,Z) 3-(2-aminoethoxyimino)-androstane-6,17-dione. These compounds were directed towards the treatment of

heart failure. The effects of alkyl hydroxyl substitution at the 6 position is not disclosed.

[0019] Tanenbaum, et. al, "Crystallographic comparison of the estrogen and progesterone receptor's ligand binding domains", Proc. Natl. Acad. Sci. USA, Biochemistry, Vol. 95,pp 5998-6003 , discloses the mechanism of ER receptors and notes that estradiol containing an aromatic ring with a 3-hydroxy substituent binds well with the ER ligand binding region. It is disclosed that a flat aromatic group without the 19 methyl substituent is favored.

[0020] US Patent 5,892,069 to D'Amato describes estradiol derivatives that inhibit tubulin polymerization during cell mitosis. Given the above, a need still exists to identify new and effective agents for treating cancer.

[0021] Another point of concern in the field is the eventual conversion of some estrogen-dependent cancers, i.e. breast cancer, to estrogen-independent types. This may be accounted for by a natural loss of differentiation by the tumor cells. Estrogen-dependent cancer cells have often been observed to eventually lose their ability to produce estrogen-binding protein receptors and degenerate into much more aggressive estrogen-independent life-threatening cancers. Indeed, the use of antiestrogens to treat estrogen-dependent tumors may lead to the clonal selection of estrogen-independent tumor cells and therefore may promote the conversion of an estrogen-dependent cancer to a non-estrogen-dependent cancer.

[0022] Cancers of other organs, such as lung and colon, may not concern estrogen-binding protein receptors and thus are considered independent of estrogens for cell replication. Such estrogen-independent tumors are not as susceptible to the antiestrogenic properties of drugs such as Tamoxifen, aromatase inhibitors. Thus other chemotherapeutic agents must be used to treat such tumors. Many compounds have been documented to be effective to varying degrees against estrogen-independent tumors.

[0023] These compounds are reviewed in many references and typically administered in combination regimen chemotherapy causing substantial side effect to the patients. The underlying principle of using general cytotoxic agents in chemotherapy is based upon the observation that malignant tumor cells replicate at a higher rate than normal body cells and are therefore correspondingly more susceptible to these compounds. Similarly, normal tissues that proliferate rapidly (for example, bone marrow and intestinal epithelium) are subject to substantial damage once exposed to these potent cytotoxic drugs, and such toxicity often limits utility.

[0024] On the other hand, slow growing tumors with a small growth fraction, for example carcinomas of the colon or lung, are often unresponsive to cytotoxic drugs. Aside from the treatment of estrogen-dependent and estrogen-independent tumors, many of the cytotoxic drugs are currently being used for other proliferative diseases with rapidly growing cells involved non-cancerous or non-malignant hyperproliferative conditions.

[0025] Also the increasing importance of effective therapeutic management of viral diseases such as AIDS, herpes, various types of hepatitis and bacterial infections, especially among immune suppressed patients, calls for alternative modes of therapy with favorable side effect profile.

[0026] Accordingly, there is not only a need for new and improved cancer chemotherapeutics that can be used to treat both estrogen-dependent and estrogen-independent tumors with minimal risk of systemic toxicity challenging the quality of life for such fragile population of patients, but also for therapeutic remedies that target non-cancerous hyperproliferative conditions which can benefit from effective doses of estradiol derivatives. The hyperproliferative cells can be normal, rapidly growing cells or abnormal cells and can include tissue having rapidly growing endogenous cells or their abnormal subpopulation, or other tissues generally exogenous to the patient.

[0027] None of the teachings of prior art provide for a therapeutic estradiol derivative with favorable side effect profile that can be used for these types of conditions.

SUMMARY OF THE INVENTION

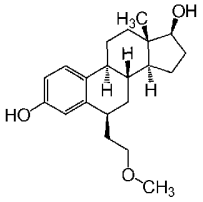
[0028] In light of the foregoing, the present invention is directed towards chemotherapeutic compounds and compositions, thereby overcoming various deficiencies and shortcomings of the prior art, including those outlined above. Accordingly, it is one object of the present invention to provide compounds useful in the treatment of estrogen-dependent conditions and tumors which provide a better patient tolerance, prognosis and compliance.

[0029] Another object of the present invention is to provide compounds for the treatment of estrogen-independent tumors with compounds having substantially less side effects than those currently available to the patients.

[0030] Yet another objective of the present invention is to provide for compounds and alternative modes of treatment of tissues afflicted with hyperproliferative conditions, including viral and bacterial infections.

[0031] The present disclosure providescompounds according to Formula IV.

[0032] Specific examples of compounds of the invention are shown below:



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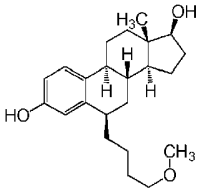

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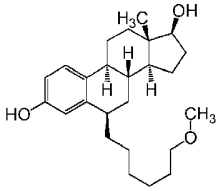

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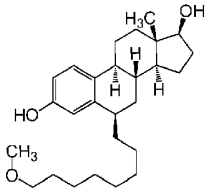
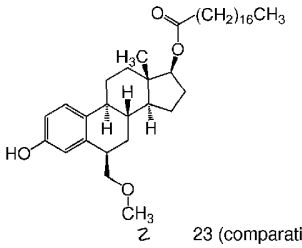
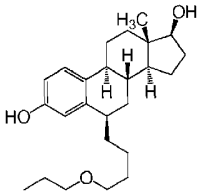


22



23 (comparative)



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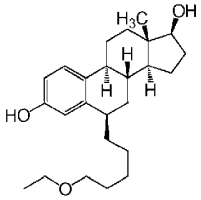

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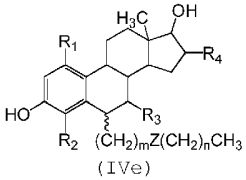
[0033] The present invention pertains to a compound of Formula IVe:



(IVe)

wherein R1, R2, R3 and R4 are hydrogen; m is an integer from 2 - 8; n is an integer from 0 - 3 and Z is -O-. In addition to this, both the C-13 methyl and C-17 hydroxyl are (S)-configured. In the forumla IVe, the

symbol represents any type of bond regardless of the stereochemistry; and the respective hydrates, solvates and pharmaceutically acceptable salts thereof.

[0034] The compounds of the present invention may be used to treat any tumor which may be either directly or indirectly effected by hormonal and/or estrogen-related activity, including but not in any way limited to solid tumors associated with breast, pancreatic, lung, colon, prostate, ovarian cancers, as well as brain, liver, spleen, kidney, lymph node, small intestine, blood cells, bone, stomach, endometrium, testicular, ovary, central nervous system, skin, head and neck, esophagus, or bone marrow cancer; as well as hematological cancers, such as leukemia, acute promyelocytic leukemia, lymphoma, multiple myeloma, myelodysplasia, myeloproliferative disease, or refractory anemia.

[0035] The compounds of the present invention may also be used in combination-based therapeutic cancer treatments in a mammalian subject. Such methods may comprise administration of a compound of Formula (IVe) in combination with other adjunct cancer therapies, such as chemotherapy, radiotherapy, gene therapy, hormone therapy and other cancer therapies known in the

art.

[0036] Any of the compounds of the present invention may be contemplated for administration to the mammalian subject in the form of a drug. In this disclosure, the term "administering" shall encompass the treatment of the various conditions described with the compound specifically disclosed.

[0037] Other objects, features, benefits and advantages of the present invention will be apparent from this summary and the following descriptions of certain embodiments, and will be readily apparent to those skilled in the art having knowledge of various chemotherapeutic compounds, methods and/or modes of operation.

DETAILED DESCRIPTION OF THE INVENTION

[0038] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs and shall be understood to have the meanings described below. Unless otherwise specified, a reference to a particular compound includes all such isomeric forms, including racemic and other mixtures thereof. Unless otherwise specified, a reference to a particular compound also includes ionic, salt, solvate (e.g., hydrate), for example, as discussed herein.

[0039] It may be convenient or desirable to prepare, purify, and/or handle a corresponding salt of the active compound, for example, a pharmaceutically-acceptable salt. Examples of pharmaceutically acceptable salts are discussed in Berge et al., 1977, "Pharmaceutically Acceptable Salts," J. Pharm. Sci., Vol. 66, pp. 1-19, and discussed herein.

[0040] Anti-proliferative compounds of the present invention have application in the treatment of cancer, and so the present invention further provides anti-cancer agents. The term "anti-cancer agent" as used herein, pertains to a compound which treats, delays progression, prolongs relapse period of, and controls symptoms of a cancer (i.e., a compound which is useful in the treatment of a cancer). The anti-cancer effect may arise through one or more mechanisms, including but not limited to, the regulation of cell proliferation, the inhibition of angiogenesis (the formation of new blood vessels), the inhibition of metastasis (the spread of a tumor from its origin), the inhibition of invasion (the spread of tumor cells into neighboring normal structures), or the promotion of apoptosis (programmed cell death), or tumor necrosis or tumor autophagy or any combinations thereof.

[0041] The invention further provides active compounds for use in a method of treatment of the human or animal body by therapy. Such a method may comprise administering to such a subject a therapeutically-effective amount of an active compound, preferably in the form of a pharmaceutical composition as discussed further herein.

[0042] The term "estrogen" as used herein encompass steroid like hormones that are naturally made and is able to cross the cell membrane to exert its activity inside the cell by binding to the estrogen receptors. Example of such compounds include but are not limited to estradiols, estrols, and esterenes.

[0043] The term "treatment," or "therapy" as used herein in the context of treating a condition, pertains generally to treatment and therapy of a mammalian subject, whether of a human or a non-human animal (e.g., in veterinary applications), in which some desired therapeutic effect is achieved, for example, the inhibition of the progress of the condition, and includes a reduction in the rate of progress, a halt in the rate of progress, amelioration of the condition, and/or cure of the condition. Treatment as a prophylactic measure is also included. Treatment includes combination treatments and therapies, in which two or more treatments or therapies are combined, for example, sequentially or simultaneously. Examples of treatments and therapies include, but are not limited to, chemotherapy (the administration of active agents, including, e.g., drugs, antibodies (e.g., as in immunotherapy) ; surgery; radiation therapy; and gene therapy.

[0044] The term "stereochemical isomer" as used herein, refers to isomers that differ from each other only in the way the atoms are oriented in space. The two stereoisomers particularly of importance in the instant invention are enantiomers and diastereomers depending on whether or not the two isomers are mirror images of each other. In the preferred embodiment, the claimed formulations comprise such compounds that isolated, resolved and are "substantially free of other isomers."

[0045] The term "therapeutically-effective amount," as used herein, pertains to that amount of an active compound, or a material, composition or dosage form comprising an active compound, which is effective for producing some desired therapeutic effect, commensurate with a reasonable benefit/risk ratio.

[0046] The term "patient" refers to animals, including mammals, preferably humans.

[0047] The term "region of a patient" refers to a particular area or portion of the patient afflicted with a proliferative disorder, cancer or tumor and in some instances to regions throughout the entire patient. Exemplary of such regions are the pulmonary region, the gastrointestinal region, the breast region, the renal region as well as other bodily regions, tissues, lymphocytes, receptors, organs and the like, including the vasculature and circulatory system, and cancerous tissue. "Region of a patient" includes, for example, regions to be treated with the disclosed compounds and compositions. The "region of a patient" is preferably internal, although it may be external.

[0048] The term "tissue" refers generally to specialized cells which may perform a particular function. The term "tissue" may refer to an individual cell or a plurality or aggregate of cells, for example, membranes, blood or organs. The term "tissue" also includes reference to an abnormal cell or a plurality of abnormal cells. Exemplary tissues include breast tissue, including breast cells, membranous tissues, including endothelium and epithelium, laminae, connective tissue, including interstitial tissue, and tumors.

[0049] The term "proliferative cell disorders" as used herein refers to disorders such as tumors, primary malignant tumors, and other hyperproliferative conditions. The terms "primary malignant tumor(s)" and "cancer(s)" are used interchangeably.

Compounds

[0050] The present invention relates to estradiol derivatives of formula (IVe) with specific modifications at position 6 of the B ring of the estradiol.

[0051] Embodiment compounds of the present invention can be used in a pharmaceutical composition. Such a composition can comprise one or more compounds selected from those discussed above, illustrated below or otherwise inferred herein, and combinations thereof. In certain embodiments, such a composition can comprise a pharmaceutically-acceptable carrier component. Without limitation, such a composition can comprise a racemic mixture of compounds. In certain embodiments, such a compound can be present as the S and R enantiomer, preferably their isolated and purified form which is substantially free of other isomers

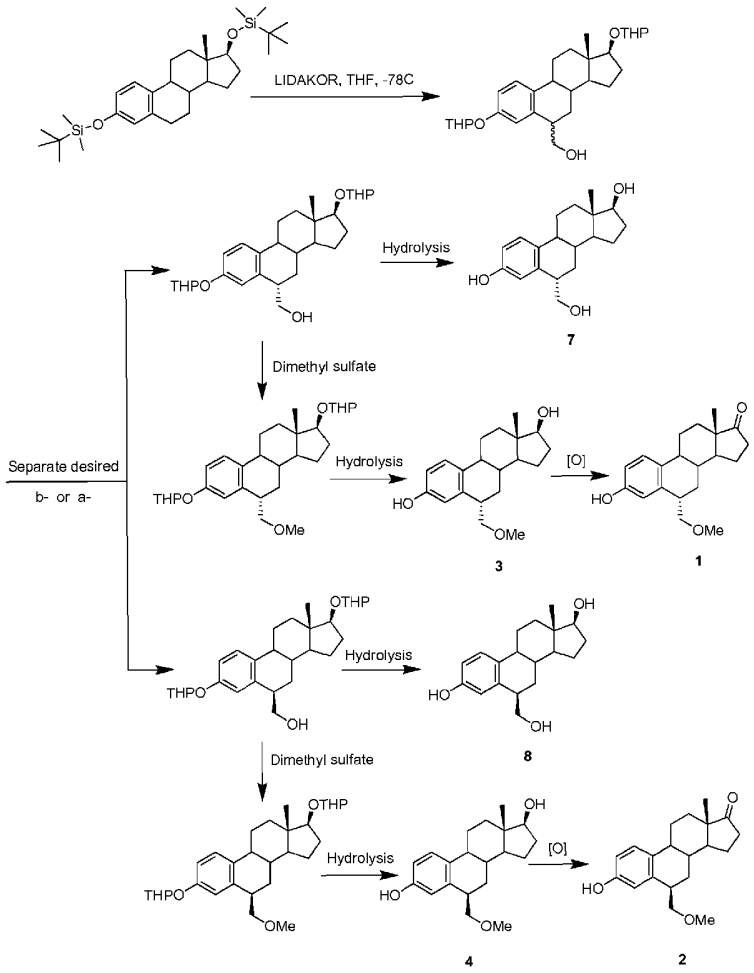
[0052] The compounds disclosed herein may have asymmetric centers and may occur as a racemate, a racemic mixture or as individual and purified diastereomers or enantiomers such as (named via ChemDraw Ultra, Version 11.0(3) or 12.0) (6*S*,8*R*,9*S*,13*S*,14*S*)-6-(methoxymethyl)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[alphenanthrene-3,17-diol (comparative compound 3); (6*R*,8*R*,9*S*,13*S*,14S)-6-(methoxymethyl)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[alphenanthrene-3,17-diol (comparative compound 4);(6*R*,8*R*,9*S*,13*S*,14*S*)-6-((aminooxy)methyl)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[alphenanthrene-3,17-diol (comparative compound 10); (6*S*,8*R*,9*S*,13*S*,14*S*)-6-((aminooxy)methyl)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[alphenanthrene-3,17-diol (comparative compound 11); (6*R*,8*R*,9*S*,13*S*,14*S*)-6-((aminooxy)methyl)-17-hydroxy-13-methyl-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[a]phenanthren-3-one (comparative compound **12**); (6*S*,8*R*,9*S*,13*S*,14*S*)-6-((aminooxy)methyl)-17-hydroxy-13-methyl-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[a]phenanthren-3-one (comparative compound **13**); (6*R*,8*R*,9*S*,13*S*,14*S*)-6-(((methoxymethyl)amino)methyl)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[a]phenanthrene-3,17-diol (comparative compound 14); (6*S*,8*R*,9*S*,13*S*,14*S*)-6-(((methoxymethyl)amino)methyl)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[alphenanthrene-3,17-diol (comparative compound 15); 1-((((6*R*,8*R*,9*S*,13*S*,14*S*)-3,17-dihydroxy-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[alphenanthren-6-yl)methyl)amino)propan-2-one (comparative compound **16**); 1-*((((*6*S*,8*R*,9*S*,13*S*,14*S*)-3,17-dihydroxy-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[alphenanthren-6-yl)methyl)amino)propan-2-one (comparative compound **17**) ; (6*R*,8*R*,9*S*,13*S*,14*S*)-6-methoxy-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[a]phenanthrene-3,17-diol (comparative compound **18**); (6*S*,8*R*,9*S*,13*S*,14*S*)-6-(2-methoxyethyl)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[a]phenanthrene-3,17-diol (compound **19**); (6*R*,8*R*,9*S*,13*S*,14*S*)-6-(4-methoxybutyl)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[a]phenanthrene-3,17-diol (compound **20**); (*6*R,8*R*,9*S*,13*S*,14*S*)-6-(6-methoxyhexyl)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[a]phenanthrene-3,17-diol (compound **21**); (6*R*,8*R*,9*S*,13*S*,14*S*)-6-(6-methoxyoctyl)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[a]phenanthrene-3,17-diol (compound **22**); (6*R*,8*R*,9*S*,13*S*,14*S*)-3-hydroxy-6-(methoxymethyl)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[alphenanthren-17-yl stearate (comparative compound **23**); (6*R*,8*R*,9*S*,13*S*,14*S)*-13-methyl-6-(4-propoxybutyl)-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[a]phenanthrene-3,17-diol (compound **30**); and (6*R*,8*R*,9*S*,13*S*,14*S*)-13-methyl-6-(5-ethoxypentyl)-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[alphenanthrene-3,17-diol (compound **31**).

[0053] This disclosure pertains to the preparation of the R or S enantiomers, and/or R or S diastereomers of 6 substituted estradiols. Methods for the preparation (e.g., asymmetric synthesis) and separation (e.g., fractional crystallization and chromatographic means) of such isomeric forms are either generally known in the art or are readily obtained by adapting the methods taught herein. Such methodologies are, for example, described in the co-pending U.S. application USSN 11/541, 987.

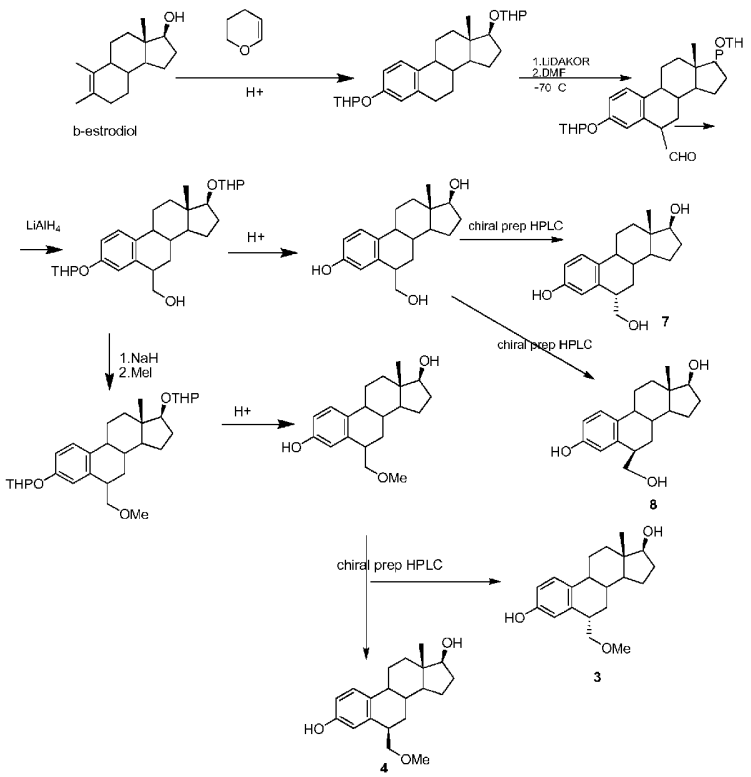
[0054] By way of illustration a method for preparing a 6-hydroxymethyl, 6-alkoxyalkyl, 6-alkylthioalkyl, 6-aminomethoxy, 6-methylaminomethoxy, or 6-methoxyamine derivatives of estradiol is disclosed herein. Reaction schemes for preparing estradiol derivatives are given below, Schemes 1-3. Such methods can comprise reaction of a t-butyldimethylsilyl derivative of estradiol with LIDAKOR/THF/formaldehyde to obtain a 6-hydroxylated compound followed by such steps as: (i) hydrolysis to obtain 6-hydroxymethyl derivative of estradiol; and/or (ii)treatment with dimethylsulfate followed by hydrolysis to obtain 6-methyloxymethyl derivative of estradiol. NDC-1088 can be obtained by further oxidation of NDC-1033 at the C-17 hydroxyl position.

[0055] In an alternative approach, 6-substituted aromatic steroids related to those of the present invention can also be prepared by a method comprising such steps as: (i) protecting an estrodial compound, (ii) acylating the protected estradiol compound at the benzylic 6-position with LIDAKOR/ButylLithium/Diisopropylamine/potassium tert-amylate, (iii) reducing the position 6 aldehyde with lithium aluminum hydride, (iv) deprotecting the protected regions of the estradiol compound. A reaction scheme for preparing estradiol derivatives is given below in Scheme 2.

Scheme 1



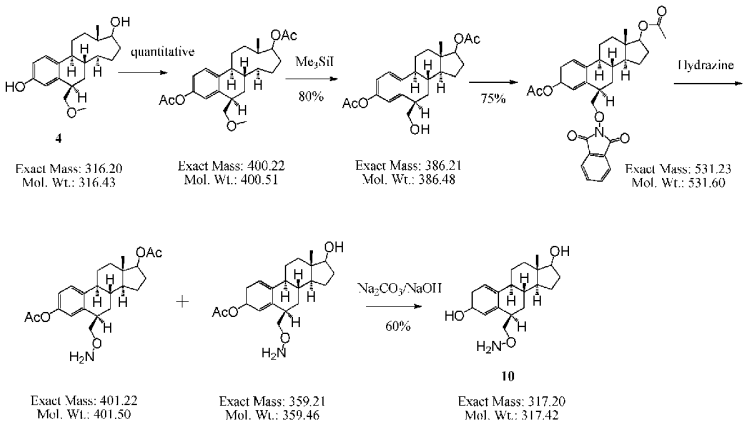
Scheme 2



[0056] Various alkyloxyalkyl derivatives, in accordance with this disclosure, involve selection of alkylating agents. Such derivatives would be understood by those skilled in art made aware of this disclosure, and is available through synthetic procedures of the sort described herein. Accordingly, without limitation, various C_1 to C_6 alkyl and substituted alkyl reagents can be used as described herein to prepare the corresponding alkyloxyalkyl derivatives.

[0057] In another aspect of this disclosure, methods of making 6-amino derivatives of the estradiol are disclosed in reaction schemes below. Accordingly, 6-methoxylated estradiols described in Schemes 1-2 are employed and converted to their respective amino derivatives.

Scheme 3



[0058] Among other things, the invention offers a new mode of action for treating estrogen dependent or independent tumors. Traditional approach employed drugs once bound to the ERs modified the ERs configuration to the extent that in effect rendered them destroyed. Accordingly, destruction of such bound ERs would cease transmission of all external and internal signals essential for vitality of the cells, creating a stop in cellular growth.

[0059] It is believed that the presently disclosed compounds are able to bind to number of receptors including the estrogen, testosterone and androgen receptors. The inventor has unexpectedly observed that upon binding, the compounds of the present invention are able to modulate the cellular first or second messenger signaling pathways and further potentiate their clinical effects through gene dependent or gene independent mechanisms, e.g. gene dependent estrogen activity has been well described in the art and those of ordinary skill in the art are able to ascertain the pathways involved inactivation of a estrogen dependent gene.

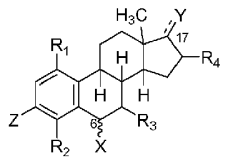
[0060] However, in the present invention, the claimed compounds are able to modulate cellular activity at a level independent of the traditional gene regulated mechanisms. In this aspect of the invention, the compounds of the instant invention are capable of binding directly to multiple steroid receptors at the plasma membrane and trigger internal cell mediated stress mechanisms involving the unfolded protein response ("UPR") at the endoplasmic reticulum. The UPR stress response subsequently lead to growth inhibition, and cell death through modulation of stress response genes such as CHOP also known as GADD153, TRIB3, etc.

[0061] In addition, administration of the compounds disclosed herein for treatment of various cancer states may comprise administration of a compound of formula IVe in combination with other adjunct cancer therapies, such as chemotherapy, radiotherapy, gene therapy, hormone therapy and other cancer therapies known in the art. Combinations of the presently disclosed compounds with other anti-cancer or chemotherapeutic agents are within the scope of the invention. Examples of such agents can be found in Cancer Principles and Practice of Oncology by V. T. Devita and S. Hellman (editors), 6th edition (Feb. 15, 2001), Lippincott Williams & Wilkins Publishers. A physician, veterinarian or clinician of ordinary skill in the art would be able to discern which combinations of agents would be useful based on the particular characteristics of the drugs and the cancer involved. Such anti-cancer agents include the following: estrogen receptor modulators, androgen receptor modulators, retinoid receptor modulators, cytotoxic agents, anti-proliferative agents, prenyl-protein transferase inhibitors, HMG-CoA reductase inhibitors, EHV protease inhibitors, reverse transcriptase inhibitors, aromatase inhibitors, and angiogenesis inhibitors.

Exemplified Compounds

[0062] Compounds of the present disclosure are illustrated in table III below:

Table III



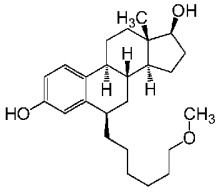
R_1, R_2, R_3, R_4: independently H, C_1-C_6 alkyl, substituted alkyl, or halogen and — is a single or double bond; not present.

Entry	X	Z	Y	C-6	C-8	C-9	C-13	C-14	C-17
Comparative Compound **18**	OMe	OH	OH	R	R	S	S	S	S
Comparative Compound **14**	$CH_2NHCH_2OCH_3$	OH	OH	R	R	S	S	S	S
Comparative Compound **15**	$CH_2NHCH_2OCH_3$	OH	OH	S	R	S	S	S	S
Comparative Compound **16**	$CH_2NHC(O)$- OCH_3	OH	OH	R	R	S	S	S	S
Comparative Compound **17**	$CH_2NHC(O)$- OCH_3	OH	OH	S	R	S	S	S	S
Compound **19**	$(CH_2)_2OMe$	OH	OH	S	R	S	S	S	S
Compound **20**	$(CH_2)_4OMe$	OH	OH	R	R	S	S	S	S
Compound **21**	$(CH_2)_6OMe$	OH	OH	R	R	S	S	S	S
Compound **22**	$(CH_2)_8OMe$	OH	OH	R	R	S	S	S	S

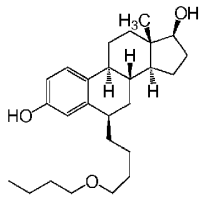
Entry	X	Z	Y	C-6	C-8	C-9	C-13	C-14	C-17
Comparative Compound **23**	Me	$C(O)-(CH_2)_{16}CH_3$	H	R	R	S	S	S	S
Compound **30**	$(CH_2)_4O(CH_2)_2-CH_3$	OH	OH	R	R	S	S	S	S
Compound **31**	$(CH_2)_5O(CH_2)-CH_3$	OH	OH	R	R	S	S	S	S

[0063] The preferred compounds in Table III include compounds **19, 20, 21, 22,** and **31.** At least one aspect of the instant invention is directed to these preferred compounds, their method of use and making.

[0064] One specific non-limiting example for treatment of an identified cancer state as described herein includes use a compound of formula IVe such as:



21



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[0065] The above active compounds may also be used as part of an *in vitro* assay, for example, in order to determine whether a candidate host is likely to benefit from treatment with the compound in question. Any active compound of the present invention may also be used as a standard, for example, in an assay, in order to identify other active compounds, other anti-proliferative agents, other anti-inflammatory agents, etc.

[0066] At least in one aspect of the instant invention, the candidate compounds were evaluated for their estrogen receptor antagonistic activity. The evaluation as to whether a compound is an estrogen receptor antagonist may be carried out by various methodologies known in the art. In the instant application, such capacity was determined by conducting the Luciferase binding assay according to the screening methods described herein.

[0067] In a more preferred embodiment of this aspect of the invention, the estrogen receptor binding capacity were assessed by transiently transfecting CV-1 cells with expression constructs for either ER(α) or ER (β) plus an ERE-tk-luciferase reporter construct. The cells were then divided into controls and candidate groups wherein the controls received no treatment, or were treated with estradiol alone (1 nM) and the candidate groups received estradiol plus a compound of the invention at varying concentrations. After 16-24 hours the cells were harvested and assayed for luciferase activity using a commercially available assay kit.

[0068] In yet another aspect of the instant invention, the IC_{50} or the half maximal inhibitory concentration of the candidate compounds were determined to assess drug potency and potential dosing regimens for *in vivo* use. One of ordinary skill in the art is readily able to ascertain such information using commonly known methodologies. As it has been well described in the art, IC_{50} represents and measures how much of a particular substance/molecule is needed to inhibit some biological process by 50%. In the instant case, the IC_{50} of the candidate compounds were determined as the concentration that led to a response of 50% compared to the vehicle control cells.

[0069] As noted herein, the salts of the compounds of this invention refer to non-toxic "pharmaceutically acceptable salts." Other salts may, however, be useful in the preparation of the compounds according to the invention or of their pharmaceutically acceptable salts. When the compounds of the present invention contain a basic group, salts encompassed within the term "pharmaceutically acceptable salts" refer to non-toxic salts which are generally prepared by reacting the free base with a suitable organic or inorganic acid. Representative salts include any such salt known in the art. Where compounds of the present invention carry an acidic moiety, suitable pharmaceutically acceptable salts thereof may include alkali metal salts, e.g., sodium or potassium salts; alkaline earth metal salts, e.g., calcium or magnesium salts; and salts formed with suitable organic ligands, e.g., quaternary ammonium salts.

[0070] To treat a mammalian subject, such as a human patient, an effective amount of one or more compounds of the present

invention, or a pharmaceutically-acceptable salt thereof, is administered to the mammalian subject so as to promote exposure to or contact of cancer cells or the targeted tumor growth. Effective dosage forms, modes of administration and dosage amounts may be determined empirically, and making such determinations is within the skill of the art. It is understood by the physician, veterinarian or clinician of ordinary skill in the art that the dosage amount will vary with the activity of the particular compound employed, course and/or progression of the disease state, the route of administration, the rate of excretion of the compound, renal and hepatic function of the patient, the duration of the treatment, the identity of any other drugs being administered to the subject, age, size and like factors well known in the medical arts. As discussed herein, the compounds of the present invention can be administered in such oral dosage forms as tablets, capsules (each of which includes sustained release or timed release formulations), pills, powders, micronized compositions, granules, elixirs, tinctures, suspensions, syrups and emulsions. Likewise, they may also be administered in intravenous (bolus or infusion), intraperitoneal, topical (e.g., ocular eyedrop), subcutaneous, intramuscular or transdermal (e.g., patch) form, all using forms well known to those of ordinary skill in the pharmaceutical arts. Again, the ordinarily skilled physician, veterinarian or clinician can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition.

[0071] Oral dosages of the present invention, when used for the indicated effects, will range between about 0.01 mg per kg of body weight per day (mg/kg/day) to about 100 mg/kg/day, preferably 0.01 to 10 mg/kg/day, and most preferably 0.1 to 5.0 mg/kg/day. For oral administration, the compositions are preferably provided in the form of tablets containing 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100 and 500 milligrams of the active ingredient for the symptomatic adjustment of the dosage to the patient to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the active ingredient, preferably, from about 1 mg to about 100 mg of active ingredient. Intravenously, the most preferred doses will range from about 0.1 to about 10 mg/kg/minute during a constant rate infusion. Compounds of the present invention may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three or four times daily.

[0072] As noted herein, the compounds of the present invention can be used in combination with other anti-cancer agents or other agents which will enhance the treatment regime for the mammalian subject. The individual components of such combinations can be administered separately at different times during the course of therapy or concurrently in divided or single combination forms to patients or regions of such patients in need of such therapy. The instant invention is therefore to be understood as embracing all such regimes of simultaneous or alternating treatment and the term "administering" is to be interpreted accordingly. It will be understood that the scope of combinations of the compounds of this invention with other agents useful to treat the targeted cancer condition includes in principle any combination with any pharmaceutical composition useful for treating disorders related to estrogen functioning.

[0073] Compounds of the present invention may be prodrugs for potent anti-proliferative agents. Compounds which exhibit low or moderate intrinsic activity may act as prodrugs, and be metabolically activated (e.g., in vivo) to generate more potent compounds. This is especially useful in cancer therapy where metabolic activation can be achieved by an enzyme that is expressed in tumors. Prodrugs, acting as a substrate, may be metabolized by CYP19, 17β-HSD, HS-demethylase or another steroidal linked enzyme to generate a potent anti-cancer agent. The *(R)* or (*S*)-6-methyloxoalkyl derivatives of exemestane suggest that it may be active against numerous forms of cancer beyond breast cancer. Activity in inhibiting tumor cell growth in cells lines derived from breast, lung, colon, prostate, endometrial and ovarian cancers was observed for the NDC-1011 enantiomer. For example, *in vitro* studies of tumor cell growth is the highest in cell lines that are CYP19 positive (MDA-MB-213 and SK-OV-3) and is reduced in those cell lines that are CYP19 negative (MCF-7 and NIH:OVCAR-3) indicates that comparative compound 24 may act as a pro-drug.

[0074] While not bound by any theory, for example, if comparative compound 24 is a pro-drug, then any number of the body's normal steroidogenic enzymes should be active towards comparative compound 24 thereby converting comparative compound 24 into the active metabolite(s). This aspect of the invention can apply in the same manner to both the S and the R diastereomers.

Compositions

[0075] As used herein, the term "composition" is intended to encompass a product comprising the specified ingredients in the specified amounts, as well as any product which results, directly or indirectly, from combination of the specified ingredients in the specified amounts.

[0076] Pharmaceutical formulations of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. Regardless of the route of administration selected, the active ingredient(s) are formulated into pharmaceutically-acceptable dosage forms by conventional methods known to those of skill in the art.

[0077] The amount of the active ingredient (s) which will be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration and all of the other factors described above. The amount of the active ingredient(s) which will be combined with a carrier material to produce a single dosage form will generally be that amount of the active ingredient(s) which is the lowest dose effective to produce a therapeutic effect.

[0078] Methods of preparing pharmaceutical formulations or compositions include the step of bringing the active ingredient(s) into association with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing the active ingredient(s) into association with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

[0079] Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or nonaqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of the active ingredient(s). The active ingredient(s) may also be administered as a bolus, electuary or paste.

[0080] In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the prodrug(s), active ingredient(s) (in their micronized form) is/are mixed with one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethyl-cellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

[0081] A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered active ingredient(s) moistened with an inert liquid diluent.

[0082] The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient(s) therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient(s) can also be in microencapsulated form.

[0083] Liquid dosage forms for oral administration of the active ingredient(s) include pharmaceutically-acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient(s), the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethylacetate, butyl alcohol, benzyl benzoate, propylene glycol, glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, amyl alcohol, tetrahydrofuryl polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

[0084] Besides inert diluents the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents. Suspensions, in addition to the active ingredient(s),may contain suspending agents as, for example, ethoxylated alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

[0085] Formulations of the pharmaceutical compositions of the invention for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing the active ingredient(s) with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, wax or salicylate and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active ingredient(s). Formulations of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

[0086] Dosage forms for the topical or transdermal administration of the active ingredient(s) include powders sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active ingredient(s) may be mixed under sterile conditions with pharmaceutically-acceptable carrier, and with any buffers, or propellants which may be required.

[0087] The ointments, pastes, creams and gels may contain, in addition to the active ingredient(s), excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof. Powders and sprays can contain, in addition to the active ingredient(s), excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

[0088] Compounds of the present invention may be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in the art. A transdermal delivery system provides for continuous administration throughout the dosage regimen. Transdermal patches have the added advantage of providing controlled delivery of the active ingredient(s) to the body. Such dosage forms can be made by dissolving, dispersing or otherwise incorporating the active ingredient(s) in a proper medium, such as an elastomeric matrix material. Absorption enhancers can also be used to increase the flux of the active ingredient(s) across the skin. The rate of such flux can be controlled by either providing a rate-controlling membrane or dispersing the active ingredient(s) in a polymer matrix or gel.

[0089] The compounds of the present invention can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines.

[0090] Another mode of delivery for the compounds of the present invention may be delivery via the use of monoclonal antibodies as individual carriers to which the compound molecules are coupled. The compounds of the present invention may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxypropylm ethacrylamide-phenol, polyhydroxy-ethylaspartamidephenol, or polyethyleneoxide-polylysine substituted with palmitoyl residues. Furthermore, the compounds of the present invention may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyglycolic acid, copolymers of polyactic and polyglycolic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates and crosslinked or amphipathic block copolymers of hydrogels.

[0091] Pharmaceutical compositions of this invention suitable for parenteral administration comprise the active ingredient(s) in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or nonaqueous solutions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

[0092] Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size, and by the use of surfactants.

[0093] These compositions may also contain adjuvants such as wetting agents, emulsifying agents and dispersing agents. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like in the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

[0094] In some cases, in order to prolong the effect of the active ingredient(s), it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the active ingredient(s) then depends upon its/their rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of parenterally-administered active ingredient(s) is accomplished by dissolving or suspending the active ingredient(s) in an oil vehicle.

[0095] Injectable depot forms are made by forming microencapsule matrices of the active ingredient(s) in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of the active ingredient (s) to polymer, and the nature of the particular polymer employed, the rate of release of the active ingredient (s) can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the active ingredient(s) in liposomes or microemulsions which are compatible with body tissue. The injectable materials can be sterilized for example, by filtration through a bacterial-retaining filter.

[0096] Preferably the composition delivered in the form of an injectable dosage form comprise a biocompatible polymer, a compatible form of the presently disclosed compounds and a biocompatible solvent which solubilizes the biocompatible polymer wherein the weight percents of the biocompatible polymer, the instant and biocompatible solvent are based on the total weight of the complete composition; further wherein sufficient amounts of said polymer are employed in said composition such that, upon delivery to a vascular site, the polymer is able to precipitate and allow release of the active compound in doses sufficient to stop tumor growth.

[0097] Still another aspect of this embodiment would observe for appropriate viscosity of said composition, preferably in the range of about 10 to 200 cSt at 40° C.

[0098] More preferably, the composition delivered locally to the solid tumor comprises a biocompatible polymer at a concentration of from about 1 to 95 weight percent, active compound at a concentration of from about 5 to about 75 weight percent, and a biocompatible solvent from about 5 to about 95 weight percent, wherein the weight percent of the all components is based on the total weight of the complete composition and further wherein the composition has a viscosity of at least 10 to about 200 and more preferably at least about 200 cSt at 40°C.

[0099] Biodegradable polymers are disclosed in the art. For example, Dunn, et al. in U.S. Patent 4,938,763, discloses the following examples of biodegradable polymers: linear-chain polymers such as polylactides, polyglycolides, polycaprolactones, polyanhydrides, polyamides, polyurethanes, polyesteramides, polyorthoesters, polydioxanones, polyacetals, polyketals, polycarbonates, polyorthocarbonates, polyphosphazenes, polyhydroxybutyrates, polyhydroxyvalerates, polyalkylene oxalates, polyalkylene succinates, poly(malic acid), poly(amino acids), polyvinylpyrrolidone, polyethylene glycol, polyhydroxycellulose, chitin, chitosan, and copolymers, terpolymers and combinations thereof. Other biodegradable polymers include, for example, gelatin, collagen, etc.

[0100] Suitable non-biodegradable biocompatible polymers include, by way of example, cellulose acetates, ethylene vinyl alcohol copolymers, hydrogels (e.g., acrylics), polyacrylonitrile, polyvinylacetate, cellulose acetate butyrate, nitrocellulose, copolymers of urethane/carbonate, copolymers of styrene/maleic acid, and mixtures thereof.

[0101] Preferred biocompatible polymers can include acrylic polymers, cellulose diacetate and ethylene vinyl alcohol copolymer, polyethylene glycol, chitosen, collagen and gelatin. Such polymers are either commercially available or can be prepared by art recognized procedures. In a preferred embodiment, the number average molecular weight, as determined by gel permeation chromatography composition is from about 5,000 to about 200,000 more preferably from about 25,000 to about 180,000 and still more preferably from about 50,000 to 100,000.

[0102] A biocompatible contrast agent may be employed within the composition provided herein to observe and monitor the clinical progress of the local site of interest. These contrast agents include water soluble contrast agents and water insoluble contrast agents. Preferably, the water insoluble contrast agent is a biocompatible material selected from the group consisting of barium sulfate, tantalum powder and tantalum oxide. In still a further preferred embodiment, the biocompatible solvent is water, dimethylsulfoxide (DMSO), ethanol, ethyl lactate or acetone.

[0103] The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a lyophilized condition requiring only the addition of the sterile liquid carrier, for example water for injection, immediately prior to use. Extemporaneous injection solutions and suspensions maybe prepared from sterile powders, granules,

nanoparticles and tablets of the type described above.

[0104] The pharmaceutical compositions of the present invention may also be used in the form of veterinary formulations, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or nonaqueous solutions or suspensions), tablets, boluses, powders, granules or pellets for admixture with feed stuffs, pastes for application to the tongue; (2) parenteral administration, for "ampule, by subcutaneous, intramuscular or intravenous injection as, for example, a sterile solution or suspension or, when appropriate, by intramammary injection where a suspension or solution is introduced into the udder of the animal via its teat; (3) topical application, for example, as a cream, ointment or spray applied to the skin; or (4) intravaginally, for example, as a pessary, cream or foam or any other methods fit to by those of ordinary skill in the art for administration to a region of interest.

[0105] The general methods given in Schemes 1, 2, and 3 for the preparation of compounds exemplified in formula IV and in Table III above are further illustrated by the following examples. Specifically, the methods given in Schemes 1 and 2 for the preparation of 6-alkoxyalkyl Estradiol compounds are illustrated by Comparative Examples 1, 2, 4 and 5 shown below, and Scheme 3 is for the preparation of 6-amino derivatives of estradiol. An example of assessing the estrogen receptor binding capacity is articulated in comparative example 4, and finally assessing the IC_{50} of the preferred compounds of this disclosure and their comparative efficacy is given in comparative example 5. Unless otherwise specified all starting materials and reagents are of standard commercial grade, and are used without further purification, or are readily prepared from such materials by routine methods. Those skilled in the art of organic synthesis will recognize that starting materials and reaction conditions may be varied to achieve the desired end product.

Comparative Example 1

Methods of Preparing 6-hydroxymethyl-androsta-1,4-diene-3,17 dione.

[0106] In a reaction system, sufficient amounts of (+)androsta-1,4-diene-3,17-dione (ADD), 12.2 equivalents pyrrolidine, catalytic acetic acid, denatured ethanol (95/5 ethanol/methanol) and 6-7% tetrahydrofuran (THF) are heated to 30 to 40°C for a minimum of 16 hours to form 1,3 -dipyrrolidinoandrosta-3,5-diene-17one. Once the ADD content reaches to a less than 3% by HPLC area, or it becomes static or the resulting dipyrrolidinoandrostadiene begins to revert to ADD, the reaction mixture is cooled to 5 $\pm$ 5°C. The resulting compound is then collected and washed with cold denatured ethanol. Yields are typically 70-80% on a dry basis with purities typically 90-95% by HPLC area percent.

[0107] The resulting 1,3-dipyrrolidinoandrosta-3,5-diene-17one is then mixed in amount of 1 equivalent with 2.6 equivalents formalin (formaldehyde) in 10 ml dichloromethane/g at room temperature. The reaction mixture is then acidified to a pH of about 2 with 2% sulfuric acid solution. Accordingly, an organic layer is formed, which is washed with 2% sulfuric acid and 1:1 water/brine. Solvent exchange into toluene (approximately 10ml/g) is then carried out wherein the product crystallizes as toluene exchange transpires. Said product is collected washed and dried to provide 6-hydroxymethyl-androsta-1,4-diene-3,17 dione. One of ordinary skill in the art can further modify the stereochemistry at position 6, if so desired, by employing known techniques in the art.

Comparative Example 2

Methods of preparing comparative compounds 3 and 4.

[0108] As outlined in Scheme 2, estradiol comparative compounds 3 and 4 are synthesized in the following manner. The protected estradiol is prepared by reaction of β-estradiol with dihydropyran in THF, using toluenesulfonic acid or camphorsulfonic acid as catalyst. As one of ordinary skill in the art can appreciate, this reaction is an equilibrium reaction and would not go to completion under such conditions. Thus, both the mono-protected estradiols can be found in the reaction mixture. Such crude reaction mixture would undergo a trituration step with acetonitrile causing the desired bis-THP estradiol to crystallize in approximately 70 % yield.

[0109] As shown in Scheme 2, the intermediate aldehyde is obtained via acylation at the benzylic 6-position with a strong base

mixture referred to as LiDAKOR: butyl lithium, diisopropylamine, and potassium tert-amylate. Under such conditions at -70°C, one of ordinary skill in the art can appreciate the abstraction of a proton at a benzylic position. The intermediate aldehyde is then purified by column chromatography to give a syrup in approximately 50 % yield. Reduction of the aldehyde with an excess of lithium aluminum hydride results in high yields of the racemic hydroxymethyl estradiol compound as a glassy foam.

[0110] For purposes of preparing comparative compounds 3 and 4, the methoxymethyl intermediate compound is prepared by methylation of the racemic hydroxymethyl estradiol compound with sodium hydride and methyl iodide. The methoxymethyl intermediate is purified by column chromatography to give a glassy foam. Deprotection of the protecting groups gives deprotected racemic 6-methoxymethyl estradiol. Separation of the enantiomers is performed using chiral preparative HPLC to give the comparative compounds 3 and 4. For comparative compound 4, a chiral purity of >95:5 *R:S* is realized. For comparative compound 3, a chiral purity of 86:14 *S:R* is realized. It is well within the level of one of ordinary skill in the art to employ NMR for determination of the absolute stereochemistry of the 6-position, where the 4-and 6-protons are diagnostic.

Comparative Example 4

Methods of preparing comparative compound 10.

[0111] Using the same methodologies as in comparative examples 1-2, comparative compound 4 is prepared. To a solution of comparative compound 4 (0.32 g, 1 mmol) in DCM (30 ml), acetic anhydride (0.6 ml, 6 mmol, 3 eq), TEA (0.5 ml, 3.6 mmol, 1.8 eq) and DMAP (50 mg) is added. The reaction solution is stirred at ambient temperature for 3 hrs. Thin Layer Chromatography ("TLC") follows the reaction to completion.

[0112] The reaction solution is washed with 1M HCl (20 ml), saturated $NaHCO_3$ (20 ml) and brine (20 ml), respectively. The DCM phase is dried over $MgSO_4$ and filtered. The filtrate is evaporated and dried in high vacuum at 60 °C for 3 hrs and at ambient temperature overnight to afford pure desired protected methoxy compound of Scheme 3 (0.4 g, white, quantitative).

[0113] To a solution of protected methoxy compound of Scheme 3 (0.35 g, 0.87 mmol) in DCM is added iodotrimethylsilane (6 ml, 44 mmol, 50 eq) at ambient temperature. The yellow solution is stirred at 38 °C overnight under argon.

[0114] The reaction solution is cooled in an ice-bath. Excess sat. $NaHCO_3$ (10 ml) is slowly added to quench the reaction. After separation of the mixture, the organic phase is washed with brine and concentrated for silica gel purification using 3% MeOH in DCM as mobile phase. A yield (80%) of 270 mg of pure hydroxyl compound of Scheme 3 is obtained.

[0115] A solution of hydroxyl compound of Scheme 3(250 mg, 0.65 mmol), triphenylphosphine (222 mg, 0.85 mmol, 1.3 eq), and N-hydroxyphthalimide (140 mg, 0.85 mmol, 1.3 eq) in THF (20 ml) is cooled in an ice-batch. To the cooled solution is added diethyl azodicarboxylate (0.6 ml, 1 mmol, 1.5 eq). The reaction mixture is warmed to ambient temperature and stirred overnight.

[0116] The reaction mixture is then evaporated, and the resulting residue is diluted with DCM (50 ml), washed with brine and concentrated for silica gel purification using 3% MeOH/DCM as mobile phase to yield 260 mg (75%) of desired white phthalimide compound of Scheme 3.

[0117] A solution of phthalimide compound of Scheme 3(580 mg, 1.1 mmol) in anhydrous DCM (30 ml) is cooled in an ice-batch. Methyl hydrazine (0.22 ml, 2.2 mmol, 4 eq) is added. The mixture is warmed to ambient temperature and stirred for 3 hrs. LC-MS follows the reaction to completion.

[0118] The reaction solution is washed with a solution of brine and sat. $NaHCO_3$ (1:1, 10 ml). The aqueous phase is washed with DCM (10 ml). The combined DCM phases are evaporated to dryness under high vacuum. LC-MS confirms that the crude product contains two major aminooxy products of Scheme 3.

[0119] The crude mixture (~ 0.7 mmol) of aminooxy compounds of Scheme 3 in MeOH (60 ml) is cooled in an ice-bath. A solution of sodium carbonate (0.5 g, 4.7 mmol) in water (10 ml) and a solution of NaOH (0.8 g, 20 mmol) in water (10 ml) is subsequently added. The reaction is warmed to ambient temperature and stirred overnight.

[0120] The pH of final reaction solution is about 14. A solution of sat. sodium bicarbonate (~10 ml) is added to adjust pH to 10.

The mixture is then evaporated to remove most of the methanol. To the resulting mixture is added DCM (100 ml) and sat. sodium bicarbonate (30 ml) for extraction. The aqueous phase is washed with DCM (2x50 ml). The combined DCM phases are evaporated to dryness to give crude mixture 400 mg.

[0121] The crude mixture is purified by silica gel column using 5% MeOH/DCM as mobile phase to afford desired final product comparative compound 10 (135 mg, off white, 60% yield, 98% HPLC purity, NMR and LC-MS confirmed).

Comparative Example 5

Methods of preparing comparative compound 21

[0122]

1. a) (8*R*, 9*S*, 13*S*, 14*S*, 17*S*) -3, 17-bis(methoxymethoxy)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6**H**-cyclopenta[*a*]phenanthrene - Chloromethyl methyl ether (7.0 mL, 92.0 mmol) is added to a solution of β-estradiol (5 g, 18.4 mmol) and diisopropylethylamine (16.0 mL 92 mmol) in 100 mL of THF. The reaction mixture is heated to reflux and stirred for 18 hours. The THF is removed *in vacuo,* and the yellow/brown oil is partitioned between water and CH_2Cl_2. The organic layer is separated, washed with brine, dried (Na_2SO_4), filtered, and evaporated *in vacuo* to give a golden oil. Purification by silica gel column chromatography (10% EtOAc/Hex) affords the title compound as a viscous, clear oil (5.7 g, 86%).
2. b) (8*R*, 9*S*, 13*S*, 14*S*, 17*S*) -3, 17-bis(methoxymethoxy)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[*a*]phenanthren-6-ol - To a solution of potassium tert-butoxide (8.87 g, 79.0 mmol) and diisopropylamine (11.2 mL, 79.0 mmol) in 80 mL of anhydrous THF cooled to -78°C under argon is added n-butyllithium (49.4 mL, 79.0 mmol, 1.6 M in hexane) dropwise. The reaction mixture is stirred at -78°C for 30-45 minutes. A solution of the compound from a) (5.7 g, 15.8 mmol) in 45 mL of THF is then added dropwise, and the reaction mixture is stirred for 3 hours at -78°C. During the addition of the compound from a), the reaction turns a deep red color. Trimethyl borate (10.6 mL, 94.8 mmol) is then added slowly, and the mixture is warmed to 0°C and stirred for 2 hours. Hydrogen peroxide (24 mL of a 30% aq. solution) is then added, and the reaction mixture is warmed to room temperature and stirred for a further 1 hour. The reaction is cooled back to 0°C and carefully quenched with a 10% aq. Na_2S2O_3 solution (70 ml). The resulting mixture is extracted with EtOAc (2x), and the combined organic extracts are dried (Na_2SO_4), filtered, and evaporated *in vacuo* to give a yellow/brown oil. Purification by silica gel column chromatography (25% EtOAc/Hex) affords the title compound as a white solid (3,5 g, 59%) .
3. c) (8*R*, 9*S*, 13*S*, 14*S*, 17*S*) -3, 17-bis(methoxymethoxy)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[*a*]phenanthren-6-one - Dess-Martin Periodinane (9.46 g, 22.3 mmol) is added portionwise to a solution of the compound from b) (7.0 g, 18.6 mmol) in 300 mL of CH_2Cl_2. The resulting reaction mixture stirred at room temperature for 3 hours. The mixture is poured into water and the layers are separated. The aqueous layer is extracted with CH_2Cl_2, and the combined organic extracts are washed with brine, dried (Na_2SO_4), filtered, and evaporated *in vacuo* to give a gooey, brown solid. Purification by silica gel column chromatography (15% EtOAc/Hex) affords the title compound as a pale yellow, viscous oil (6.0 g, 86%).
4. d) ethyl 2-(((8*R*,9*S*,13*S*,14*S*,17*S*)-3,17-bis(methoxymethoxy)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[*a*]phenanthren-6-ylidene)acetate - Triethyl phosphonoacetate (4.1 mL, 20.8 mmol) is added to a mixture of sodium hydride (832 mg, 20.8 mmol) in 25 mL of THF at room temperature. After approximately 10 minutes, a solution of the compound from c) (3.9 g, 10.4 mmol) in 10 mL of THF is added dropwise. The resulting reaction mixture is heated to reflux in a sealed tube for 72 hours. The mixture is concentrated *in vacuo* and purified by silica gel column chromatography (gradient from 5% EtOAc/Hex to 40% EtOAc/Hex) to give the title compound as a clear, viscous oil (3.4 g, 74%).
5. e) 2-((8*R*,9*S*,13*S*,14*S*,17*S*)-3,17-bis(methoxymethoxy)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[*a*]phenanthren-6-ylidene)ethanol- A solution of the compound from d) (3.1 g, 6.97 mmol) in 65 mL of THF is treated with lithium aluminum hydride (5.2 mL, 10.46 mmol, 2 **M** in THF) dropwise at 0°C. The cold bath is removed, and the reaction mixture is stirred at room temperature for 15 minutes. The reaction is cooled back to 0°C and quenched by the careful addition of 1.3 mL of water, followed by 2.6 mL of 2N NaOH, and then 1.3 mL of water. The mixture is stirred vigorously until a white solid forms. The mixture is filtered, and the filtrate is concentrated *in vacuo* to give the title compound as a clear oil (2.8 g, 99%).
6. f) 2-((6*S*,8*R*,9*S*,13*S*,14*S*,17*S*)-3,17-bis(methoxymethoxy)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-CyClopenta[*a*]phenanthren-6-yl)acetaldehyde - A mixture of the compound from e) (3.09 g, 7.68 mmol) and 10% Pd/C (500 mg) in 100 mL of ethyl acetate is stirred under 40 psi of H_2 (g) for 5 hours at room temperature. The mixture is filtered through Celite, and the Celite is washed well with ethyl acetate. The filtrate is concentrated *in vacuo* to give a pale yellow oil

(3.1 g). The oil is dissolved in 100 mL of dichloromethane, and Dess-Martin Periodinane (3.9 g, 9.22 mmol) is added portionwise. The resulting reaction mixture is stirred at room temperture for 30 minutes. The mixture is poured into water and extracted with CH_2Cl_2. The combined organic extracts are washed with brine, dried (Na_2SO_4), filtered, and evaporated *in vacuo* to give a brown solid. Purification by silica gel column chromatography (15% EtOAc/Hex) affords the title compound as a clear oil (2.0 g, 65%) .

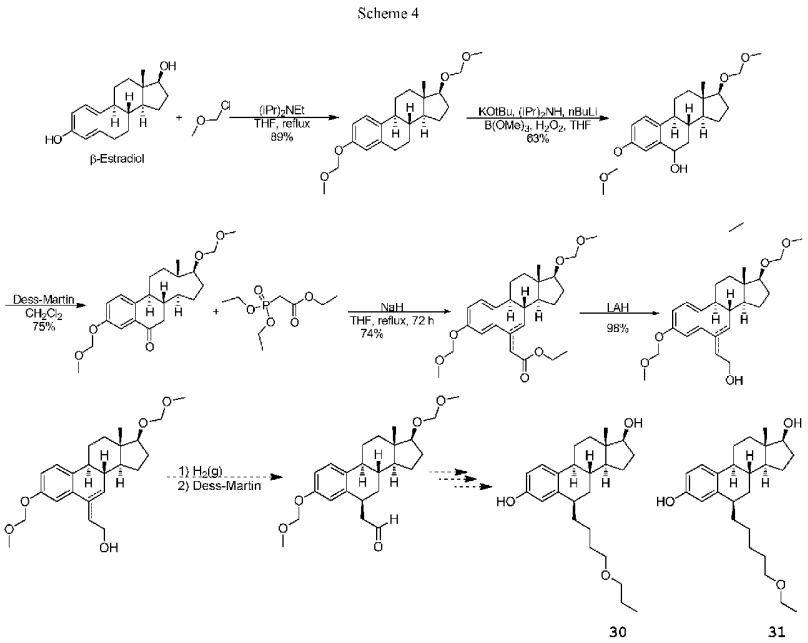
7. g) 4-((6*R*,8*R*,9*S*,13*S*,14*S*,17*S*)-3,17-bis(methoxymethoxy)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[*a*]phenanthren-6-yl)but-2-en-1-ol - Lithium bis(trimethylsilyl)amide (18.4 mL, 18.4 mmol, 1.0 M in THF) is added dropwise to a suspension of (2-hydroxyethyl) triphenylphosphonium bromide (3.37 g, 8.70 mmol) in 60 mL of THF at 0°C. After 1 hour, the golden brown solution is treated with a solution of the compound from f) (1.4 g, 3.48 mmol) in 10 mL of THF dropwise. The resulting reaction mixture is stirred at 0°C for 40 minutes and then quenched with saturated aqueous NH_4Cl. The resulting mixture is extracted with EtOAc (2x), and the combined organic extracts are dried (Na_2SO_4), filtered, and evaporated to give a brown oil. Purification by silica gel column chromatography (gradient from 20% EtOAc/Hex to 75% EtOAc/Hex) affords the title compound as a yellow, viscous oil (680 mg, 45%).
8. h) 4-((6R,8R,9S,13S,14S,17S)-3,17-bis(methoxymethoxy)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[*a*]phenanthren-6-yl)but-2-enal - Dess-Martin Periodinane (437 mg, 1.03 mmol) is added to a solution of the compound from g) (370 mg, 0.86 mmol) in 15 mL of CH_2Cl_2 at room temperature. The resulting reaction mixture is stirred for 10 minutes and then poured into water. The layers are separated and the aqueous layer is extracted with CH_2Cl_2 (2x). The combined organic extracts are washed with brine, dried (Na_2SO_4), filtered, and evaporated *in vacuo* to give a brown oil. Purification by silica gel column chromatography (gradient from 5% EtOAc/CH2Cl2 to 10% EtOAc/CH_2Cl_2) affords the title compound as a pale yellow, viscous oil (358 mg, 86%).
9. i) 6-((6*R*,8*R*,9*S*,13*S*,14*S*,17*S*)-3,17-bis(methoxymethoxy)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[*a*]phenanthren-6-yl)hexa-2,4-dien-1-ol - Lithium bis(trimethylsilyl)amide (4.3 mL, 4.29 mmol, 1.0 M in THF) is added dropwise to a suspension of (2-hydroxyethyl) triphenylphosphonium bromide (786 mg, 2.03 mmol) in 14 mL of THF at 0°C. After 30 minutes, the golden brown solution is treated with a solution of the compound from h) (345 mg, 0.81 mmol) in 2 mL of THF dropwise. The resulting reaction mixture is stirred at 0°C for 20 minutes and quenched with saturated aqueous NH_4Cl-The resulting mixture is extracted with EtOAc (2x), and the combined organic extracts are dried (Na_2SO_4), filtered, and evaporated to give a brown oil. Purification by silica gel column chromatography (gradient from 5% EtOAc/CH_2Cl_2 to 40% EtOAc/CH_2Cl_2) affords the title compound as a yellow, viscous oil (140 mg, 38%).
10. j) (6*R*,8*R*,9*S*,13*S*,14*S*,17*S*)-6-(6-methoxyhexa-2,4-dien-1-yl)-3,17-bis(methoxymethoxy)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[*a*]phenanthrene - A solution of the compound in i) (135 mg, 0.3 mmol) is cooled to 0°C, and sodium hydride (120 mg, 3.0 mmol) is added portionwise. After 5-10 minutes, iodomethane (0.19 mL, 3.0 mmol) is added dropwise, and the resulting reaction mixture is warmed to room temperature and stirred for 4 hours. EtOAc is added and the reaction is carefully quenched with water. The layers are separated and the organic layer is dried (Na_2SO_4), filtered, and evaporated to give a brown oily residue. Purification by silica gel column chromatography (gradient from 5% EtOAc/Hex to 20% EtOAc/Hex) affords the title compound as a clear oil (92 mg, 65%).
11. k) (6*R*,8*R*,9*S*,13*S*,14*S*,17*S*)-6-(6-methoxyhexyl)-3,17-bis(methoxymethoxy)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[*a*]phenanthrene - A mixture of the compound in j) (90 mg, 0.19 mmol) and 10% Pd/C (100 mg) in 5-10 mL of ethyl acetate is stirred under a balloon of H_2 (g) for 16 hours at room temperature. The mixture is filtered through Celite, and the Celite is washed well with ethyl acetate. The filtrate is concentrated *in vacuo* to give the title compound as a clear oil (90 mg, 99%).
12. l) (6*R*,8*R*,9*S*,13*S*,14*S*)-6-(6-methoxyhexyl)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[*a*]phenanthrene-3,17-diol (comparative compound **21**) - A solution of the compound from k) (90 mg, 0.19 mmol) in 1.5 mL each of 6 N HCl and THF is stirred for 5 hours at room temperature. The reaction mixture is diluted with water and extracted with EtOAc (2x). The combined organic extracts are dried (Na_2SO_4), filtered, and evaporated *in vacuo* to give a clear, oily residue. Purification by silica gel column chromatography (gradient from CH2Cl2 to 30% EtOAc/CH2Cl2) afforded comparative Compound **21** as a white solid foam (38 mg, 52%).

Example 6

Methods of preparing Compounds 30 and 31

[0123] Compounds 30 and 31 are prepared according to Scheme 4 (actually yields are provided).

Scheme 4



Example 7

Methods of determining estrogen receptor binding capacity using Luciferase activity.

[0124] Estrogen receptor-negative CV-1 kidney cells are maintained in Dulbecco's modified Eagle's medium with 4.5 g/L glucose supplemented with 10% fetal bovine serum and 100 units/ml penicillin-streptomycin at 37° C in a humidified 5% CO_2 atmosphere.

The cells are then plated in 6-well dishes at a density of 2 x 10^5 cells per well in phenol-red free Dulbecco's modified Eagle's medium containing 10% charcoal-dextran-stripped fetal bovine serum. CV-1 cells are transfected using LipofectAMINE reagent according to the manufacturer's protocol. Transfections containing 1.5 ug of reporter plasmid (containing ERE-tk-luciferase containing a single ERE cloned upstream of the thymidine kinase promoter and luciferase gene) and 0.5 ug of either ERα or ERβ expression vector (containing CMV-ERα or CMV-ERβ full length coding sequence respectively). The next day, cells receive no treatment (controls) or are treated with estradiol alone (1 nM) or estradiol plus a compound of the invention (at varying concentrations). After 16-24 hours, cells are harvested and assayed for luciferase activity.

[0125] At the outset, cell monolayers are washed twice with ice-cold phosphate-buffered saline and incubated for 15 minutes in 250 µl of 1X cell culture lysis reagent (Promega, Madison, WI). Cell extracts are transferred to a fresh tube and assayed using the luciferase assay system (Promega). For each assay, 10 µl of extract is diluted with 90 µl of 1X cell culture lysis reagent. Luminescence is read using an AutoLumat LB953 luminometer.

[0126] A compound or a salt thereof, which is identified by the binding assay described herein, is a compound that inhibits the binding of estrodial at the ligand binding site of the estrogen receptors. Specifically, it is a compound or a salt thereof that is envisioned to cause cell proliferation statasis and accordingly exerts its pharmacological activity.

[0127] CV-1 cells are transfected with two plasmid constructs, the reporter construct ERE-tk-luciferase and a CMV-ER-β contruct. Transfected control (Ctrl) CV-1 cells receive no treatment while estradiol treated cells receive estradiol E2) added alone at 10^{-9} M (1 nM). In the case of the compounds of the invention, each compound respectively is either added alone at 10^{-8} M (10 nM) or at 10^{-8} M plus 10^{-9} M estradiol (E2).

Example 8

Method of determining the IC_{50} values of the candidate compounds.

[0128] The cell lines listed are maintained at approximately 5% CO_2, 37°C, 95% relative humidity in the media appropriate for that cell line. The cells are subcultured every two to three days and plated in clear bottom 96-well plates at a density of 1 x 10^4 cells/well and incubated at ca. 5% CO_2, 37°C overnight prior to initiation of the assay. To begin cell viability assays, the media in the cell plate (100 µL) is replaced with fresh media (100 µL). The test articles are serially diluted 1:2 in fresh media in duplicate and added to the cells (100 µL) at final sample concentrations of 0.46, 1.37, 4.12, 12.35, 37.04, 111.1, 333.3 and 1000 µM (≤ 1% DMSO) in a total volume of 200 µL. Wells containing no cells and wells containing cells lysed with 0.1% Triton-X are used for baseline controls. Tamoxifen is used as a known control for each assay and DMSO only will be run as vehicle control. The samples are incubated at ca. 37 °C in humidified 5% CO_2 atmosphere for 72 hours. The plate is monitored once a day during the incubation period, paying special attention to the level of confluence. If the cells approach confluence prior to the end of the 72 hour incubation period, the experiment is terminated and cell viability measured as described below.

[0129] Cell viability is determined using a commercially available kit to determine ATP levels by luminescence. Briefly, the cell plate has the media removed and replaced with 100 µL of fresh media, and the buffer and lyophilized substrate are equilibrated to room temperature. The buffer is used to reconstitute the substrate just prior to addition to the wells of the cell plate (100 µL per well). The plate is placed into the Infinite M200 plate reader, allowed to shake for 10 minutes followed by a 10 minute wait period. The plate is then read using an integration time of 0.5 sec with no attenuation.

[0130] The mean baseline controls (wells with Triton X-100 or no cells) are subtracted from the total luminescence to give the net luminescence for that well. This total is compared to the control of DMSO only. An IC_{50} is calculated as the concentration that led to a response of 50% compared to the vehicle control cells. Accordingly, those of ordinary skill in the art can appreciate that the R configuration (at C-6) of the instantly claimed composition are superior to other stereoisomers.

[0131] Table IV gives the IC_{50} in various cell lines for the compounds diclosed herein.

[0132] The results for compounds 3, 4, 10, 18 and 23 are provided below for the purposes of comparison.

Table IV

Compound	IC50 lung (A549)	IC50 ovary (Ovcar-3)	IC50 Pancreas (Capan-1)
18	139 uM (91%)	207 uM (89%)	192 uM (85%)
4	70 uM (97%)	100 uM (95%)	32 uM (93%)
3	84 uM (95%)	94 uM (90%)	168 uM (80%)
19	80 uM (98%)	96 uM (98%)	32 uM (90%)
20	34 uM (99%)	60 uM (99%)	16 uM (99%)
21	17 uM (100 %)	24 uM (100 %)	23 uM (100 %)
22	13 uM (100%)	20 uM (100%)	22 uM (100%)
10	170 uM (87%)	317 uM (52%)	277 uM (66%)
23	1000 uM (7%)	1000 uM (35%)	775 uM (65%)
30	5 uM (100%)	29 uM (100%)	10 uM (100%)
31	7 uM (100%)	28 uM (100%)	15 uM (100%)

REFERENCES CITED IN THE DESCRIPTION

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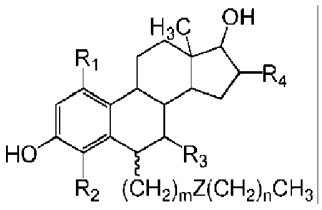
Patentkrav

1. Forbindelse med formlen:



hvori

R_1, R_2, R_3 og R_4 er brint;

m er et heltal fra 2 til 8;

n er et heltal fra 0 til 3;

Z er - O -;

hvori både C-13 metyl og C-17 hydroxyl er (S)-konfigureret;

og

symbolet ~~~ repræsenterer enhver type binding uanset stereokemi; og de respektive hydrater, solvater og farmaceutisk acceptable salte deraf.

2. Farmaceutisk sammensætning omfattende en forbindelse ifølge krav 1 og en farmaceutisk acceptabel bærer.