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(54) Title: ANTIANGIOGENIC AGENTS

(57) Abstract: Compositions and methods for treating mammalian diseases or conditions characterized by abnormal cell mitosis and/or abnormal or undesirable angiogenesis, by administering an effective amount of analogs of 2-methoxyestradiol. Compounds that inhibit cell proliferation are preferred. Preferred compositions may also exhibit a change (increase or decrease) in estrogen receptor binding, or improved absorption, transport (*e.g.*, through blood-brain barrier and cellular membranes), biological stability, or decreased toxicity.

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ANTIANGIOGENIC AGENTS

FIELD OF THE INVENTION

The present invention relates to treating disease states characterized by abnormal cell mitosis, to treating disease states characterized by abnormal angiogenesis or to treating disease states characterized by a combination of these events. More particularly, the present invention relates to analogs of 2-methoxyestradiol ($2ME_2$) and their effect on diseases characterized by abnormal cell mitosis and/or abnormal or undesirable angiogenesis.

BACKGROUND OF THE INVENTION

Angiogenesis is the generation of new blood vessels into a tissue or organ. Under normal physiological conditions, humans and animals undergo angiogenesis only in very specific, restricted situations. For example, angiogenesis is normally observed in wound healing, fetal and embryonal development, and formation of the corpus luteum, endometrium and placenta.

Angiogenesis is controlled through a highly regulated system of angiogenic stimulators and inhibitors. The control of angiogenesis has been found to be altered in certain disease states and, in many cases, pathological damage associated with the diseases is related to uncontrolled angiogenesis. Both controlled and uncontrolled angiogenesis are thought to proceed in a similar manner. Endothelial cells and pericytes, surrounded by a basement membrane, form capillary blood vessels. Angiogenesis begins with the erosion of the basement membrane by enzymes released by endothelial cells and leukocytes. Endothelial cells, lining the lumen of blood vessels, then protrude through the basement membrane. Angiogenic stimulants induce the endothelial cells to migrate through the eroded basement membrane. The migrating cells form a "sprout" off the parent blood vessel where the endothelial cells undergo mitosis and proliferate. The endothelial sprouts merge with each other to form capillary loops, creating a new blood vessel.

Persistent, unregulated angiogenesis occurs in many disease states, tumor metastases, and abnormal growth by endothelial cells. The diverse pathological disease states in which unregulated angiogenesis is present have been grouped together as angiogenic-dependent or angiogenic-associated diseases.

One example of a disease dependent on angiogenesis is ocular neovascular disease. This disease is characterized by invasion of new blood vessels into the structures of the eye, such as the retina or cornea. It is the most common cause of blindness and is involved in approximately twenty eye diseases. In age-related macular degeneration, the associated visual problems are caused by an ingrowth of choroidal capillaries through defects in Bruch's membrane with proliferation of fibrovascular tissue beneath the retinal pigment epithelium. Angiogenic damage is also associated with diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, neovascular glaucoma, and retrolental fibroplasia. Other diseases associated with corneal neovascularization include, but are not limited to, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, and pterygium keratitis sicca. Other diseases associated with undesirable angiogenesis include Sjögren's syndrome, acne rosacea, phylectenulosis, syphilis, *Mycobacteria* infections, lipid degeneration, chemical burns, bacterial ulcers, fungal ulcers, *Herpes simplex* infection, *Herpes zoster* infections, protozoan infections, Kaposi's sarcoma, Mooren's ulcer, Terrien's marginal degeneration, marginal keratolysis, rheumatoid arthritis, systemic lupus, polyarteritis, trauma, Wegener's sarcoidosis, scleritis, Stevens-Johnson's disease, pemphigoid, and radial keratotomy.

Diseases associated with neovascularization include, but are not limited to, retinal/choroidal neovascularization, diabetic retinopathy, macular degeneration, sickle cell anemia, sarcoidosis, syphilis, pseudoxanthoma elasticum, Paget's disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis/vitritis, *Mycobacteria* infections, Lyme's disease, systemic lupus erythematosis, retinopathy of prematurity, Eales' disease, Behcet's disease, infections causing retinitis or choroiditis, presumed ocular histoplasmosis, Best's disease, myopia, optic pits, Stargardt's disease, pars

planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma and post-laser complications. Other eye-related diseases include, but are not limited to, diseases associated with rubeosis (neovascularization of the iris and of the angle) and diseases caused by the abnormal proliferation of fibrovascular or fibrous tissue, including all forms of prolific vitreoretinopathy.

Another angiogenesis associated disease is rheumatoid arthritis. The blood vessels in the synovial lining of the joints undergo angiogenesis. In addition to forming new vascular networks, the endothelial cells release factors and reactive oxygen species that lead to pannus growth and cartilage destruction. Angiogenesis may also play a role in osteoarthritis. The activation of the chondrocytes by angiogenic-related factors contributes to the destruction of the joint. At a later stage, the angiogenic factors promote new bone growth. Therapeutic intervention that prevents the cartilage destruction could halt the progress of the disease and provide relief for persons suffering with arthritis.

Chronic inflammation may also involve pathological angiogenesis. Such diseases as ulcerative colitis and Crohn's disease show histological changes with the ingrowth of new blood vessels into inflamed tissues. Bartonellosis, a bacterial infection found in South America, can result in a chronic stage that is characterized by proliferation of vascular endothelial cells. Another pathological role associated with angiogenesis is found in atherosclerosis. The plaques formed within the lumen of blood vessels have been shown to have angiogenic stimulatory activity.

The hypothesis that tumor growth is angiogenesis-dependent was first proposed in 1971. (Folkman, *New Eng. J. Med.*, 285:1182-86 (1971)). In its simplest terms, this hypothesis states: "Once tumor 'take' has occurred, every increase in tumor cell population must be preceded by an increase in new capillaries converging on the tumor." Tumor 'take' is currently understood to indicate a prevascular phase of tumor growth in which a population of tumor cells occupying a few cubic millimeters volume, and not exceeding a few million cells, can survive on existing host microvessels. Expansion of tumor volume beyond this phase requires the induction of new capillary blood vessels. For example,

pulmonary micrometastases in the early prevascular phase in mice would be undetectable except by high power microscopy on histological sections.

Examples of the indirect evidence which support this concept include:

(1) The growth rate of tumors implanted in subcutaneous transparent chambers in mice is slow and linear before neovascularization, and rapid and nearly exponential after neovascularization. (Algire, *et al.*, *J. Nat. Cancer Inst.*, 6:73-85 (1945)).

(2) Tumors grown in isolated perfused organs where blood vessels do not proliferate are limited to 1-2 mm^3 but expand rapidly to >1000 times this volume when they are transplanted to mice and become neovascularized. (Folkman, *et al.*, *Annals of Surgery*, 164:491-502 (1966)).

(3) Tumor growth in the avascular cornea proceeds slowly and at a linear rate, but switches to exponential growth after neovascularization. (Gimbrone, Jr., *et al.*, *J. Nat. Cancer Inst.*, 52:421-27 (1974)).

(4) Tumors suspended in the aqueous fluid of the anterior chamber of a rabbit eye remain viable, avascular, and limited in size to < 1 mm^3. Once they are implanted on the iris vascular bed, they become neovascularized and grow rapidly, reaching 16,000 times their original volume within 2 weeks. (Gimbrone, Jr., *et al.*, *J. Exp. Med.*, 136:261-76).

(5) When tumors are implanted on a chick embryo chorioallantoic membrane, they grow slowly during an avascular phase of >72 hours, but do not exceed a mean diameter of 0.93 + 0.29 mm. Rapid tumor expansion occurs within 24 hours after the onset of neovascularization, and by day 7 these vascularized tumors reach a mean diameter of 8.0 + 2.5 mm. (Knighton, *British J. Cancer*, 35:347-56 (1977)).

(6) Vascular casts of metastases in a rabbit liver reveal heterogeneity in size of the metastases, but show a relatively uniform cut-off point for the size at which vascularization is present. Tumors are generally avascular up to 1 mm in diameter, but are neovascularized beyond that diameter. (Lien, *et al.*, *Surgery*, 68:334-40 (1970)).

(7) In transgenic mice that develop carcinomas in the beta cells of the pancreatic islets, pre-vascular hyperplastic islets are limited in size to < 1 mm.

At 6-7 weeks of age, 4-10% of the islets become neovascularized, and from these islets arise large vascularized tumors of more than 1000 times the volume of the pre-vascular islets. (Folkman, *et al., Nature,* 339:58-61 (1989)).

(8) A specific antibody against VEGF (vascular endothelial growth factor) reduces microvessel density and causes "significant or dramatic" inhibition of growth of three human tumors which rely on VEGF as their sole mediator of angiogenesis (in nude mice). The antibody does not inhibit growth of the tumor cells *in vitro.* (Kim, *et al., Nature,* 362:841-44 (1993)).

(9) Anti-bFGF monoclonal antibody causes 70% inhibition of growth of a mouse tumor which is dependent upon secretion of bFGF as its only mediator of angiogenesis. The antibody does not inhibit growth of the tumor cells *in vitro.* (Hori, *et al., Cancer Res.*, 51:6180-84 (1991)).

(10) Intraperitoneal injection of bFGF enhances growth of a primary tumor and its metastases by stimulating growth of capillary endothelial cells in the tumor. The tumor cells themselves lack receptors for bFGF, and bFGF is not a mitogen for the tumor cells *in vitro.* (Gross, *et al., Proc. Am. Assoc. Cancer Res.*, 31:79 (1990)).

(11) A specific angiogenesis inhibitor (AGM-1470) inhibits tumor growth and metastases *in vivo,* but is much less active in inhibiting tumor cell proliferation *in vitro.* It inhibits vascular endothelial cell proliferation half-maximally at 4 logs lower concentration than it inhibits tumor cell proliferation. (Ingber, *et al., Nature,* 48:555-57 (1990)). There is also indirect clinical evidence that tumor growth is angiogenesis dependent.

(12) Human retinoblastomas that are metastatic to the vitreous develop into avascular spheroids that are restricted to less than 1 mm^3 despite the fact that they are viable and incorporate ^3H-thymidine (when removed from an enucleated eye and analyzed *in vitro).*

(13) Carcinoma of the ovary metastasizes to the peritoneal membrane as tiny avascular white seeds (1-3 mm^3). These implants rarely grow larger until one or more of them become neovascularized.

(14) Intensity of neovascularization in breast cancer (Weidner, *et al., New Eng. J. Med.*, 324:1-8 (1991); Weidner, *et al., J Nat. Cancer Inst.*, 84:1875-

87 (1992)) and in prostate cancer (Weidner, *et al.*, *Am. J. Pathol.*, 143(2):401-09 (1993)) correlates highly with risk of future metastasis.

(15) Metastasis from human cutaneous melanoma is rare prior to neovascularization. The onset of neovascularization leads to increased thickness of the lesion and an increased risk of metastasis. (Srivastava, *et al.*, *Am. J. Pathol.*, 133:419-23 (1988)).

(16) In bladder cancer, the urinary level of an angiogenic protein, bFGF, is a more sensitive indicator of status and extent of disease than is cytology. (Nguyen, *et al.*, *J. Nat. Cancer Inst.*, 85:241-42 (1993)).

Thus, it is clear that angiogenesis plays a major role in the metastasis of cancer. If this angiogenic activity could be repressed or eliminated, then the tumor, although present, would not grow. In the disease state, prevention of angiogenesis could avert the damage caused by the invasion of the new microvascular system. Therapies directed at control of the angiogenic processes could lead to the abrogation or mitigation of these diseases.

Angiogenesis has been associated with a number of different types of cancer, including solid tumors and blood-borne tumors. Solid tumors with which angiogenesis has been associated include, but are not limited to, rhabdomyosarcomas, retinoblastoma, Ewing's sarcoma, neuroblastoma, and osteosarcoma. Angiogenesis is also associated with blood-borne tumors, such as leukemias, any of various acute or chronic neoplastic diseases of the bone marrow in which unrestrained proliferation of white blood cells occurs, usually accompanied by anemia, impaired blood clotting, and enlargement of the lymph nodes, liver and spleen. It is believed that angiogenesis plays a role in the abnormalities in the bone marrow that give rise to leukemia tumors and multiple myeloma diseases.

One of the most frequent angiogenic diseases of childhood is the hemangioma. A hemangioma is a tumor composed of newly formed blood vessels. In most cases the tumors are benign and regress without intervention. In more severe cases, the tumors progress to large cavernous and infiltrative forms and create clinical complications. Systemic forms of hemangiomas,

hemangiomatoses, have a high mortality rate. Therapy-resistant hemangiomas exist that cannot be treated with therapeutics currently in use.

Angiogenesis is also responsible for damage found in heredity diseases such as Osler-Weber-Rendu disease, or heredity hemorrhagic telangiectasia. This is an inherited disease characterized by multiple small angiomas, tumors of blood or lymph vessels. The angiomas are found in the skin and mucous membranes, often accompanied by epitaxis (nose bleeds) or gastrointestinal bleeding and sometimes with pulmonary or hepatitic arteriovenous fistula.

What is needed, therefore, is a composition and method that can inhibit angiogenesis. What is also needed is a composition and method that can inhibit the unwanted growth of blood vessels, especially in tumors.

Angiogenesis is also involved in normal physiological processes, such as reproduction and wound healing. Angiogenesis is an important step in ovulation and also in implantation of the blastula after fertilization. Prevention of angiogenesis could be used to induce amenorrhea, to block ovulation, or to prevent implantation by the blastula.

In wound healing, excessive repair or fibroplasia can be a detrimental side effect of surgical procedures and may be caused or exacerbated by angiogenesis. Adhesions are a frequent complication of surgery and lead to problems such as small bowel obstruction.

Several compounds have been used to inhibit angiogenesis. Taylor, *et al.* (*Nature*, 297:307 (1982)) have used protamine to inhibit angiogenesis. The toxicity of protamine limits its practical use as a therapeutic. Folkman, *et al.* (*Science*, 221:719 (1983), and U.S. Pat. Nos. 5,001,116 and 4,994,443) have disclosed the use of heparin and steroids to control angiogenesis. Steroids, such as tetrahydrocortisol, which lack glucocorticoid and mineralocorticoid activity, have been found to be angiogenic inhibitors.

Other factors found endogenously in animals, such as a 4 kDa glycoprotein from bovine vitreous humor and a cartilage derived factor, have been used to inhibit angiogenesis. Cellular factors, such as interferon, inhibit angiogenesis. For example, interferon alpha or human interferon beta have been shown to inhibit tumor-induced angiogenesis in mouse dermis stimulated by

human neoplastic cells. Interferon beta is also a potent inhibitor of angiogenesis induced by allogeneic spleen cells. (Sidky, *et al., Cancer Res.*, 47:5155-61(1987)). Human recombinant interferon (alpha/A) was reported to be successfully used in the treatment of pulmonary hemangiomatosis, an angiogenesis-induced disease. (White, *et al., New Eng. J. Med.*, 320:1197-1200 (1989)).

Other agents that have been used to inhibit angiogenesis include ascorbic acid ethers and related compounds. (Japanese Kokai Tokkyo Koho No.58-13 (1978)). Sulfated polysaccharide DS 4152 also inhibits angiogenesis. (Japanese Kokai Tokkyo Koho No. 63-119500). Additional anti-angiogenic compounds include Angiostatin® (U.S. Patent Nos. 5,639,725; 5,792,845; 5,885,795; 5,733,876; 5,776,704; 5,837,682; 5,861,372, and 5,854,221) and Endostatin (U.S. Patent No. 5,854,205).

Another compound which has been shown to inhibit angiogenesis is thalidomide. (D'Amato, *et al., Proc. Natl. Acad. Sci.*, 90:4082-85 (1994)). Thalidomide is a hypnosedative that has been successfully used to treat a number of diseases, such as rheumatoid arthritis (Gutierrez-Rodriguez, *Arthritis Rheum.*, 27 (10):1118-21 (1984); Gutierrez-Rodriguez, *et al., J. Rheumatol.*, 16(2):158-63 (1989)), and Behcet's disease (Handley, *et al., Br. J. Dermatol.*, 127 Suppl, 40:67-8 (1992); Gunzler, *Med. Hypotheses*, 30(2):105-9 (1989)).

Although thalidomide has minimal side effects in adults, it is a potent teratogen. Thus, there are concerns regarding its use in women of child-bearing age. Although minimal, there are a number of side effects that limit the desirability of thalidomide as a treatment. One such side effect is drowsiness. In a number of therapeutic studies, the initial dosage of thalidomide had to be reduced because patients became lethargic and had difficulty functioning normally. Another side effect limiting the use of thalidomide is peripheral neuropathy, in which individuals suffer from numbness and dysfunction in their extremities.

Thus, improved methods and compositions are needed that are easily administered and capable of inhibiting angiogenesis. Additionally, what is

needed are safe and effective treatments that cause minimal unwanted side effects.

2-Methoxyestradiol is an endogenous metabolite of estradiol (E2). When administered orally, it exhibits anti-tumor and anti-proliferative activity with little toxicity. *In vitro* data suggests that 2-methoxyestradiol does not engage the estrogen receptor for its anti-proliferative activity and is not estrogenic over a wide range of concentrations, as assayed by estrogen dependent MCF-7 cell proliferation. However, the presence of metabolizing enzymes, such as demethylases, *in vivo* and *in vitro*, may metabolize this compound to products, such as 2-hydroxyestradiol, which has been shown to be estrogenic by several approaches. What is needed is a means to improve the bioavailability of estradiol derivatives or 2-methoxyestradiol and to reduce the formation of estrogenic 2-methoxyestradiol metabolites. Other forms of metabolism include conversion of the 17-hydroxy function to the corresponding ketone. Conjugation (either glucuronidation or sulfation) is another major form of metabolism of steroids. What is also needed is a means to modify estradiol derivatives or 2-methoxyestradiol in such a way as to prevent conversion into an estrogenic derivative, metabolic conjugation and/or conversion to estrones.

SUMMARY OF THE INVENTION

The present invention provides certain analogs of 2-methoxyestradiol that are effective in treating diseases characterized by abnormal mitosis and/or abnormal angiogenesis. Specifically the present invention relates to analogs of 2-methoxyestradiol that have been modified at the 2, 3 and 17 positions thereof. Compounds within the general Formulae I and II (shown below) that inhibit cell proliferation are preferred. Compounds within Formulae I and II that inhibit angiogenesis are also preferred. Preferred compositions may also exhibit a change (increase or decrease) in estrogen receptor binding, or improved absorption, transport (*e.g.*, through blood-brain barrier and cellular membranes), biological stability, or decreased toxicity. The invention also provides compounds useful in the method, as described by the general formulae of the claims.

A mammalian disease characterized by undesirable cell mitosis, as defined herein, includes but is not limited to excessive or abnormal stimulation of endothelial cells (*e.g.*, atherosclerosis), solid tumors and tumor metastasis, benign tumors, for example, hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, vascular malfunctions, abnormal wound healing, inflammatory and immune disorders, Bechet's disease, gout or gouty arthritis, abnormal angiogenesis accompanying rheumatoid arthritis, skin diseases, such as psoriasis, diabetic retinopathy and other ocular angiogenic diseases such as retinopathy of prematurity (retrolental fibroplasic), macular degeneration, corneal graft rejection, neovascular glaucoma and Osler Weber syndrome (Osler-Weber-Rendu disease). Other undesired angiogenesis involves normal processes including ovulation and implantation of a blastula. Accordingly, the compositions described above can be used to block ovulation and implantation of a blastula or to block menstruation (induce amenorrhea).

It is known that 2-methoxyestradiol ($2ME_2$), an endogenous metabolite of estradiol with no intrinsic estrogenic activity, is a potent antiproliferative agent that induces apoptosis in a wide variety of tumor and non-tumor cell lines. When administered orally, it exhibits antitumor and antiangiogenic activity with little or no toxicity. Currently, $2ME_2$ is in several clinical trials under the name PANZEM ®.

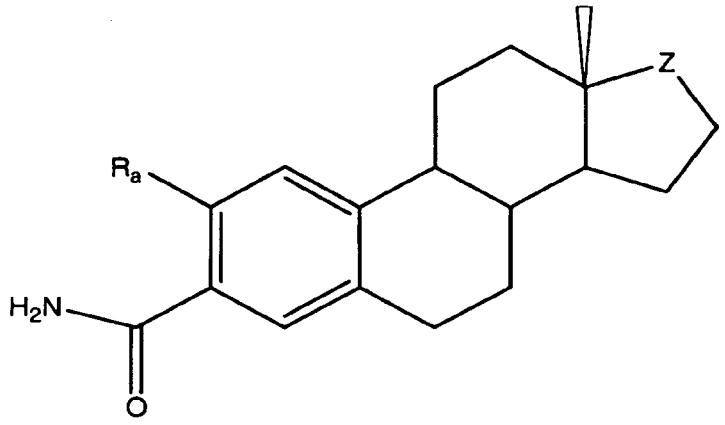
A novel series of compounds has been prepared that retains the biological activities of $2ME_2$ but is believed to have reduced metabolism. Most of these analogs lack the hydroxyl moiety at position 17 and cannot be metabolized to 2-methoxyestrone or conjugated at that position. These 17-position analogs retain antiproliferative activity in HUVEC and tumor cells. Replacement of the 2-methoxy group by other moieties, such as an ethoxy or a propynyl group, retain antiproliferative activity, but these functionalities cannot be de-methylated to yield the estrogenic 2-hydroxyl derivatives. Also disclosed are compounds and methods for altering the chemical nature of positions 3 and 17 of 2-methoxyestradiol for preventing conversion to 2-methoxyestrone and/or the conjugation of 2-methoxyestradiol (or metabolites) with other molecules and subsequent loss during excretion of the resulting compounds.

Other features and advantages of the invention will be apparent from the following detailed description of preferred embodiments thereof.

DETAILED DESCRIPTION OF THE INVENTION

As described below, compounds that are useful in accordance with the invention include novel 2-methoxyestradiol derivatives that exhibit anti-mitotic, anti-angiogenic and/or anti-tumor properties. Preferred compounds of the invention are 2-methoxyestradiol derivatives modified at the 2-, 3- or 17-positions or at combinations of the 2-, 3-, and 17-positions. Preferred compounds are those of the general Formulae I or II:

(I)



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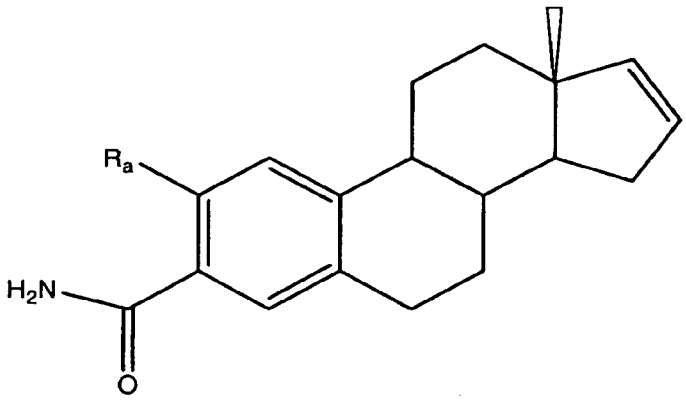
(II)



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wherein R_a is selected from $-OCH_3$, $-OCH_2CH_3$ or $-CCCH_3$; and Z is selected from $>C(H_2)$, $>C(H)-CH_3$, $>C=CH_2$, $>C=CHCH_3$ (cis or trans), $>C=O$, $>C(H)-OH$, >C(H)-O-alkyl, >C(H)-O-sulfamate or >C(H)-dioxolane. Alkyl is defined herein

as a linear, branched and/or cyclic hydrocarbon chain containing 1-10 carbons. Preferred species according to the present invention are described below.

In an alternate disclosed embodiment of the present invention, compounds according to the present invention are those of Formula I, wherein R_a is $-OCH_3$; and Z is selected from $>C(H_2)$, $>C(H)-CH_3$, $>C=CH_2$, $>C=CHCH_3$ (cis or trans), >C=O, >C(H)-OH, >C(H)-O-alkyl, >C(H)-O-sulfamate, or >C(H)-dioxolane.

In another alternate disclosed embodiment of the present invention, compounds according to the present invention are those of Formula I, wherein R_a is $-OCH_2CH_3$; and Z is selected from $>C(H_2)$, $>C(H)-CH_3$, $>C=CH_2$, $>C=CHCH_3$ (cis or trans), >C=O, >C(H)-OH, >C(H)-O-alkyl, >C(H)-O-sulfamate, or >C(H)-dioxolane.

In a further alternate disclosed embodiment of the present invention, compounds according to the present invention are those of Formula I, wherein R_a is $-CCCH_3$; and Z is selected from $>C(H_2)$, $>C(H)-CH_3$, $>C=CH_2$, $>C=CHCH_3$ (cis or trans), >C=O, >C(H)-OH, >C(H)-O-alkyl, >C(H)-O-sulfamate or >C(H)-dioxolane.

In each of the cases where stereoisomers are possible, both R and S stereoisomers are envisioned as well as any mixture of stereoisomers.

In yet another alternate disclosed embodiment of the present invention, compounds according to the present invention are those of Formula II, wherein R_a is $-OCH_3$.

In another alternate disclosed embodiment of the present invention, compounds according to the present invention are those of Formula II, wherein R_a is $-OCH_2CH_3$.

In a further alternate disclosed embodiment of the present invention, compounds according to the present invention are those of Formula II, wherein R_a is $-CCCH_3$.

Those skilled in the art will appreciate that the invention extends to other compounds within the formulae given in the claims below, having the described characteristics. These characteristics can be determined for each test compound using the assays detailed below and elsewhere in the literature.

Although not wishing to be bound by theory, it is believed that 2-methoxyestrone ($2ME_1$) is formed through the same enzymatic pathway as estrone is formed from estradiol. Although not wishing to be bound by theory, it is further believed that the enzymes responsible for this reaction on estradiol are the 17β-hydroxysteroid dehydrogenases (17β-HSD), which utilize NADP+ as a co-factor (Han *et al.*, *J. Biol. Chem.* 275:2, 1105-1111 (2000)). Each of the four members of this enzyme family, types 1, 2, 3, and 4 has distinct activities. It appears that 17β-HSD type 1 catalyzes the reductive reaction (estrone to estradiol), while 17β-HSD type 2 catalyzes the oxidation reaction (estradiol to estrone), and type 3 catalyzes 4-androstenedione to testosterone. It is also believed that an additional metabolic deactivation pathway results in conjugation of 2-methoxyestradiol or 2-methoxyestrone with molecules, such as sulfate or glucuronic acid, and subsequent loss via excretion. In this invention, positions 3 and 17 of 2-methoxyestradiol, and derivatives thereof, may be modified to prevent these metabolic pathways from occurring.

It is well known that orally-delivered steroids, such as estradiol (E_2) and ethynyl-E_2, are extensively metabolized during passage through the gastrointestinal tract and by first-pass metabolism in the liver. Two major metabolic pathways that lead to rapid deactivation and excretion are well studied *viz.*, oxidation at the D-ring 17-hydroxy group of E_2 to form estrone and/or conjugation with sulfate and/or glucuronate at the hydroxyls of position 3 on the A-ring and position 17 on the D-ring.

Several studies have been conducted to determine structure activity relationship ("SAR") of $2ME_2$ analogs (D'Amato, R. J.; Lin, C. M.; Flynn, E.; Folkman, J.; Hamel, E. "Inhibition of Angiogenesis and Breast Cancer in Mice by the Microtubule Inhibitors 2-Methoxyestradiol and Taxol," *Cancer Res.* 1997, 57, 81-86; Cushman, M.; He, M.-H.; Katzenellenbogen, J. A.; Lin, C. M.; Hamel, E. "Synthesis, Antitubulin and Antimitotic Activity, and Cytotoxicity of Analogs of 2-Methoxyestradiol, an Endogenous Mammalian Metabolite of Estradiol that inhibits Tubulin Polymerization by Binding to the Colchicine Binding Site," *J. Med. Chem.* 1995, 38, 2041-2049; Wang, Z.; Yang, D.; Mohanakrishnan, A. K.; Fanwick, P. E.; Nampoothiri, P.; Hamel, E.; Cushman, M. "Synthesis of B-Ring

Homologated Estradiol Analogs that Modulate Tubulin Polymerization and Microtubule Stability," *J. Med. Chem.*, 2000, 43, 2419-2429; Cushman, et. al., *J. Med. Chem.* (1997) 40, 2323-2334.; Wang et al., *Synthetic Comm* 1998, 28, 4431; Cushman et al., *J. Med. Chem.* 2002, 45, 4748; Miller, T. A.; Bulman, A. L.; Thompson, C. D.; Garst, M. E.; Macdonald, T. L., "The Synthesis and Evaluation of Functionalized Estratropinones: Potent Inhibitors of Tubulin Polymerization," *Biooorg & Med. Chem. Letters* 1997, 7, 1851-1856.; Miller et al., *J. Med. Chem.* 1997, 7, 1851.), but none to reduce or stop its metabolic pathway.

In the preferred embodiment of the invention, 2-methoxyestradiol, and derivatives thereof, are modified at the 2-, 3- and 17-positions or combinations thereof.

Anti-Proliferative Activity *In Vitro*

The process or processes by which $2ME_2$ affects cell growth remains unclear, however, a number of studies have implicated various mechanisms of action and cellular targets. $2ME_2$ induced changes in the levels and activities of various proteins involved in the progression of the cell cycle. These include cofactors of DNA replication and repair, e.g., proliferating cell nuclear antigen (PCNA) (Klauber, N., Parangi, S., Flynn, E., Hamel, E. and D'Amato, R.J. (1997), Inhibition of angiogenesis and breast cancer in mice by the microtubule inhibitors 2-methoxyestradiol and Taxol., Cancer Research 57, 81-86; Lottering, M-L., de Kock, M., Viljoen, T.C., Grobler, C.J.S. and Seegers, J.C. (1996) 17β-Estradiol metabolites affect some regulators of the MCF-7 cell cycle. Cancer Letters 110, 181-186); cell division cycle kinases and regulators, e.g., $p34^{cdc2}$ and cyclin B (Lottering *et al.* (1996); Attalla, H., Mäkelä, T.P., Adlercreutz, H. and Andersson, L.C. (1996) 2-Methoxyestradiol arrests cells in mitosis without depolymerizing tubulin. Biochemical and Biophysical Research Communications 228, 467-473; Zoubine, M.N., Weston, A.P., Johnson, D.C., Campbell, D.R. and Banerjee, S.K. (1999) 2-Methoxyestradiol-induced growth suppression and lethality in estrogen- responsive MCF-7 cells may be mediated by down regulation of p34cdc2 and cyclin B1 expression. Int J Oncol 15, 639-646);

transcription factor modulators, e.g., SAPK/JNK (Yue, T-L., Wang, X., Louden, C.S., Gupta, L.S., Pillarisetti, K., Gu, J-L., Hart, T.K., Lysko, P.G. and Feuerstein, G.Z. (1997) 2-Methoxyestradiol, an endogenous estrogen metabolite induces apoptosis in endothelial cells and inhibits angiogenesis: Possible role for stress-activated protein kinase signaling pathway and fas expression. Molecular Pharmacology 51, 951-962; Attalla, H., Westberg, J.A., Andersson, L.C., Aldercreutz, H. and Makela, T.P. (1998) 2-Methoxyestradiol-induced phosphorylation of bcl-2: uncoupling from JNK/SAPK activation. Biochem and Biophys Res Commun 247, 616-619); and regulators of cell arrest and apoptosis, e.g., tubulin (D'Amato, R.J., Lin, C.M., Flynn, E., Folkman, J. and Hamel, E. (1994) 2-Methoxyestradiol, and endogenous mammalian metabolite, inhibits tubulin polymerization by interacting at the colchicine site. Proc. Natl. Acad. Sci. USA 91, 3964-3968; Hamel, E., Lin, C.M., Flynn, E. and D'Amato, R.J. (1996) Interactions of 2-methoxyestradiol, and endogenous mammalian metabolite, with unpolymerized tubulin and with tubulin polymers. Biochemistry 35, 1304-1310), $p21^{WAF1/CIP1}$ (Mukhopadhyay, T. and Roth, J.A. (1997) Induction of apoptosis in human lung cancer cells after wild-type p53 activation by methoxyestradiol. Oncogene 14, 379-384), bcl-2 and FAS (Yue *et al.* (1997); Attalla *et al.* (1998)), and p53 (Kataoka, M., Schumacher, G., Cristiano, R.J., Atkinson, E.N., Roth, J.A. and Mukhopadhyay, T. (1998) An agent that increases tumor suppressor transgene product coupled with systemic transgene delivery inhibits growth of metastatic lung cancer *in vivo*. Cancer Res 58, 4761-4765; Mukhopadhyay *et al.* (1997); Seegers, J.C., Lottering, M-L., Grobler C.J.S., van Papendorp, D.H., Habbersett, R.C., Shou, Y. and Lehnert B.E. (1997) The mammalian metabolite, 2-methoxyestradiol, affects p53 levels and apoptosis induction in transformed cells but not in normal cells. J. Steroid Biochem. Molec.Biol. 62, 253-267). The effects on the level of cAMP, calmodulin activity and protein phosphorylation may also be related to each other. More recently, $2ME_2$ was shown to upregulate Death Receptor 5 and caspase 8 in human endothelial and tumor cell lines (LaVallee TM, Zhan XH, Johnson MS, Herbstritt CJ, Swartz G, Williams MS, Hembrough WA, Green SJ, Pribluda VS. 2-Methoxyestradiol up-regulates death receptor 5 and induces apoptosis through activation of the extrinsic pathway.

Cancer Res. (2003) 63#2:468-75). Additionally, 2ME2 has been shown to interact with superoxide dismutase (SOD) 1 and SOD 2 and to inhibit their enzymatic activities (Huang, P., Feng, L., Oldham, E. A., Keating, M. J., and Plunkett, W. 2000. Superoxide dismutase as a target for the selective killing of cancer cells, Nature. 407:390-5.). All cellular targets described above are not necessarily mutually exclusive to the inhibitory effects of $2ME_2$ in actively dividing cells.

The high affinity binding of $2ME_2$ to sex hormone-binding globulin ("SHBG") has been mechanistically associated to its efficacy in a canine model of prostate cancer, in which signaling by estradiol and 5□-androstan-3□,17□-diol were inhibited by $2ME_2$ (Ding, V.D., Moller, D.E., Feeney, W.P., Didolkar, V., Nakhla, A.M., Rhodes, L., Rosner, W. and Smith, R.G., Sex hormone-binding globulin mediates prostate androgen receptor action via a novel signaling pathway, Endocrinology 139, 213-218 (1998)).

The more relevant mechanisms described above have been extensively discussed in Victor S. Pribluda, Theresa M. LaVallee and Shawn J. Green, *2-Methoxyestradiol:* A *novel endogenous chemotherapeutic and antiangiogenic* in The New Angiotherapy, Tai-Ping Fan and Robert Auerbach eds., Human Press Publisher.

Assays relevant to these mechanisms of action and inhibition of cell proliferation are well-known in the art. For example, anti-mitotic activity mediated by effects on tubulin polymerization activity can be evaluated by testing the ability of an estradiol derivative to inhibit tubulin polymerization and microtubule assembly *in vitro.* Microtubule assembly can be followed in a Gilford recording spectrophotometer (model 250 or 2400S) equipped with electronic temperature controllers. A reaction mixture typically contains 1.0 M monosodium glutamate (pH 6.6), 1.0 mg/ml (l0μM) tubulin, 1.0 mM $MgCl_2$, 4% (v/v) dimethylsulfoxide and 20-75μM of a composition to be tested. The reaction mixtures are incubated for 15 min. at 37°C and then chilled on ice. After addition of l0 μl 2.5 mM GTP, the reaction mixture is transferred to a cuvette at 0°C, and a baseline established. At time zero, the temperature controller of the

spectrophotometer is set at 37°C. Microtubule assembly is evaluated by increased turbity at 350 nm. Alternatively, inhibition of microtubule assembly can be followed by transmission electron microscopy as described in Example 2 of U.S. Patent Nos. 5,504,074, 5,661,143, and 5,892,069, the disclosures of which are incorporated herein by reference.

Other such assays include counting of cells in tissue culture plates or assessment of cell number through metabolic assays or incorporation into DNA of labeled (radiochemically, for example ^{3}H-thymidine, or fluorescently labeled) or immuno-reactive (BrdU) nucleotides. In addition, antiangiogenic activity may be evaluated through endothelial cell migration, endothelial cell tubule formation, or vessel outgrowth in *ex-vivo* models such as rat aortic rings.

Indications

The invention can be used to treat any disease characterized by abnormal cell mitosis and/or abnormal angiogenesis. Such diseases include, but are not limited to, abnormal stimulation of endothelial cells (*e.g.*, atherosclerosis); solid tumors; blood-borne tumors, such as leukemias; tumor metastasis; benign tumors, for example, hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; vascular malfunctions; abnormal wound healing; inflammatory and immune disorders; Bechet's disease; gout or gouty arthritis; abnormal angiogenesis accompanying: rheumatoid arthritis; skin diseases, such as psoriasis; diabetic retinopathy, and other ocular angiogenic diseases, such as retinopathy of prematurity (retrolental fibroplasia), macular degeneration, corneal graft rejection, neovascular glaucoma; liver diseases and Oster Webber Syndrome (Osler-Weber Rendu disease).

Other undesired angiogenesis involves normal processes including ovulation and implantation of a blastula. The compositions described above can be used as a birth control agent by reducing or preventing uterine vascularization required for embryo implantation. Thus, the present invention provides an effective birth control method when an amount of Formulae I or II sufficient to prevent embryo implantation is administered to a female. In one aspect of the birth control method, an amount of Formulae I or II sufficient to block embryo

implantation is administered before or after intercourse and fertilization have occurred, thus providing an effective method of birth control, possibly a "morning after" method. While not wanting to be bound by this theory, it is believed that inhibition of vascularization of the uterine endometrium interferes with implantation of the blastocyte. Similar inhibition of vascularization of the mucosa of the uterine tube interferes with implantation of the blastocyte, preventing the occurrence of a tubal pregnancy. The compositions described above can also be used to block ovulation or to block menstruation (induce amenorrhea).

Diseases associated with neovascularization can be treated according to the present invention. Such diseases include, but are not limited to, ocular neovascular disease, diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, neovascular glaucoma and retrolental fibroplasias, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, Sjögren's syndrome, acne rosacea, phylectenulosis, syphilis, *Mycobacteria* infections, lipid degeneration, chemical burns, bacterial ulcers, fungal ulcers, *Herpes simplex* infections, *Herpes zoster* infections, protozoan infections, Kaposi's sarcoma, Mooren's ulcer, Terrien's marginal degeneration, marginal keratolysis, trauma, rheumatoid arthritis, systemic lupus, polyarteritis, Wegener's sarcoidosis, Scleritis, Steven-Johnson disease, pemphigoid, radial keratotomy, and corneal graph rejection.

Other diseases associated with neovascularization can be treated according to the present invention. Such diseases include, but are not limited to, sickle cell anemia, sarcoid, pseudoxanthoma elasticum, Paget's disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis/vitritis, Lyme's disease, systemic lupus erythematosis, Eales' disease, Bechet's disease, infections causing a retinitis or choroiditis, presumed ocular histoplasmosis, Best's disease, myopia, optic pits, Stargart's disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, and post-laser complications. Other diseases include, but are not limited to, diseases associated with rubeosis (neovascularization of the iris and the angle) and diseases caused

by the abnormal proliferation of fibrovascular or fibrous tissue including all forms of proliferative vitreoretinopathy, whether or not associated with diabetes.

The present invention may also be used to treat angiogenesis-dependent cancers including, but not limited to, any one or combination of rhabdomyosarcoma, retinoblastoma, Ewing sarcoma, neuroblastoma, and osteosarcoma. Other angiogenesis-dependent cancers treatable with the present invention include, but are not limited to, breast cancer, prostrate cancer, renal cell cancer, brain cancer, ovarian cancer, colon cancer, bladder cancer, pancreatic cancer, stomach cancer, esophageal cancer, cutaneous melanoma, liver cancer, small cell and non-small cell lung cancer, testicular cancer, kidney cancer, bladder cancer, cervical cancer, lymphoma, parathyroid cancer, penile cancer, rectal cancer, small intestine cancer, thyroid cancer, uterine cancer, Hodgkin's lymphoma, non-Hodgkin's lymphoma, lip cancer, oral cancer, skin cancer, leukemia or multiple myeloma.

As mentioned above, another disease that can be treated according to the present invention is rheumatoid arthritis. It is believed that the blood vessels in the synovial lining of the joints undergo angiogenesis. In addition to forming new vascular networks, the endothelial cells release factors and reactive oxygen species that lead to pannus growth and cartilage destruction. The factors involved in angiogenesis may actively contribute to, and help maintain, the chronically inflamed state of rheumatoid arthritis.

Other diseases that can be treated according to the present invention are hereditary hemorrhagic telangiectasia, osteoarthritis, chronic inflammation, Crohn's disease, ulcerative colitis, Bartonellosis, inflammatory or immune mediated bowel disease and acquired immune deficiency syndrome.

The present invention can be used to treat eye conditions in humans or animals, wherein the eye conditions include, but are not limited to, ocular neovascular disease, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasias, epidemic keratoconjunctivitis, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, myopia, chronic retinal detachment, optic pits, Terrien's marginal degeneration, hyperviscosity

syndromes, chronic uveitis, chronic vitritis, presumed ocular histoplasmosis, retinitis, choroiditis, proliferative vitreoretinopathy, scleritis, Eales' disease, Best's disease, trachoma, or post-laser complications.

The present invention can be used to treat inflammatory or immune mediated diseases in humans or animals, wherein the inflammatory or immune mediated diseases include, but are not limited to, rheumatoid arthritis, osteoarthritis, ulcerative colitis, Crohn's disease, Mooren's ulcer, arthritis, sarcoidosis, inflammatory or immune mediated bowel disease, systemic lupus, Wegener's syndrome, Stevens-Johnson disease, Behcet's disease, pemphigoid, Lyme's disease, asthma or acquired immune deficiency syndrome.

The present invention can be used to treat infectious diseases in humans or animals, wherein the infectious diseases include, but are not limited to syphilis, a bacterial infection, a *Mycobacterial* infection, a bacterial ulcer, a fungal ulcer, a *Herpes simplex* infection, a *Herpes zoster* infection, a protozoan infection, a Bartonellosis infection, or toxoplasmosis.

The present invention can be used to treat blood or blood vessel diseases in humans or animals, wherein the blood or blood vessel diseases include, but are not limited to, vein occlusion, artery occlusion, carotid obstructive disease, polyarteritis, atherosclerosis, Osler-Weber-Rendu disease, sickle cell anemia, leukemia, acute or chronic neoplastic disease of the bone marrow, hemangiomas, hereditary hemorrhagic telangiectasia, disease of the bone marrow, anemia, impaired blood clotting or enlargement of the lymph nodes, liver, or spleen. The present invention can also be used to treat chronic neoplastic disease of the bone marrow, wherein those diseases include, but are not limited to, multiple myeloma and myelo dysplastic syndrome.

The present invention can be used to treat skin conditions in a humans or an animals, wherein the skin conditions include, but are not limited to, abnormal wound healing, acne rosacea, chemical burns of the skin, dermatitis or psoriasis.

In addition, the invention can be used to treat a variety of post-menopausal symptoms, osteoporosis, cardiovascular disease, myocardial angiogenesis, plaque neovascularization, hemophiliac joints, angiofibroma, wound granulation, intestinal adhesions, scleroderma, hypertrophic scars; *i.e.*,

keloids. They are also useful in the treatment of diseases that have angiogenesis as a pathologic consequence, such as cat scratch disease, and *Helicobacter pylori* ulcers. The invention can also be used to treat Alzheimer's disease, to reduce the incidence of stroke, and as an alternative to prior estrogen replacement therapies. The compounds of the present invention can work by estrogenic and non-estrogenic biochemical pathways.

Additionally, the compounds of the present invention can be used to treat endometriosis. Endometriosis is the abnormal growth of endometrial cells; the same cells that line the uterus that are shed monthly in the menstrual process. Wayward endometrial cells can position themselves in the lower abdomen on areas such as the cul-de-sac, the recto-vaginal septum, the stomach, the fallopian tubes, the ovaries, and the bladder. During menstruation, the normal uterine lining is sloughed off and expelled through the vagina, but transplanted endometrial tissue has no means of exiting the body; instead the endometrial tissue and cells adhere and grow where positioned. The results are internal bleeding, inflammation, and scarring. One of the serious consequences of endometrial scarring is infertility. The endometrial growths are generally not malignant or cancerous. Among other complications, the growths can rupture and can spread the endometriosis to new areas of the lower abdomen. Endometriosis is a progressive disease. The growths or lesions are first seen as clear vesicles, then become red, and finally progress to black lesions over a period of seven to ten years.

Pharmaceutical Preparations

Also contemplated by the present invention are implants or other devices comprised of the compounds or drugs of Formulae I or II or prodrugs thereof where the drug or prodrug is formulated in a biodegradable or non-biodegradable polymer for sustained release. Non-biodegradable polymers release the drug in a controlled fashion through physical or mechanical processes without the polymer itself being degraded. Biodegradable polymers are designed to gradually be hydrolyzed or solubilized by natural processes in the body, allowing gradual release of the admixed drug or prodrug. The drug or prodrug can be chemically

linked to the polymer or can be incorporated into the polymer by admixture. Both biodegradable and non-biodegradable polymers and the process by which drugs are incorporated into the polymers for controlled release are well known to those skilled in the art. Examples of such polymers can be found in many references, such as Brem et al., *J. Neurosurg* 74: pp. 441-446 (1991). These implants or devices can be implanted in the vicinity where delivery is desired, for example, at the site of a tumor or a stenosis.

Because anything not formed in the body as a natural component may elicit extreme and unexpected responses, such as blood vessel closure due to thrombus formation or spasm, and because damage to blood vessels by the act of insertion of a vascular stent may be extreme and unduly injurious to the blood vessel surface, it is prudent to protect against such events. Restenosis is a re-narrowing or blockage of an artery at the same site where treatment, such as an angioplasty or stent procedure, has already taken place. If restenosis occurs within a stent that has been placed in an artery, it is technically called "in-stent restenosis," the end result being a narrowing in the artery caused by a build-up of substances that may eventually block the flow of blood. The compounds that are part of the present invention are especially useful to coat vascular stents to prevent restenosis. The coating should preferably be a biodegradable or non-biodegradable polymer that allows for a slow release of a compound of the present invention thereby preventing the restenosis event.

The present invention also relates to conjugated prodrugs and uses thereof. More particularly, the invention relates to conjugates of steroid compounds, such as compounds of Formulae I or II, and the use of such conjugates in the prophylaxis or treatment of conditions associated with enhanced angiogenesis or accelerated cell division, such as cancer, and inflammatory conditions, such as asthma and rheumatoid arthritis and hyperproliferative skin disorders including psoriasis. The invention also relates to compositions including the prodrugs of the present invention and methods of synthesizing the prodrugs.

In one aspect, the present invention provides a conjugated prodrug of an estradiol compound, preferably compounds of Formulae I or II, conjugated to a biological activity modifying agent.

Alternatively, the conjugated prodrug according to the present invention includes the compounds of Formulae I or II conjugated to a peptide moiety.

The incorporation of an estradiol compound, such as the compounds of Formulae I or II, into a disease-dependently activated pro-drug enables significant improvement of potency and selectivity of this anti-cancer and anti-inflammatory agent.

In addition to the compounds of the present invention, the pharmaceutical composition of this invention may also contain, or be co-administered (simultaneously or sequentially) with, one or more pharmacological agents of value in treating one or more disease conditions referred to hereinabove.

A person skilled in the art will be able by reference to standard texts, such as Remington's Pharmaceutical Sciences 17th edition, to determine how the formulations are to be made and how these may be administered.

In a further aspect of the present invention there is provided use of compounds of Formulae I or II or prodrugs thereof according to the present invention for the preparation of a medicament for the prophylaxis or treatment of conditions associated with angiogenesis or accelerated cell division or inflammation.

In a further aspect of the present invention there is provided a pharmaceutical composition comprising compounds of Formulae I or II or prodrugs thereof according to the present invention, together with a pharmaceutically acceptable carrier, diluent or excipient.

The pharmaceutical composition may be used for the prophylaxis or treatment of conditions associated with angiogenesis or accelerated cell division or inflammation.

In a still further aspect of the present invention there is provided a method of prophylaxis or treatment of a condition associated with angiogenesis or accelerated or increased amounts of cell division hypertrophic growth or inflammation, said method including administering to a patient in need of such

prophylaxis or treatment an effective amount of compounds of Formulae I or II or prodrugs thereof according to the present invention, as described herein. It should be understood that prophylaxis or treatment of said condition includes amelioration of said condition.

By "an effective amount" is meant a therapeutically or prophylactically effective amount. Such amounts can be readily determined by an appropriately skilled person, taking into account the condition to be treated, the route of administration and other relevant factors. Such a person will readily be able to determine a suitable dose, mode and frequency of administration.

Pharmaceutically acceptable salts of the compounds of the Formulae I or II may be prepared in any conventional manner for example from the free base and acid. *In vivo* hydrolysable esters, amides and carbamates of Formulae I or II may be prepared in any conventional manner.

Administration

The compositions described above can be provided as physiologically acceptable formulations using known techniques, and these formulations can be administered by standard routes. In general, the combinations may be administered by the topical, oral, rectal or parenteral (*e.g.*, intravenous, subcutaneous or intramuscular) route. In addition, the combinations may be incorporated into polymers allowing for sustained release, the polymers being implanted in the vicinity of where delivery is desired, for example, at the site of a tumor or within or near the eye. The dosage of the composition will depend on the condition being treated, the particular derivative used, and other clinical factors such as weight and condition of the patient and the route of administration of the compound. However, for oral administration to humans, a dosage of 0.01 to 100 mg/kg/day, especially 0.01-20 mg/kg/day, is generally preferred.

Formulations contemplated as part of the present invention include nanoparticles formulations made by methods disclosed in U.S. Patent Application No. 10/392,403 (Publication No. 2004/0033267) which is hereby incorporated by reference in its entirety. By forming nanoparticles, the compositions disclosed herein are shown to have increased bioavailability. Preferably, the particles of the

compounds of the present invention have an effective average particle size of less than about 2 microns, less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 run, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 100 nm, less than about 75 nm, or less than about 50 nm, as measured by light-scattering methods, microscopy, or other appropriate methods well known to those of ordinary skill in the art.

The formulations in accordance with the present invention can be administered in the form of tablet, a capsule, a lozenge, a cachet, a solution, a suspension, an emulsion, a powder, an aerosol, a suppository, a spray, a pastille, an ointment, a cream, a paste, a foam, a gel, a tampon, a pessary, a granule, a bolus, a mouthwash, or a transdermal patch.

The formulations include those suitable for oral, rectal, nasal, inhalation, topical (including dermal, transdermal, buccal and sublingual), vaginal, parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intraocular, intratracheal, and epidural) or inhalation administration. The formulations may conveniently be presented in unit dosage form and may be prepared by conventional pharmaceutical techniques. Such techniques include the step of bringing into association the active ingredient and a pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil emulsion, etc.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface-active or dispersing agent. Molded tablets may be made by molding, in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide a slow or controlled release of the active ingredient therein.

Formulations suitable for topical administration in the mouth include lozenges comprising the ingredients in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the ingredient to be administered in a suitable liquid carrier.

Formulations suitable for topical administration to the skin may be presented as ointments, creams, gels and pastes comprising the ingredient to be administered in a pharmaceutical acceptable carrier. A preferred topical delivery system is a transdermal patch containing the ingredient to be administered.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

Formulations suitable for nasal administration, wherein the carrier is a solid, include a coarse powder having a particle size, for example, in the range of 20 to 500 microns which is administered in the manner in which snuff is taken; *i.e.*, by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations, wherein the carrier is a liquid, for administration, as for example, a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing, in addition to the active ingredient, ingredients such as carriers as are known in the art to be appropriate.

Formulation suitable for inhalation may be presented as mists, dusts, powders or spray formulations containing, in addition to the active ingredient, ingredients such as carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in freeze-dried (lyophilized) conditions requiring only the addition of a sterile liquid carrier, for example, water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kinds previously described.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose, as herein above recited, or an appropriate fraction thereof, of the administered ingredient.

It should be understood that in addition to the ingredients, particularly mentioned above, the formulations of the present invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include flavoring agents.

The present invention includes compositions and methods for treating mammalian disease characterized by pathogenic angiogenesis by administering compounds of Formulae I or II. The 2-methoxyestradiol, and derivatives thereof, are modified at the 2-, 3- and 17-positions or combinations thereof. Combinations which are physically impossible are not contemplated by this invention, such as a carbon atom containing 5 bonds.

100% pure isomers are contemplated by this invention, however a stereochemical isomer (labeled as α or β, or as R or S) may be a mixture of both in any ratio, where it is chemically possible by one skilled in the art. Also contemplated by this invention are both classical and non-classical bioisosteric

atom and substituent replacements, such as are described by Patani and Lavoie ("Bio-isosterism: a rational approach in drug design" Chem. Rev. (1996) p. 3147-3176) and are well known to one skilled in the art. Such bioisosteric replacements include, for example, but are not limited to, substitution of =S or =NH for =O.

Improved Estradiol Derivative Synthesis

Known compounds that are used in accordance with the invention and precursors to novel compounds according to the invention can be purchased, *e.g.*, from Sigma Chemical Co., Steraloids or Research Plus. Other compounds according to the invention can be synthesized according to known methods from publicly available precursors.

The 2-position of estradiol can be modified according to known methods, such as those described in U.S. Pat. No. 6,136,992 (2000) (incorporated herein by reference); Siya Ram et al., 2-Alkoxy estradiols and derivatives thereof (incorporated herein by reference); Cushman et al., *J. Med. Chem.* (1995) 38, 2041-2049; Cushman, et. al., J. Med. Chem. (1997) 40, 2323-2334; U.S. Pat. No. 6,448,419 (2002); Paaren et al., Wang *et al*, *Synthetic Comm* 1998, 28, 4431, Cushman et al., *J. Med. Chem.* 1995, 38, 2041, Cushman et al., *J. Med. Chem.* 2002, 45, 4748), or other publications, incorporated herein by reference.

The synthetic routes for the series of analogs disclosed by this invention are summarized in Schemes 1-8. Schemes 1-4 show the preparation of singly and doubly substituted templates that are required as precursors for the triply substituted analogs. Schemes 3 and 5 demonstrate how 3-carboxamides can be prepared from the templates. These synthetic routes present one potential way to prepare this series of analogs, and other synthetic routes (including modifying the order of synthetic steps or reagents) are possible to those skilled in the art.

In Scheme 1, the 2-methoxy, 2-ethoxy or 2-propynyl derivatives, which are either commercially available or can be readily prepared by literature methods, are oxidized using the Oppenauer oxidation. The resulting ketone can be deoxygenated using the Wolf-Kishner reduction (Shapiro R. J. et al., *J. Org. Chem.* 1964, *86*, 2825-2832.) or olefinated (Schow et al., *J. Org. Chem.* 1979, *44*,

22; Krubner et al., *J. Org. Chem.* 1968, *33*, 1715.) using the Wittig reaction. Both the 17-methylene and 17-ethylene estrane analogs can be reduced to the alkane using catalytic reduction (Pd on activated carbon, H_2).

In specific cases, the nature of protecting groups or the order of reactions may have to be altered to reach the desired products. These changes to the general synthetic schemes would be well understood to one skilled in the art. For instance, in the case where the desired 2-functionality is a propyne and the desired 17-functionality is an alkyl group, catalytic hydrogenation can not be carried out on the 17-olefin since the 2-alkyne would also be reduced. Scheme 2 presents a synthetic route to prepare analog 18. In this example, the 17-methyl is introduced as in Scheme 1 starting with estrone, and subsequently the 2-propyne is incorporated using a literature method (Cushman et al., *J. Med. Chem.* 2002, *45*, 4748).

Scheme 3 demonstrates the preparation of compounds 33-38. 2-Methoxyestradiol can be converted selectively to 3-triflate-2-methoxyestra-17-ol using 4-nitrophenyl trifluorosulfonate (*J. Org. Chem.* 1999, 64, 178). The 17-hydroxy can be converted to the sulfamate with sulfamoyl chloride and either sodium hydride (Howarth et al., *J. Med. Chem.* 1994, *37*, 219) or 2,6-di-*tert*-butyl-4-methylpyridine (Coibanu et al., *J. Med. Chem.* 1999, *42*, 2280) or alkylated using the Williamson ether synthesis. The ether or sulfamate can then be converted to the carboxamide using the Morera procedure (*Tetrahedron Letters*, 1998, *39*, 2835-2838). The triflate is a versatile synthetic intermediate and can be used to incorporate a wide range of functional groups at position 3. Alternate paths to the carboxamide could also utilize a carboxylic acid (Shi et al., *Chem. & Biol.* 2001, *8*, 501); then conversion to a carboxamide with thionyl chloride and ammonia gas (Tomas de, Paulis, et. at., *J. Med. Chem.* 1986, *29*, 61). Alternatively, an ester can be used which can be transformed to a carboxamide with ammonia. Nitriles (Weissman et al., *J. Org. Chem.* 2005, *70*, 1509) can also be converted to amides (hydrolysis: NaOH and H_2O_2). Generally speaking, aryl halides and triflates are interchangeable in Pd catalyzed reactions, such as the ones contemplated herein.

Scheme 4 demonstrates one possible route for preparation of 2-methoxy-1,3,5(10)16-estratetraene-3-ol using the Shapiro reaction. See U.S. Pat. No. 5,783,571, incorporated herein by reference.

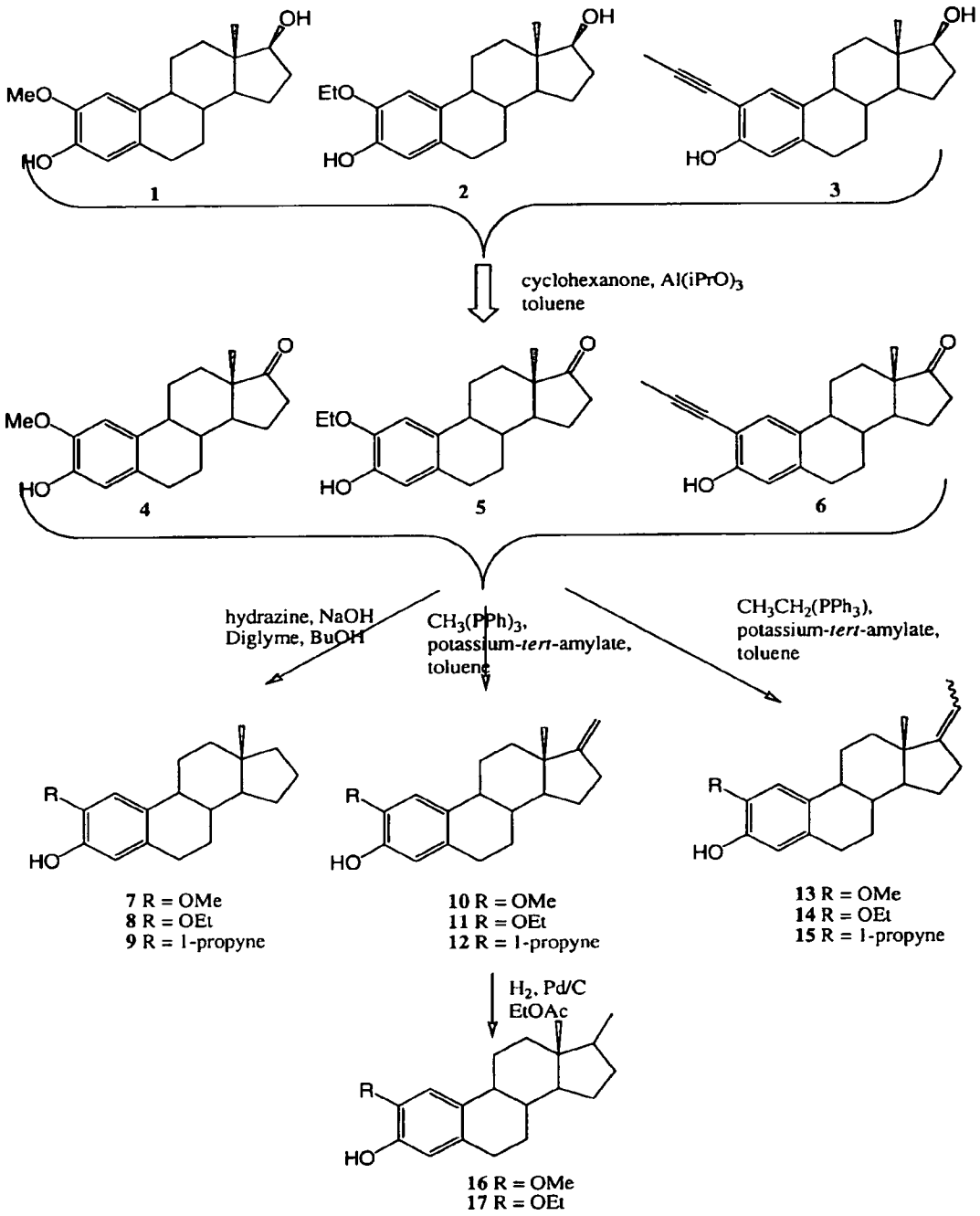
Scheme 5 demonstrates one possible route using triflic anhydride and pyridine to convert templates 4-12, 16, 17, 23 and 39-41 to the corresponding triflates (Echvarren et al., *J. Am. Chem.* 1987, *109*, 5478). These triflates can be converted to the respective carboxamides using Morera's procedure as discussed above. Ketones 54, 55 and 56 can be converted to the 17-hydroxy analogs by mild reduction with sodium borohydride as shown in Scheme 6.

Scheme 7 demonstrates one possible route for preparation of 17-(1,3-dioxolane)-2-methoxyestra-1,3,5(10)-triene-3-caroxamide using the Oppenauer Oxidation reaction.

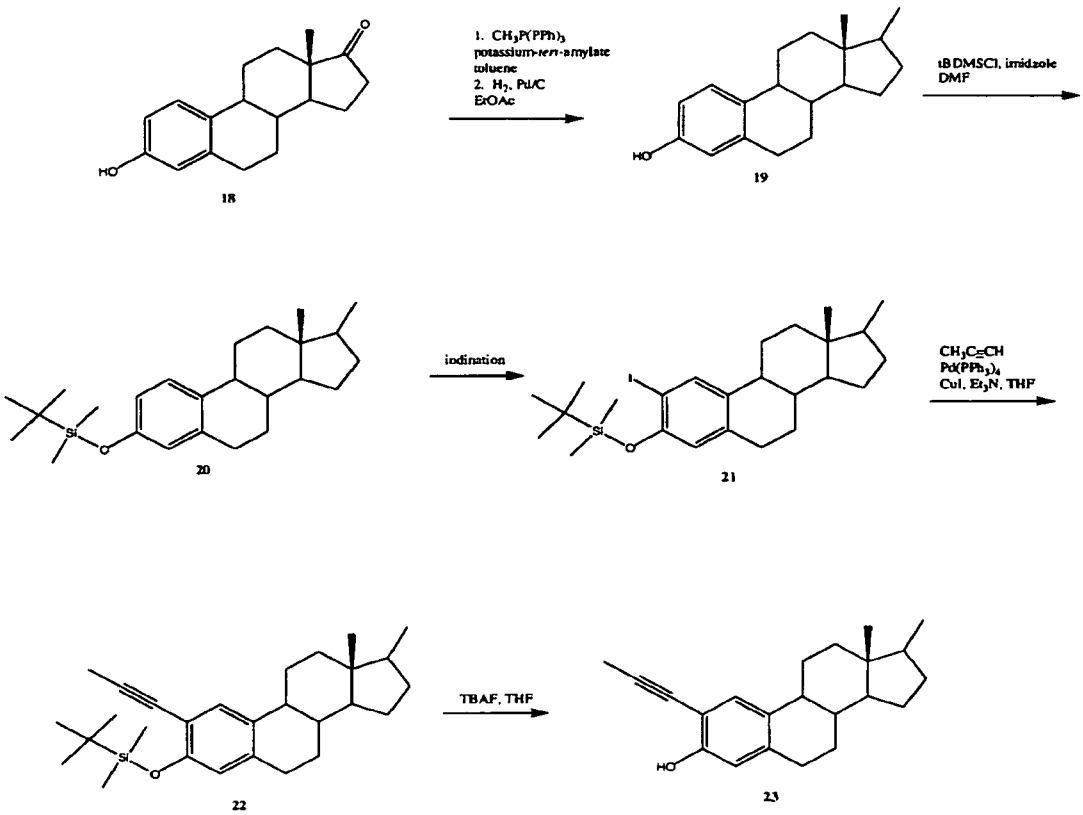
Scheme 8 demonstrates one possible route for preparation of 2,17β-(bismethoxy)estra-1,3,5(10)-triene-3-carboxamide.

To one skilled in the art, there are multiple alternative synthetic paths that can be used to prepare these compounds. While the synthetic pathways discussed above have been put to practice, these paths are not the only viable routes available to one skilled in the art. All of these compounds can be prepared from either estrone or estradiol, and other reactions, such as the Barton deoxygenation, Clemmensen reduction, Tebbe reaction and alcohol dehydration, among other possible reactions, can be used to incorporate the 17-modification. Carboxamides and esters can be prepared from the corresponding carboxylic acids using known chemical or enzymatic amidation techniques.

Scheme I

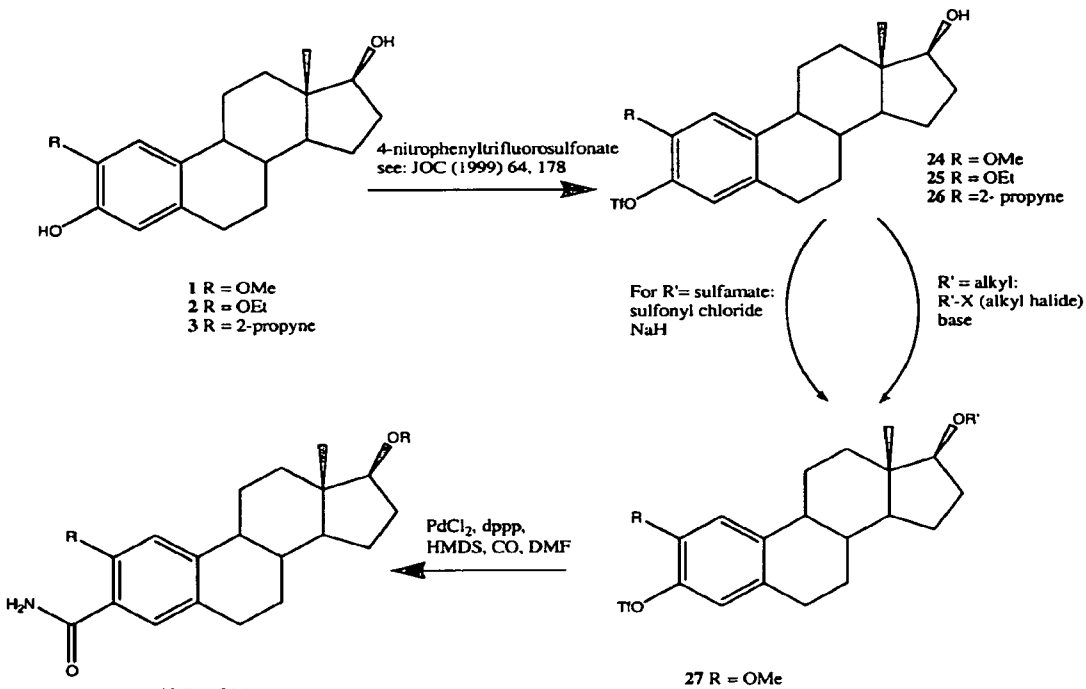

MeO
HO
OH
1
EtO
HO
OH
2
HO
OH
3
cyclohexanone, Al(iPrO)3
toluene
MeO
HO
O
4
EtO
HO
O
5
HO
O
6
hydrazine, NaOH
Diglyme, BuOH
CH3(PPh)3,
potassium-tert-amylate,
toluene
CH3CH2(PPh3),
potassium-tert-amylate,
toluene
R
HO
7 R = OMe
8 R = OEt
9 R = 1-propyne
R
HO
10 R = OMe
11 R = OEt
12 R = 1-propyne
R
HO
13 R = OMe
14 R = OEt
15 R = 1-propyne
H2, Pd/C
EtOAc
R
HO
16 R = OMe
17 R = OEt

Scheme 2


1. $CH_3P(PPh)_3$
potassium-*tert*-amylate
toluene
2. H_2, Pt/C
EtOAc
tBDMSCl, imidzole
DMF
iodination
$CH_3C{\equiv}CH$
$Pd(PPh_3)_4$
CuI, Et_3N, THF
TBAF, THF
HO
Si
O
I
18
19
20
21
22
23

5

Scheme 3



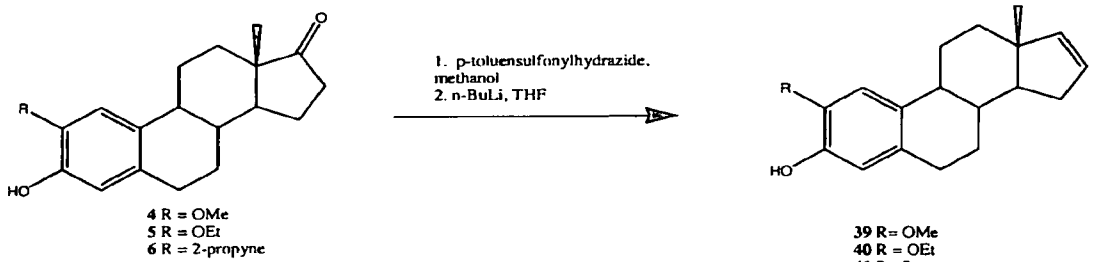
33 R = OMe
34 R = OEt
35 R = 2-propyne
when R' = sulfamate

36 R = OMe
37 R = OEt
38 R = 2-propyne
when R' = O alkyl

27 R = OMe
28 R = OEt
29 R = 2-propyne
when R' = sulfamate

30 R = OMe
31 R = OEt
32 R = 2-propyne
when R' = O alkyl

Scheme 4

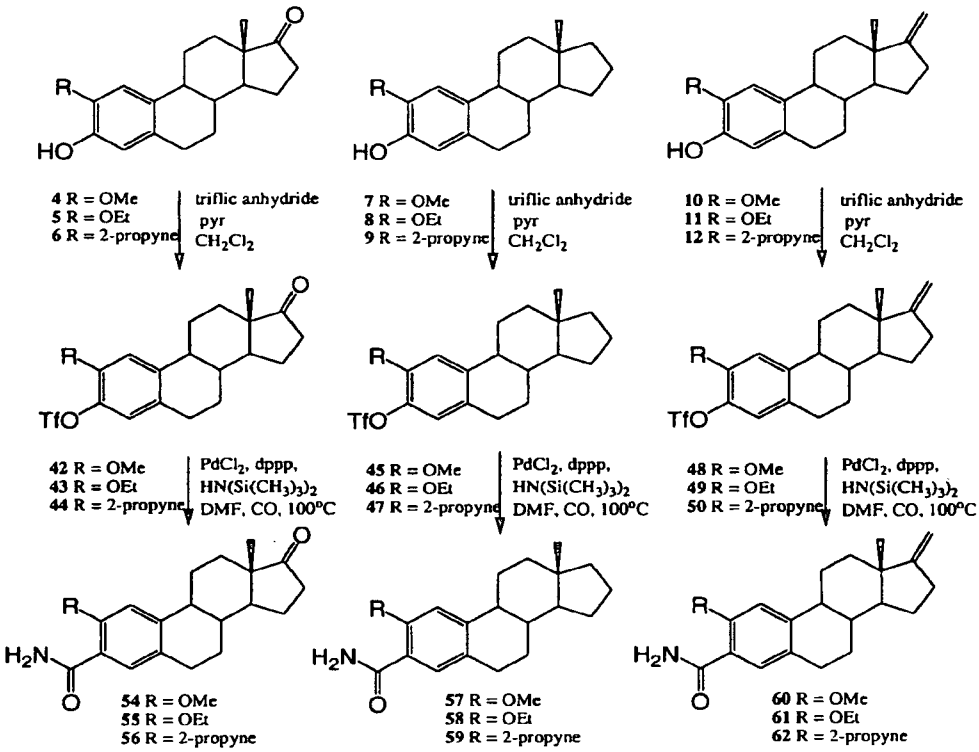


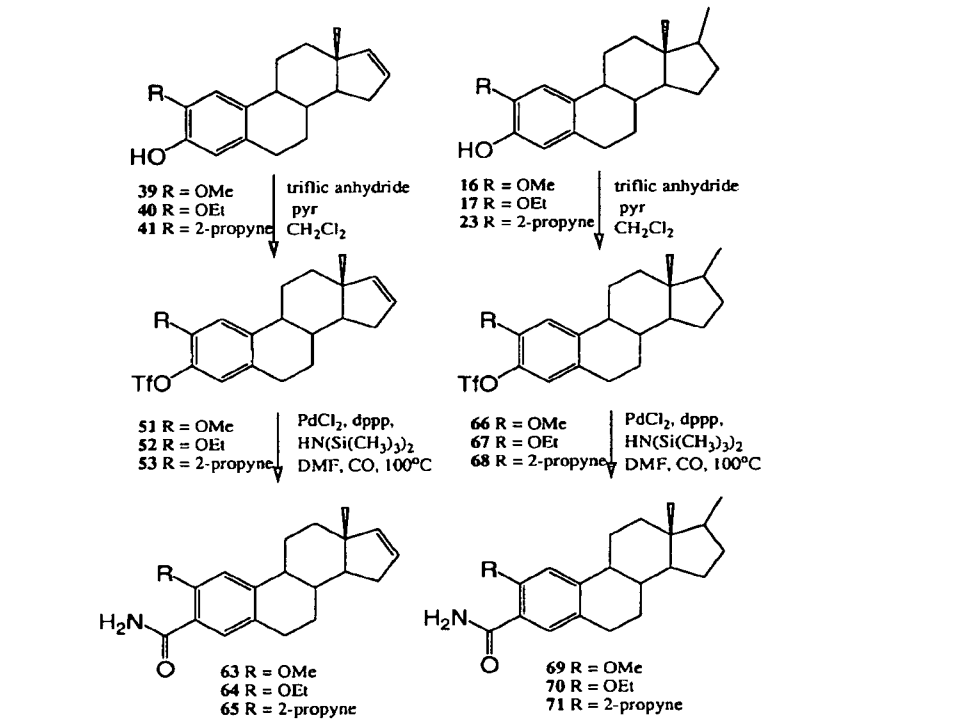
4 R = OMe
5 R = OEt
6 R = 2-propyne

39 R= OMe
40 R = OEt
41 R= 2-propyne

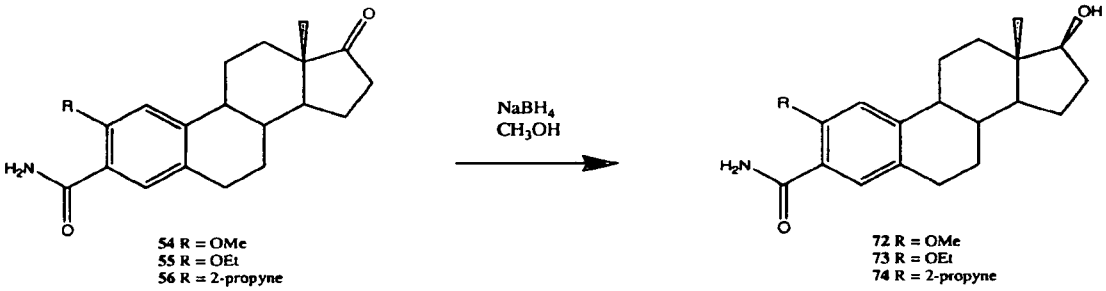
5

10 Scheme 5


4 R = OMe
5 R = OEt
6 R = 2-propyne
triflic anhydride
pyr
CH_2Cl_2
7 R = OMe
8 R = OEt
9 R = 2-propyne
triflic anhydride
pyr
CH_2Cl_2
10 R = OMe
11 R = OEt
12 R = 2-propyne
triflic anhydride
pyr
CH_2Cl_2
42 R = OMe
43 R = OEt
44 R = 2-propyne
$PdCl_2$, dppp,
$HN(Si(CH_3)_3)_2$
DMF, CO, 100°C
45 R = OMe
46 R = OEt
47 R = 2-propyne
$PdCl_2$, dppp,
$HN(Si(CH_3)_3)_2$
DMF, CO, 100°C
48 R = OMe
49 R = OEt
50 R = 2-propyne
$PdCl_2$, dppp,
$HN(Si(CH_3)_3)_2$
DMF, CO, 100°C
54 R = OMe
55 R = OEt
56 R = 2-propyne
57 R = OMe
58 R = OEt
59 R = 2-propyne
60 R = OMe
61 R = OEt
62 R = 2-propyne


39 R = OMe
40 R = OEt
41 R = 2-propyne
triflic anhydride
pyr
CH_2Cl_2
16 R = OMe
17 R = OEt
23 R = 2-propyne
triflic anhydride
pyr
CH_2Cl_2
51 R = OMe
52 R = OEt
53 R = 2-propyne
$PdCl_2$, dppp,
$HN(Si(CH_3)_3)_2$
DMF, CO, 100°C
66 R = OMe
67 R = OEt
68 R = 2-propyne
$PdCl_2$, dppp,
$HN(Si(CH_3)_3)_2$
DMF, CO, 100°C
63 R = OMe
64 R = OEt
65 R = 2-propyne
69 R = OMe
70 R = OEt
71 R = 2-propyne

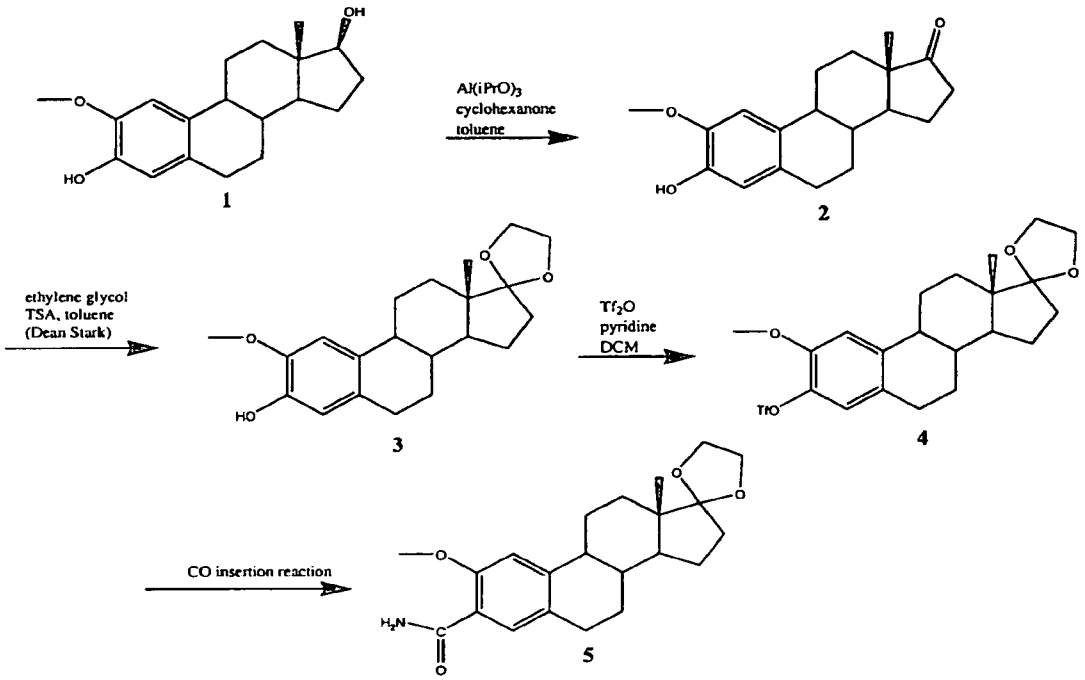
Scheme 6



54 R = OMe
55 R = OEt
56 R = 2-propyne

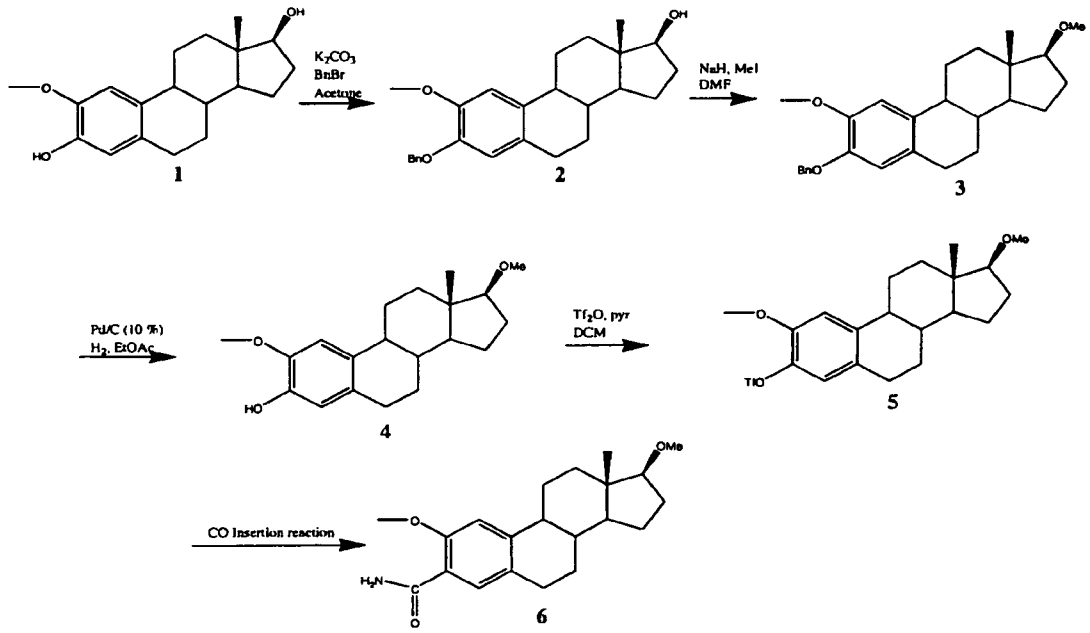
72 R = OMe
73 R = OEt
74 R = 2-propyne

Scheme 7



1 2 3 4 5

Scheme 8



EXPERIMENTAL EXAMPLES

Representative Oppenauer Oxidation:

Preparation of 2-methoxyestra-1,3,5(10)-trien-17-one (4)

2-Methoxyestradiol (Scheme 1, Compound 1) (10 g, 33.1 mmol) was placed in a 1 L round bottom flask that was equipped with a 25 mL Dean-Stark trap and a reflux condenser. The entire apparatus had been flame dried under an argon atmosphere. Toluene (400 mL) was added to dissolve the starting material. Aluminum isopropoxide (34.6 g, 169 mmol) and cyclohexanone (135 mL, 1.3 mol) were added and the entire reaction mixture was heated at reflux (145°-150°C) for 20h. Saturated aqueous sodium bicarbonate solution (200 mL) was added after the reaction mixture was allowed to cool to room temperature. The organic material was extracted with dichloromethane (3 x 300 mL). The aqueous emulsion was acidified with 3 N HCl (~20 mL) until the emulsion separated and the aqueous layer was extracted with ethyl acetate (2 x 75 mL). The combined organic extracts were dried over magnesium sulfate and condensed using a rotary evaporator. The cyclohexanone and cyclohexanol were removed by vacuum distillation. When the distillation pot was cool enough, hexane was added and 2-

methoxyestrone (Scheme 1, Compound 4) precipitated out of solution. 7.72 g (25.7 mmol, 78 %) of product was obtained. ^{1}H NMR (300 MHz, $CDCl_3$) □ 6.81 (s, 1H), 6.68 (s, 1H), 5.46 (s, 1H), 3.88 (s, 3H), 2.89-2.76 (m,2H), 2.61-1.24 (m, 13H), 0.94 (s, 3H).

Representative Wolf-Kishner Reduction:

Preparation of 2-Methoxyestra-1,3,5(10)-triene-3-ol (7)

2-Methoxyestrone (Scheme 1, Compound 4) (450 mg, 1.5 mmol) was suspended in ethylene glycol (10 mL) and n-butanol (2 mL). Hydrazine hydrate (0.145 mL, 3 mmol) was added and the mixture was heated to 130°C for 1 h. The reaction was cooled to 70°C, KOH (253 mg, 4.5 mmol) was added, then the reaction was heated at 200°C for 1 h. The mixture was cooled to room temperature, poured into ice and HCl (6N, 6 mL) was added. The resulting solid was filtered off. The solid was dissolved in acetone, which was then evaporated to give a product (Scheme 1, Compound 7) (310 mg, 72 % yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.93 (1H, s, aromatic), 6.75 (1H, s, aromatic), 5.35 (1H, s, phenol), 3.95 (3H, s, methoxy), 2.95 (2H, dd, J = 5.0, 3.0 Hz), 2.25 (2H, m,), 1.95 (2H, m), 1.95-1.05 (11H, m), 0.8 (3H, s)

Representative Wittig Olefination:

Preparation of 17(20)-methyleneestra-1,3,5(10)-triene-3-ol (10)

Potassium-*tert*-amylate (1.54 M, toluene, 4.35 mL 6.69 mmol), (prepared as in Schow et al., *J. Org. Chem.* 1979, *44*, 3760) (potassium-*tert*-butoxide is an alternate base) was added to a suspension of methyl triphenylphosphonium bromide (2.39 g, 6.69 mmol) in anhydrous benzene and refluxed for 30 min. 2-Methoxyestrone (Scheme 1, Compound 4) (300 mg, 1 mmol) in warm benzene (5 mL) was added and the mixture was refluxed for 3 h. The reaction was cooled to room temperature, poured into 100 mL water, and washed with ether (2 x 100 mL). The combined organics were washed with 6 M HCl (1 x 100 mL), $NaHCO_3$ (saturated, 1 x 100 mL), water (1 x 100 mL), and brine (1 x 100 mL). The combined organics were then dried with sodium sulfate, filtered and rotoevaporated to give a semi-solid, yellowish oil. This product was purified by

silica gel column chromatography using 95:5 chloroform:methanol as an eluent. 220 mg 17(20)-methyleneestra-1,3,5(10)-triene-3-ol (Scheme 1, Compound 10) (0.738 mmol, 73 % yield) was obtained. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.83 (s, 1H), 6.67 (s, 1H), 5.44 (br s, 1H), 4.70 (t, J=2.26 Hz, 2H), 3.89 (s, 3H), 2.86-2.74 (m, 2H), 2.64-2.49 (m, 1H), 2.39-2.17 (m, 3H), 2.02-1.78 (m, 3H), 1.65-1.19 (m, 4H), 0.85 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.2, 144.9, 143.8, 132.3, 130.0, 115.0, 108.5, 101.2, 77.6, 56.5, 53.9, 44.7, 39.2, 36.2, 29.9, 29.5, 28.1, 27.3, 24.3, 19.0. Elemental analysis ($C_{20}H_{26}O_2$) calculated C=80.48, H=8.79; found C=80.60, H=8.77.

Representative Shapiro Reaction (Scheme 4):

Preparation of 2-Methoxyestrone p-toluensulfonylhydrazone

2-Methoxyestrone (Scheme 4, Compound 4) (1.011 g, 2.3 mmol) and *p*-toluensulfonylhydrazide (775 mg, 4.17 mmol) were dissolved in anhydrous methanol (10 mL) in a flame dried 50 ml round bottomed flask. The mixture was refluxed for 24 h, after which the contents were transferred to a 50 ml Erlenmeyer flask and cooled to room temperature. Solvent was removed under reduced pressure to give an orangish foam, which was used without further purification (1.5757 g, quantitative yield). Selected ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.87 (d, J=8 Hz, 2H), 7.23 (d, J=8 Hz, 2H), 6.92 (br s, 1H), 6.80 (s, 1H), 6.66 (s, 1H), 5.45 (br s, 1H), 3.88 (s, 3H), 2.85-2.70 (m, 2H), 2.45 (s, 3H), 2.41-1.2 (m, 13H), 0.85 (s, 3H).

Preparation of 2-methoxy-1,3,5(10)16-estratetraene-3-ol (39)

2-Methoxyestrone *p*-tosylhydrazone (1.5757 g, 3.3 mmol) was dissolved in anhydrous tetrahydrofuran (50 mL) in a flame dried 250 mL round bottomed flask and cooled to -10°C. The mixture was stirred and n-butyl lithium (5.3 mL, 2.5 M in hexanes) was added dropwise. The mixture was warmed to room temperature over 3 days. Ice (~5 g) was added, followed by saturated ammonium chloride (~50 mL). The mixture was transferred to a 250 mL separatory funnel, shaken, and separated. The aqueous layer was washed with ether (50 mL) and the organics were combined. The organics were washed with saturated sodium

bicarbonate (50 mL) and brine (50 mL). The organics were dried with magnesium sulfate, filtered and solvent was removed under reduced pressure. The resulting solid was dissolved in acetone (~10 mL) and solvent was removed under reduced pressure two times. Crystalline 2-methoxy-1,3,5(10)16-estratetraene-3-ol (Scheme 4, Compound 39) was obtained (845 mg, 90 % yield). H-NMR (300 MHz, $CDCl_3$) δ 6.81 (s, 1H), 6.68 (s, 1H), 5.97-5.91 (m, 1H), 5.80-5.74 (m, 1H), 5.43 (s, 1H), 3.88 (s, 3H), 2.90-2.71 (m, 2H), 2.38-1.34 (m, 11H), 0.82 (s, 3H).

Representative Preparation of Triflic esters:
Preparation of 2-Methoxy-17-oxoestra-1,3,5(10)-trien-3-yl (trifluoromethyl)sulfonate (42)

A 2-L 3-neck round-bottomed flask fitted with an overhead stirrer, dropping funnel, and nitrogen inlet was charged with 2-methoxyestrone (Scheme 5, Compound 4) (35 g, 0.116 mol), anhydrous methylene chloride (1300 mL), and anhydrous pyridine (330 mL), and cooled externally with an ice-water bath. Trifluoromethanesulfonic anhydride (32.5 mL, 0.193 mol) was then added dropwise over 1 hour, while the temperature was maintained below 5°C. The mixture was stirred an additional hour, and poured into 1 N HCl (2 L). The organic layer was removed, the aqueous layer was extracted with additional methylene chloride (300 mL), and the combined organic layers were extracted with 1 N HCl (2 L). The aqueous layer was extracted with methylene chloride (300 mL), and the total combined organic layers were washed with 1 N HCl (1 L) at which point the pH of the aqueous phase was acidic (pH paper). The organic layer was washed with brine (1 L) and dried over sodium sulfate (370 g). Suction-filtration through a plug of silica gel 60 (150 g) and concentration afforded a brown foam (39.7 g) which was purified by flash column chromatography using 550 g of silica gel 60, eluted with 3:1 hexanes/methylene chloride (2 L) then methylene chloride (4 L). Product-containing fractions were concentrated affording 36.05 g (72%) of 2-methoxy-17-oxoestra-1,3,5(10)-trien-3-yl(trifluoromethyl)sulfonate (Scheme 5, Compound 42) as a white solid, which was used without further purification. H NMR (300 MHz, $CDCl_3$) δ 6.96 (s, 1H),

6.95 (s, 1H), 3.90 (s, 3H), 2.87 (dd, J = 4.2, 6.4 Hz) 2.61-2.95 (m, 8H), 1.74-1.36 (m, 5H), 0.94 (s, 3H).

Preparation of 2-Methoxyestra-1,3,5(10)-trien-3-yl(trifluoromethyl)sulfonate(45)

A 2-L 3-neck round-bottomed flask, fitted with an overhead stirrer, a Claisen adaptor with a nitrogen inlet and a thermocouple probe, and a septum, was charged with 2-methoxyestra-1,3,5(10)-trien-3-ol (Scheme 5, Compound 7) (18 g, 62.84 mmol, anhydrous dichloromethane (750 mL) and pyridine (170 mL). The resulting solution was stirred under nitrogen, extremely cooled using an ice-water bath, and treated with trifluoromethanesulfonic anhydride (16 mL, 94.26 mmol) over 1.5 hours while maintaining the internal reaction temperature below 2°C. The resulting dark mixture was stirred an additional 1 h at which point TLC analysis (dichloromethane) indicated complete loss of starting material and the formation of a new upper-R_f spot. The reaction mixture was poured into 1 N hydrochloric acid (2000 mL) and the phases were partitioned. The aqueous phase was extracted with dichloromethane (2 x 300 mL) and the combined organic layers were washed with brine (200 mL), dried over sodium sulfate (500 g), filtered, concentrated to dryness, dissolved in a mixture of 1:1 dichloromethane-hexanes, and suction-filtered through a plug of silica gel 60 (150 g), eluting with an additional 1000 mL of the same solvent system. The colorless filtrate was concentrated to afford 24.3 g (92%) of the desired compound (Scheme 5, Compound 45), after drying to a constant weight under high vacuum (1 torr) at ambient temperature. The compound was used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98 (s, 1 H), 6.90 (s, 1 H), 3.88 (s, 3 H), 2.85 (m, 2 H), 2.32 (m, 2 H), 1.95 (m, 2 H), 1.80-1.58 (m, 6 H), 1.42-1.05 (m, 8 H), 0.80 (s, 3 H).

Preparation of 2-Methoxy-17-methyleneestra-1,3,5(10)-trien-3-yl (trifluoromethyl)sulfonate (48)

A 3-L 3-neck round-bottomed flask fitted with an overhead stirrer, dropping funnel, nitrogen inlet and temperature probe, was charged with 17(20)-methyleneestra-1,3,5(10)-triene-3-ol (Scheme 5, Compound 10) (47.0 g, 0.158

mol), anhydrous methylene chloride (1.75 L), and anhydrous pyridine (450 mL), then cooled externally with an ice-water bath. Trifluoromethanesulfonic anhydride (40.0 mL, 0.238 mol) was added dropwise over 1 hour while maintaining the temperature below 5°C. After an additional 4 hours, TLC analysis indicated complete conversion of the starting material (new upper R_f spot, methylene chloride). The reaction mixture was poured into 1 N HCl (3 L). The organic layer was removed, the aqueous layer was extracted with methylene chloride (3 x 500 mL), and the combined organic layers were extracted with 1 N HCl (2 L). The aqueous layer was extracted with methylene chloride (500 mL) and the combined organic layers were washed with 1 N HCl (1 L). The aqueous layer was extracted with methylene chloride (500 mL) at which point the pH of the aqueous phase was acidic (pH paper). The combined organic layer was washed with brine (1 L) and dried over sodium sulfate. Suction-filtration through a plug of silica gel (305 g) and concentration afforded 66.1 g (97%) of triflate (Scheme 5, Compound 48) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98 (s, 1H), 6.92 (s, 1H), 4.71 (s, 2H), 3.91 (s, 3H), 2.91-2.72 (m, 2H), 2.66-1.18 (m, 13H), 0.85 (s, 3H).

Preparation of 2-methoxy-3-triflic-1,3,5(10)16-estratetraene (51)

2-Methoxy-1,3,5(10)16-estratetraene-3-ol (Scheme 5, Compound 39) (2.258 g, 8.89 mmol) was dissolved in anhydrous dichloromethane (80 mL) and anhydrous pyridine (22 mL, 272 mmol) and cooled to 0°C. Triflic anhydride (12.6 mL, 74.6 mmol) was added dropwise and the mixture was stirred for 18 h with warming to rt. The mixture was poured into water (200 mL) and washed with dichloromethane (2 x 200 mL). The organics were washed with 2 M HCl (2 x 200 mL) water (200 mL) and brine (200 mL). The organics were dried with Na_2SO_4, filtered and the solvent was reduced pressure. The crude product was purified with a Biotage FLASH SiO_2 column (99:1 hexanes:ethyl acetate). 3.195 g of 2-methoxy-3-triflic-1,3,5(10)16-estratetraene (Scheme 5, Compound 51) was obtained (7.68 mmol, 86 % yield). H-NMR (300 MHz, $CDCl_3$ δ 6.96 (s, 1H), 6.93 (s, 1H), 5.93 (ddd, J = 4.5, 1.7, 1 Hz, 1H), 5.80-5.75 (m, 1H), 3.90 (s, 1H), 2.90-2.78 (m, 2H), 2.39-1.21 (m, 11H), 0.82 (s, 3H).

Representative carboxamidations:

(General procedure based on Tetrahedron Lett 1998 39, 2835-2838)

Preparation of 2-methoxy-17-oxoestra-1,3,5(10)-triene-3-carboxamide (54)

A 1-L 3 neck round-bottomed flask equipped with an overhead stirrer, reflux condenser, nitrogen inlet and thermocouple, was charged with 2-methoxy-17-oxoestra-1,3,5(10)-triene-3-yl(trifluoromethyl)sulfonate (Scheme 5, Compound 42) (15 g, 34.7 mmol), 1,3-bis(diphenylphosphino)propane (1.54 g, 3.73 mmol), palladium (II) chloride (0.33 g, 1.9 mmol), 1,1,1,3,3,3-hexamethyldisilazane (31 mL, 150 mmol) and anhydrous dimethylformamide (110 mL). Carbon monoxide was bubbled through the yellow solution while stirring for 5 minutes. The reaction was heated to 110°C under an atmosphere of carbon monoxide (balloon) and allowed to stir for 15 h. During this period the reaction became dark. The reaction was quenched by treating with methanol (25 mL) and stirred for 10 min. The mixture was poured into ethyl acetate (1L) and washed with 2N sulfuric acid (750 mL). The aqueous layer was extracted with ethyl acetate (2 x 500 mL) and the combined organic phases were washed with 2N sulfuric acid (250 mL) and saturated aqueous sodium bicarbonate (500 mL) then dried over 500 g of sodium sulfate. This mixture was suction-filtered through a bed of silica gel 60 (67 g) and concentrated to dryness affording crude product (11.2 g, 98 % recovery). The crude material was purified by Flash column chromatography (360 g of silica gel 60, eluting with methylene chloride, then 1:5 ethyl acetate:methylene chloride). Concentration of the pure fractions (R_f = 0.3, 1:5 ethyl acetete:methylene chloride, UV detection) and drying in a vacuum oven at 50°C, and removal of residual solvents afforded pure product (Scheme 5, Compound 54) (4.97 g, 44 % yield) as an off-white to pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.82 (br s, 1H),7.05 (s, 1H), 5.92 (br s, 1H), 4.02 (s, 3 H), 3.05 (dd, 2 H, J = 5), 2.15 (m 2H,) 2.02-1.65 (m, 6 H), 1.55-1.15 (m, 8 H), 0.80 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 220.6, 167.2, 155.9, 145.5, 132.8, 129.4, 118.3, 108.4, 55.9, 50.5, 47.9, 44.9, 37.8, 35.8, 31.6, 28.26, 26.4, 25.8, 21.6, 13.8.

Preparation of (2-Methoxyestra-1,3,5(10)-trien-3-yl)-3-carboxamide (57)

A 500 mL round-bottomed flask fitted with an overhead stirrer, a Claisen adaptor with a thermocouple probe and carbon monoxide inlet, and a vacuum inlet, was charged with 2-methoxyestra-1,3,5(10)-trien-3-yl (trifluoromethyl) sulfonate (Scheme 5, Compound 45) (24 g, 57.35 mmol), anhydrous dimethylformamide (185 mL), palladium (II) chloride (0.500 g, 2.87 mmol), 1,3-bis(diphenylphospino)propane (2.37 g, 5.74 mmol), and 1,1,1,3,3,3-hexamethyldisilazane (48 mL, 229 mmol). The resulting yellow solution was stirred and evacuated, flushed with carbon monoxide (balloon) several times, then heated to 102°C for 12 h. Additional palladium (II) chloride (0.500 g), 1,3-bis(diphenylphospino)propane (2.40 g), and hexamethyldisilazane (30 mL) were added and the mixture was re-evacuated, charged with carbon monoxide, and heated at 102°C for an additional 12 h. Methanol (50 mL) was added and, after several minutes, the dark solution was partitioned with ethyl acetate (1000 mL) and 2 N sulfuric acid (1000 mL). The aqueous phase was extracted with ethyl acetate (2 x 250 mL), the combined organic extracts were washed with additional sulfuric acid (500 mL), and the aqueous phase was back-extracted with ethyl acetate (2 x 250 mL), and the total combined dark organic layers were washed with saturated aqueous sodium bicarbonate (500 mL) and dried over sodium sulfate (400 g). Suction-filtration through a plug of silica gel 60 (136 g) and concentration afforded 19 g of crude product as a red paste. This was purified by flash chromatography using 430 g of silica gel, and eluting with 10% ethyl acetate-dichloromethane. The product-containing fractions were concentrated, then taken up in acetone and re-concentrated (2 x 2000 mL). The yellow solid was then slurried in *n*-heptane (200 mL) overnight and isolated by suction-filtration. Removal of residual solvent, and drying to a constant weight over 3 h in a vacuum oven at 70°C and 0.5 torr, afforded 5.07 g (28% overall) of (Scheme 5, Compound 57) as an off-white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.82 (br s, 1H), 7.99 (s, 1H), 6.95 (s, 1 H), 4.05 (s, 3 H), 2.95 (dd, 2 H, J = 5), 2.15 (m 2H,) 2.02-1.65 (m, 6 H), 1.55-1.15 (m, 8 H), 0.80 (s, 3 H). C NMR (125 MHz, $CDCl_3$) δ 167.6, 156.1, 147.1, 132.9, 130.0, 116.0, 108.7, 56.2, 54.0, 45.2, 41.2, 40.7, 39.0, 38.6, 28.8, 28.2, 26.7, 25.5, 20.8, 17.8.

Preparation of 2-Methoxy-17-methyleneestra-1,3,5(10)-triene-3-carboxamide (60)

A 250 mL three-necked flask equipped with an overhead stirrer, thermocouple, and nitrogen inlet, was charged with 2-Methoxy-17-methyleneestra-1,3,5(10)-trien-3-yl (trifluoromethyl)-sulfonate (Scheme 5, Compound 48) (20.0 g, 46.5 mmol), palladium (II) chloride (0.41 g, 2.3 mmol), 1,3-bis(diphenylphosphino) propane (1.9 g, 4.6 mmol), 1,1,1,3,3,3-hexamethyldisilazane (38.8 mL, 186 mmol) and anhydrous dimethylformamide (150 mL). The resulting orange solution was evacuated and back-filled with nitrogen three times, then evacuated and back-filled with carbon monoxide three times. The reaction was warmed to 100°C and stirred under a carbon monoxide atmosphere (balloon) for 18 hours, during which time the solution became dark red. The heat was removed, methanol (40 mL) was added, and stirred for 10 minutes. The solution was poured into ethyl acetate (1 L) and extracted with 2 N H_2SO_4 (1 L). The aqueous layer was extracted with ethyl acetate (3 x 500 mL). Each of the ethyl acetate extracts was washed with 2 N H_2SO_4 (500 mL). The combined organic phases were washed with saturated aqueous sodium bicarbonate (1 L) and dried over sodium sulfate (500 g). This suspension was suction-filtered through a bed of silica gel 60 (102 g), and the filtrate concentrated to dryness affording 16.02 g (106% recovery) of crude (Scheme 5, Compound 60). The crude product was purified on silica gel 60 (500 g, flash column) eluting with methylene chloride (2 L) then 2% methanol-methylene chloride (4 L) then 4% methanol-methylene chloride (4 L). Chromatography failed to remove all impurities so fractions containing product were combined, concentrated to dryness, and repurified on silica gel 60 (500 g, flash column) eluting with methylene chloride (2 L) then 1:5 ethyl acetate-methylene chloride. Concentration of the pure fractions (TLC, 1:5 ethyl acetate-methylene chloride, R_f = 0.3, UV detection) and drying in a vacuum oven at 80°C, afforded 12.3 g (81% overall) of (Scheme 5, Compound 60) as a light-yellow solid. Removal of methylene chloride yielded 11.36 g of (Scheme 5, Compound 60) (75% overall yield) as a light-yellow solid. ^1H NMR (300 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.63

(br s, 1H), 6.95 (s, 1H), 5.70 (br s, 1H), 4.71 (s, 2H), 3.97 (s, 3H), 2.97-2.82 (m, 2H), 2.65-1.19 (m, 13H), 0.86 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 167.2, 161.4, 155.8, 146.3, 132.6, 129.6, 118.0, 108.4, 100.9, 55.9, 53.5, 44.9, 44.2, 38.2, 35.6, 29.4, 28.4, 27.4, 26.4, 23.9, 18.5.

Preparation of 2-methoxy-1,3,5(10)16-estratetraene-3-carboxamide

2-Methoxy-3-triflic-1,3,5(10)16-estratetraene (Scheme 5, Compound 51) (3.195 g, 7.68 mmol) was dissolved in anhydrous N,N-dimethylformamide (25 mL). Palladium (II) chloride (68 mg, 0.384 mmol), 1,3-bis(diphenylphosphino)propane (316 mg, 0.768 mmol) and 1,1,1,3,3,3-hexamethyldisilazane (6.65 mL, 30.72 mmol) were then added and CO gas was bubbled through this solution for 5 min. The reaction flask was fitted with a reflux condenser and a balloon filled with CO was fitted on top. The yellowish mixture was placed in a 100°C oil bath and stirred for 18 h during which the reaction turned reddish/purple. The reaction flask was cooled to room temperature and methanol (3.5 mL) was added and stirred 10 min. The mixture was poured into ethyl acetate (300 mL) and washed with H_2SO_4 (2N, 200 mL). The aqueous layer was washed with ethyl acetate (2 x 150 mL) and the combined organic layers were washed with H_2SO_4 (2N, 150 mL). The organics were washed with saturated $NaHCO_3$ (2x100mL), dried with Na_2SO_4, filtered and solvent was removed under reduced pressure. The crude product was purified using a Biotage FLASH SiO_2 column (1:1 hexanes:ethyl acetate). The product obtained from the column was dissolved in acetone and the solvent was removed under reduced pressure to give 1.718 g of 3 -carboxamide-2-methoxy-1,3,5(10)16-estratetraene (Scheme 5, Compound 63) (5.52 mmol, 72 % yield). ^1H-NMR (300 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.76 (br s, 1H), 6.92 (s, 1H), 5.97-5.91 (m, 1H), 5.81-5.75 (m, 1H), 5.68 (br s, 1H), 3.97 (s, 3H), 2.96-2.84 (m, 2H), 2.46-1.20 (m, 11H), 0.83 (s, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 172.0, 156.2, 147.1, 144.1, 133.1, 130,1, 129.9, 108.6, 56.3, 55.9, 46.0, 45.9, 37.2, 36.3, 32.2, 28.7, 28.3, 26.8, 17.5. FT-IR (ATR, neat, cm^{-1}) 3502, 3378, 2926, 1660, 1609, 1573, 1259, 1184, 1015. Elemental analysis ($C_{20}H_{25}NO_2$): calculated C, 77.14; H, 8.09; N, 4.50; found: C, 76.90; H, 8.12; N, 4.64.

Preparation of 17-(1,3-dioxolane)-2-methoxyestra-1,3,5(10)-triene-3-carboxamide

17-(1,3-dioxolane)-2-methoxyestra-1,3,5(10)-triene-3 carboxamide (Compound **5**) was prepared as depicted in Scheme 7. 2-Methoxyestradiol (Scheme 7, Compound **1**) was oxidized to 2-methoxyestrone (Scheme 7, Compound **2**) using the Oppenauer Oxidation. The resulting ketone was converted to the 1,3-dioxolane (Scheme 7, Compound **3**) using ethylene glycol in toluene and a catalytic amount of p-toluensulfonic acid under Dean-Stark conditions. The dioxolane (Scheme 7, Compound **3**) was then converted to the triflate (Scheme 7, Compound **4**) with triflic anhydride and pyridine. Compound **5** was prepared from the triflate precursor by a palladium mediated CO insertion reaction. Spectral data for compound **5**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.91 (s, 1H), 7.74 (br s, 1H), 6.91 (s, 1H), 6.10 (s, 1H), 3.94 (s, 3H), 4.01-3.85 (m, 4H), 2.94-2.73 (m, 2H), 2.37-2.25 (m, 2H), 2.10-1.22 (m, 11 H), 0.90 (s, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 167.3, 155.8, 146.3, 132.7, 129.7, 119.0, 118.1, 108.5, 65.3, 64.5, 55.9, 49.5, 46.0, 38.5, 37.8, 34.2, 30.6, 28.4, 26.9, 25.8, 22.4, 14.3. FT-IR (ATR, neat) 3430, 3146, 2922, 1662 cm^{-1}.

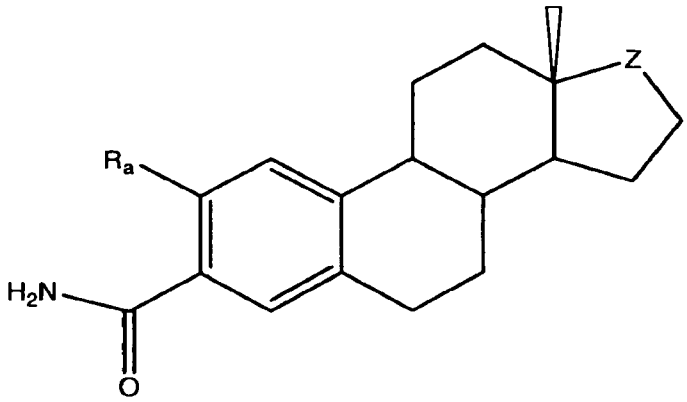
Preparation of 2,17β-(bismethoxy)estra-1,3,5(10)-triene-3-carboxamide

2,17β-(bismethoxy)estra-1,3,5(10)-triene-3-carboxamide (Compound **6**) was prepared as depicted in Scheme 8. 2-Methoxyestradiol was protected as the 3-benzyl ether to give compound **2**. The 17-alcohol was methylated using sodium hydride and methyl iodide to give compound **3**. The benzyl group was removed using catalytic hydrogenation to give compound **4** (for a related synthesis see: Prokai *et al J. Med. Chem.* **2001**, *44*, 110). Compound **4** was then converted to the triflate using triflic anhydride and pyridine to give compound **5**, which yielded carboxamide **6** by a palladium mediated CO insertion reaction. Spectral data for compound **6**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.76 (br s, 1H), 6.92 (s, 1H), 5.71 (br s, 1H), 3.97 (s, 3H), 3.40 (s, 3H), 3.34 (t, J = 8 Hz, 1H), 2.95-2.77 (m, 2H), 2.35-1.67 (m, 13H), 0.82 (s, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$) d 167.1, 155.8, 146.3, 132.8, 129.7, 118.0, 108.4, 90.6, 57.9, 55.9,

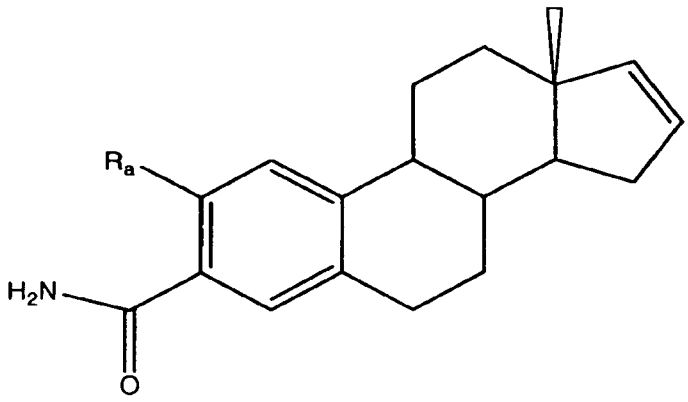
50.4, 44.8, 43.1, 38.0, 37.9, 28.4, 27.7, 27.1, 26.3, 23.1, 11.6. FT-IR (ATR, neat) 3430, 3165, 2916, 1657 cm^{-1}.

EXPERIMENTAL DATA

The following Examples refer to compounds of the following general Formulae:

(I) 

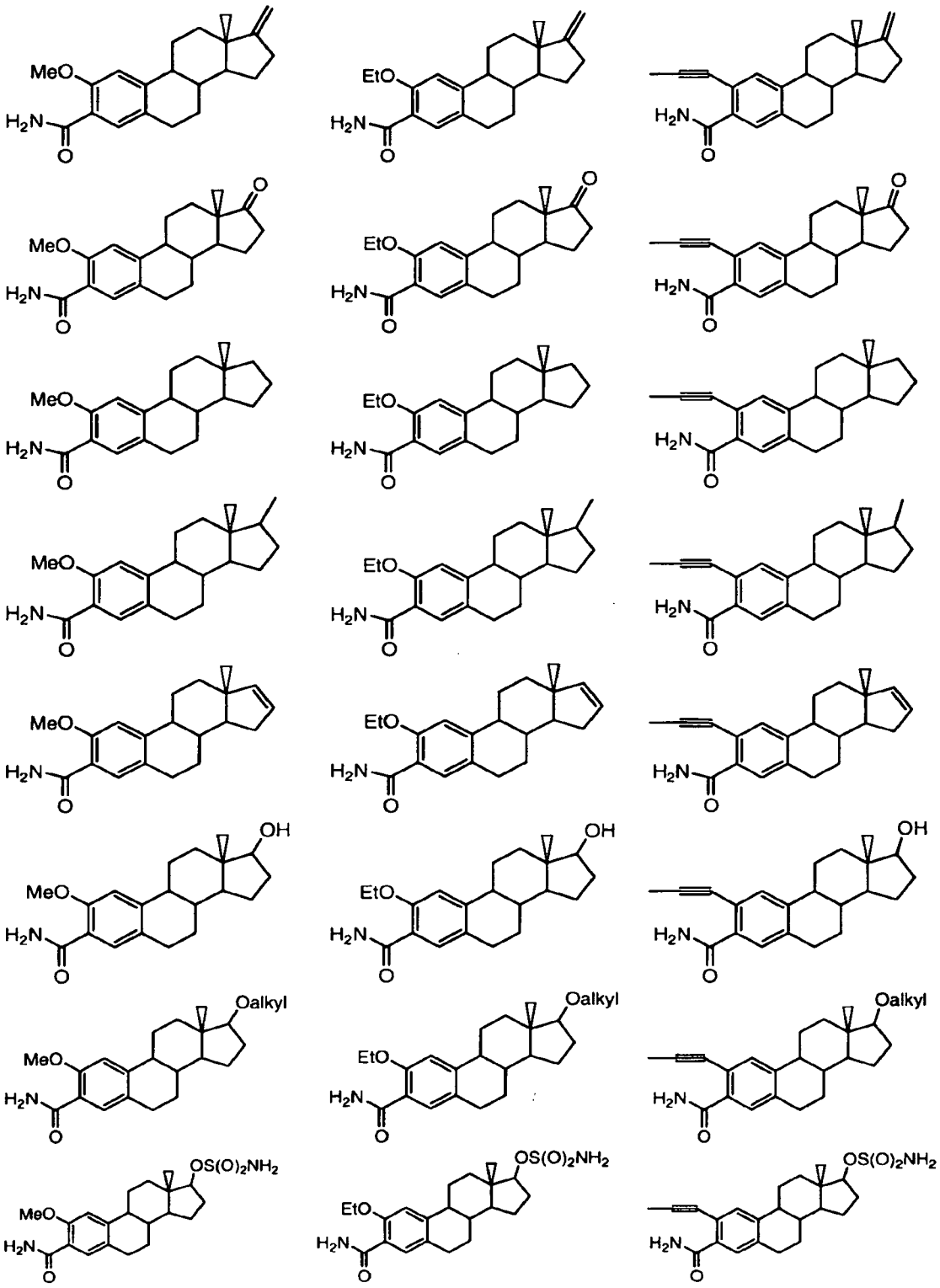
; or

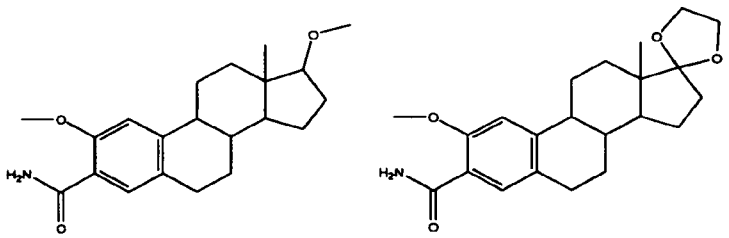
(II) 

,

wherein R_a is selected from $-OCH_3$, $-OCH_2CH_3$ or $-CCCH_3$; and Z is selected from $>C(H_2)$, $>C(H)-CH_3$, $>C=CH_2$, $>C=CHCH_3$ (*cis* or *trans*), >C=O, >C(H)-OH, >C(H)-O-alkyl, >C(H)-O-sulfamate, or >C(H)-dioxolane, wherein alkyl is a linear, branched and/or cyclic hydrocarbon chain comprising 1 to 10 carbons. Preferred species from the foregoing genus that are useful in the present invention include, but are not limited to, the compounds shown in Table I.

TABLE I





For the compounds shown in Table I above, alkyl is defined as a linear, branched and/or cyclic (or combination thereof) hydrocarbon chain, which can be saturated or unsaturated, comprising 1 to 10 carbons.

Each of the foregoing compounds from Table I is found to have anti-mitotic properties, anti-angiogenic properties, anti-tumor properties or combinations thereof.

EXAMPLE 1

Anti-tumor and anti-angiogenic activity measured in vitro as inhibition of proliferation and measure of estrogenicity.

Cell Culture: Human umbilical vein endothelial cells (HUVEC) were obtained from Clonetics (San Diego, CA), MCF7 cells were the kind gift of Dr. Dorraya El Ashry (University of Michigan), MDA-MB-231 a human breast carcinoma, PC3 a human prostate carcinoma, and U87-MG a human glioma, cell lines were obtained from ATCC. HUVEC cultures were maintained for up to 5 passages in EGM containing bovine brain extract (Clonetics) and 1X antibiotic-antimycotic (BioWhittaker, Walkersville, MD). MDA-MB-231, PC3, U87-MG and MCF-7 cells were maintained in DMEM/F12 (1:1) containing 10% (v/v) fetal bovine serum (Hyclone Laboratories, Logan, UT) and 1X antibiotic-antimycotic. MCF7 cells were used between passage 60 and passage 100.

Proliferation Assays: Proliferation was measured by cell counting using a Coulter Z1 cell counter (Coulter Corporation, Hialeah, FL) or by evaluation of DNA synthesis. Each condition was done in triplicate and experiments were repeated at least twice. To determine estrogenicity of the analogs, MCF7 estrogen-dependent proliferation assays were performed. The cells were seeded in complete media at 10,000 cells/well in a 24 well plate after allowing the cells to

adhere overnight and the seeding density was determined by cell counts. Cells were washed with PBS (37°C) and starved by placing them in IMEM-phenol red free media containing 2% charcoal-dextran fetal bovine-stripped serum (Valley Biomedical, Winchester, VA) and 1X antibiotic-antimycotic. After 3 days of starvation, cells were treated with or without increasing concentrations of compounds, replacing the media every 2-3 days and counted after 7 days of treatment. In order to ensure that only the added test compounds stimulated growth, experiments were performed only with cell cultures that duplicated less than once during the starvation period. Values are expressed as MCF7 stimulation index (SI) relative to 2ME2 (defined as 1.00). A value less than 1.00 indicates reduced estrogenicity. Estradiol is used as a positive control.

To determine anti-tumor and anti-angiogenic activities of the analogs, proliferation assays were performed by evaluating detection of DNA synthesis by use of the 5-bromo-2'-deoxyuridine (BrdU) cell proliferation colorimetric ELISA kit from Roche (Indianapolis, IN) according to the manufacturer's instructions. For BrdU assays, the cells were seeded at 1,000 cells/well (MDA-MB-231, anti-tumor activity) or 3,000 cells/well (HUVEC, anti-angiogenic activity) in a 96 well plate, allowed to attach overnight and then exposed to the compound for 48 h. IC_{50} value is the concentration at which cell proliferation is inhibited by 50%. The results are shown in Table II below.

TABLE II

Compound number (from schemes 1-6)	MW	MDA-MB-231 (IC50 μM)	U87-MG (IC50 μM)	PC3 (IC50 μM)	HUVEC (IC50 μM)	MCF7 SI Relative to $2ME_2$
1	302.2	0.69± 0.14	1.48 ± 0.62	1.08± 0.50	0.68 ± 0.15	1.00
63	311.2	0.19 ± 0.04	0.23 ± 0.08	0.21 ± 0.10	0.13 ± 0.05	0.28
60	325.2	0.19 ± 0.08	0.25 ± 0.02	0.25 ± 0.01	0.12 ± 0.06	0.26
57	313.2	2.37 ± 0.80	10.01 ± 2.49	5.70 ± 0.58	0.96 ± 0.26	0.31
54	327.2	2.20 ± 0.43	2.23 ± 0.40	4.64 ± 0.90	5.46 ± 1.57	0.26

Pharmokinetics (PK): PO Cmax is the maximal concentration of drug achieved after oral administration in rats and AUC is the area under the curve. These parameters are an indication of oral bioavailability. PK parameters were determined using a cassette-dosing approach. A group of new chemical entities (NCEs) (usually 3-6 molecules) was dissolved in DMSO or ethanol and diluted with a solution of a dextrin polymer to the final working concentration (usually 0.2 - 2 mg/mL). Groups of three rats with in-dwelling catheters were dosed either orally (5 mg/kg) or i.v. (1 mg/kg) with the cassette of NCEs. Blood was collected at nine time points from 2 minutes to 24 hours. Plasma was separated and aliquots of the replicate samples were pooled for analysis. Plasma proteins were precipitated by addition of organic solvent (most often methanol or acetonitrile). For some analogs, an acid modifier was added to the precipitation mixture to enhance recovery. Precipitated proteins were removed by centrifugation and the supernatant was injected onto a C18 reversed phase column attached to an LC-MS-MS mass spectrometer. Analytes were partly resolved by a rapid (5 minute) gradient of organic modifier. Analytes were measured as either positive or negative ions, depending on ionization characteristics, in the SRM (or MS-MS) mode. Quantification was carried out by correlation of peak heights or areas for each analyte to a set of concentration standards of the same analytes diluted into blank plasma. Concentration vs. time data was analyzed by WinNonLin. The results are shown in Table III below.

TABLE III

Compound Number (from schemes 1-6)	MW	Rat PO Cmax (nM)	Rat PO AUC (nM)
63	311.2	127	961
60	325.2	185	1401
57	313.2	130	624
54	327.2	47	185

Determination of CYP450 inhibition and IC_{50} value using human microsomes: CYP450 inhibition provides a measure of potential issues with co-medication, since CYP450 enzymes metabolize many drugs. Microsomal incubation mixtures (500 µL) were prepared in HEPES buffer (50mM HEPES, 15mM $MgCl_2$, 0.1mM EDTA, pH 7.6) containing a NADPH regenerating system (1mM NADPH, 10mM glucose-6-phosphate 1 IU glucose-6-phosphate dehydrogenase), pooled human liver microsomes (0.5 mg protein), and 5 µL of 7 concentrations of compounds and positive control inhibitors (see Tables IV, V and VI).

TABLE IV
Microsomal incubation conditions

System	Compounds	Final concentration	Volume added (µL)
HEPES buffer	HEPES	50 mM	440
	$MgCl_2$	15 mM	
	EDTA	100 µM	
NADPH regenerating system	NADPH	1 mM	25
	glucose-6-phosphate	10 mM	
	glucose-6-phosphate dehydrogenase	1 IU	
Microsomes	pooled human liver microsomes	1 mg/mL	25
Substrate cocktail* in 50/50 (v/v) acetonitrile/water	Phenacetin	10 µM	5
	Tolbutamide	100 µM	
	Omeprazole	10 µM	
	Bufuralol	10 µM	
	Midazolam	10 µM	
Test compound or positive control inhibitor			5

* CYP1A2 activity is monitored by phenacetin O-deethylation, CYP2C9 by tolbutamide methyl-hydroxylation, CYP2C19 by omeprazole hydroxylation,

CYP2D6 by bufuralol 1'-hydroxylation, and CYP3A by midazolam hydroxylation.

TABLE V

Positive inhibitor controls and test compound concentrations.

CYP	+/ve control inhibitor	Final concentrations in incubation mixture (μM)
1A2	Furafylline	0.069 -50
2C9	Sulfaphenazole	0.0069 - 5
2C19	Tranylcypromine	0.069 - 50
2D6	Quinidine	0.0069 - 5
3A4	Troleandomycin	0.069 - 50
	Test compound	0.034 - 25

After addition of the compounds or positive control inhibitors, the samples were pre-incubated at 37°C for approximately 5 minutes. Subsequently, the P450 substrate cocktail were added to the reaction mixture and incubated for 20 minutes at 37°C. Reactions were terminated by the addition of 250 μL of methanol containing 5 μM dextrorphan as an internal standard (IS). The samples were vortexed briefly, placed on ice, and then centrifuged at approximately 12000x g for 5 minutes to remove debris. The supernatant from each sample was then transferred to a separate vial for LC-MS/MS analysis. Analysis to determine the amount of substrate specific metabolites (relative to the control samples) was carried out using reverse phase HPLC with tandem mass spectrometric detection (LC-MS/MS). Analysis of the data was carried out using Micromass MetaboLynx and QuanLynx software Versions 3.5 to determine the amount of the specific substrate metabolites produced in each sample (relative to the control samples). The results produced were plotted versus the different concentrations of the test compounds or positive inhibitor control compound, and the IC_{50} values calculated using Prism non-linear software. The results are shown in Table VI below.

TABLE VI

Compound Number (from schemes 1-6)	CYP450 inhib.	Hepatocyte Rat 60/120' (1uM)	Hepatocyte Rat 60/120' (5uM)	Hepatocyte Human 60/120' (1uM)	Hepatocyte Human 60/120' (5uM)
1	CYP1A2	0/0	39/24	0/0	20/0
63	None	77/67	78/87	52/70	92/71
60	None	99/87	87/NR	NR/91	96/93
57	None	91/85	95/83	111/98	117/118
54	None	78/NR	95/101	99/102	NR/103

NR = no reaction

Hepatocyte Incubations: An *in vitro* measure of metabolism using cells from rat and human liver. Rat and human hepatocyte suspensions (10^6 cells per mL of serum-free (H)CL15 culture medium) were incubated with 2 concentrations (1 and 5 μM) of each of the compounds. Stock solutions were prepared in DMSO, and were further diluted in culture medium to the final test concentrations. 7-Ethoxycoumarin (7-EC) were included as a positive control substrate for P450. A 0.1 M 7-EC stock solution was prepared in DMSO, and the final assay concentration was 100 μM. The reactions were initiated by the addition of the substrates, incubated at 37°C, and terminated after 0, 60 and 120 minutes by addition of acetonitrile. The positive 7-EC control reactions were stopped with perchloric acid or $ZnSO_4$ and BaOH after 60-minutes of incubation. The reaction mixture was centrifuged to remove the cell debris, and the supernatant was used for subsequent analysis by HPLC/LC-MS. If necessary, the supernatants were flash frozen in liquid N_2 and stored at – 70°C until further analysis. HPLC was used to analyze product formation from 7-EC. Analyses to determine the concentration of the test compounds were carried out using reverse phase HPLC with tandem mass spectrometric detection (LC-MS/MS). Samples

were analyzed to measure the disappearance of parent compound over time. The rate of metabolism was estimated by fitting a curve to the data for each concentration against time using GraphPad Prism Version 3.02 software. The results are shown in Table VI above.

EXAMPLE 2

Summary of *in vitro* antiproliferative activity and rat pharmokinetic data with ENMD-1565 (17-dioxolane) and ENMD-1756 (17-methoxy).

Antiproliferative activity of the NCEs were assessed in tumor cell lines (MDA-MB-231 a breast carcinoma, PC3 a prostate carcinoma and U87-MG a glioma) and endothelial cells (HUVEC).

TABLE VII

IC50 value (μM)

Compound	MDA-MB-231	PC3	U87-MG	HUVEC
ENMD-1565 $C_{22}H_{29}NO_4$ pKa : 16.04 - 0.56 26,26 MW : 371.47	1.21±0.47	1.91±0.36	1.27±0.44	0.91±0.26
ENMD-1756 $C_{21}H_{29}NO_3$ pKa : 16.04 - 0.56 25,25 MW : 343.46	0.89±0.21	1.14±0.45	0.71±0.09	0.62±0.09

Pharmokinetics of the NCEs were assessed by LC/MS after oral delivery of 10 mg/kg to female Sprague Dawley rats using a cyclodextrin formulation.

TABLE VIII

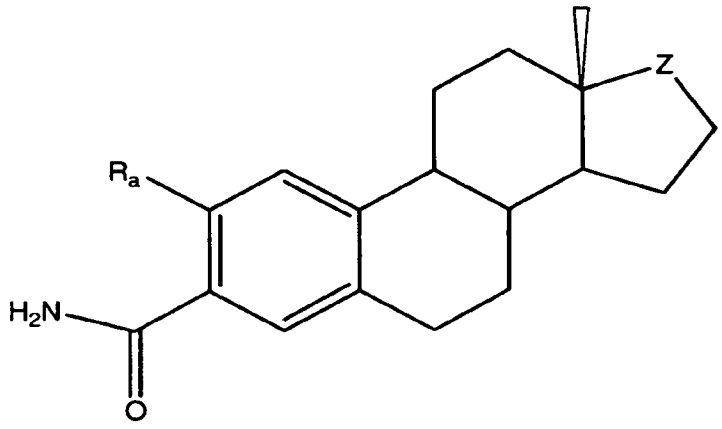
Compound	T ½ (h)	Tmax (h)	Cmax (ng/ml)	AUCall (h*ng/ml)
ENMD-1565 $C_{22}H_{29}NO_4$ pKa : 16.04 -0.56 26,26 MW : 371.47	1.63	1	1379	4657
ENMD-1756 $C_{21}H_{29}NO_3$ pKa : 16.04 -0.56 25,25 MW : 343.46	0.89	0.5	665	1442

All of the publications mentioned herein are hereby incorporated by reference in their entireties. The above examples are merely demonstrative of the present invention, and are not intended to limit the scope of the invention or the appended claims.

CLAIMS

We claim:

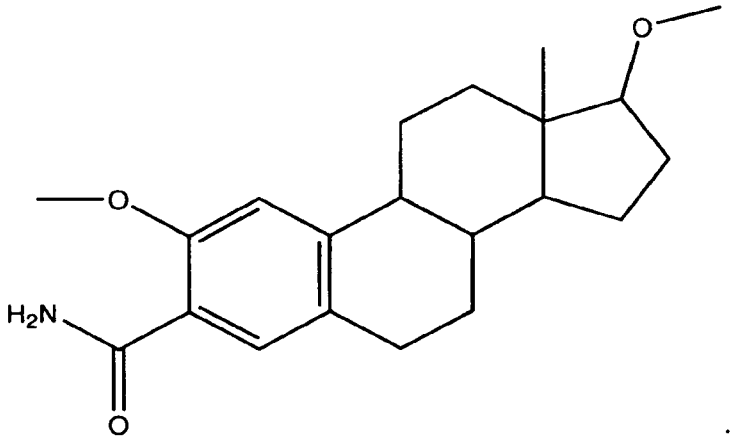
1. A compound of the formula:



,

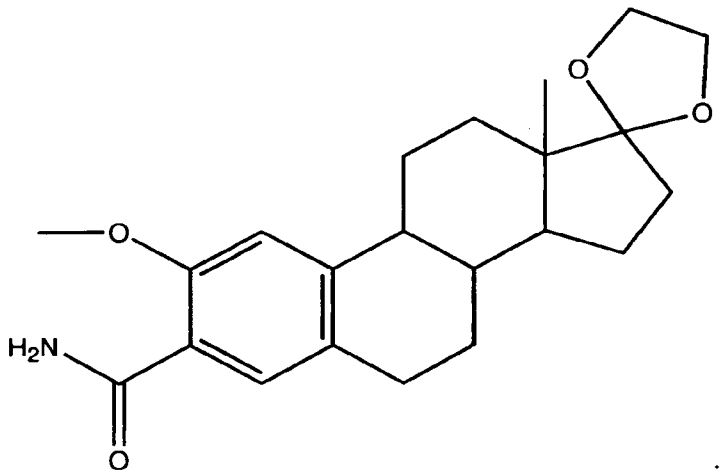
wherein R_a is selected from $-OCH_3$, $-OCH_2CH_3$ or $-CCCH_3$; and Z is selected from >C(H)-OH, >C(H)-O-alkyl, >C(H)-O-sulfamate, or >C(H)-dioxolane, where alkyl is a linear, branched and/or cyclic hydrocarbon chain comprising 1 to 10 carbons.

2. The compound of Claim 1, wherein the compound is:



.

3. The compound of Claim 1, wherein the compound is:

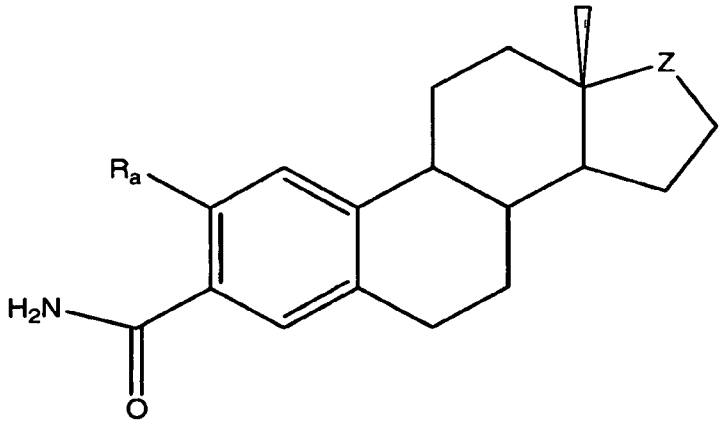


4. The compound of Claim 1, wherein the average particle size is less than about 1900 nm.

5. The compound of Claim 1, wherein the average particle size is less than about 1500 nm.

6. The compound of Claim 1, wherein the average particle size is less than about 500 nm.

7. A method of inhibiting angiogenesis in a human or animal comprising administering to the human or animal an effective amount of a compound having the formula



,

wherein R_a is selected from $-OCH_3$, $-OCH_2CH_3$ or $-CCCH_3$; and Z is selected from >C(H)-OH, >C(H)-O-alkyl, >C(H)-O-sulfamate, or >C(H)-dioxolane, where alkyl is a linear, branched and/or cyclic hydrocarbon chain comprising 1 to 10 carbons.

8. The method of Claim 7, wherein the administration of the compound is in a daily dose, a daily sub-dose, or any appropriate fraction thereof to the human or animal.

9. The method of Claim 7, wherein the amount of the compound administered is approximately 0.1 to approximately 300 mg/kg/day.

10. The method of Claim 7, wherein the amount of the compound administered is approximately 0.5 to approximately 50 mg/kg/day.

11. The method of Claim 7, wherein the amount of the compound administered is approximately 1 to approximately 10 mg/kg/day.

12. The method of Claim 7, wherein the administration of the compound is oral, parenteral, transdermal, topical, intravenous, subcutaneous, intramuscular, intradermal, ophthalmic, epidural, intratracheal, sublingual, buccal, rectal, vaginal, nasal or inhalation.

13. The method of Claim 7, wherein the compound is administered in a composition comprising an additive selected from an anti-oxidant, a buffer, a bacteriostat, a liquid carrier, a solute, a suspending agent, a thickening agent, a flavoring agent, a gelatin, glycerin, a binder, a lubricant, an inert diluent, a preservative, a surface active agent, a dispersing agent, a biodegradable polymer, or any combination thereof.

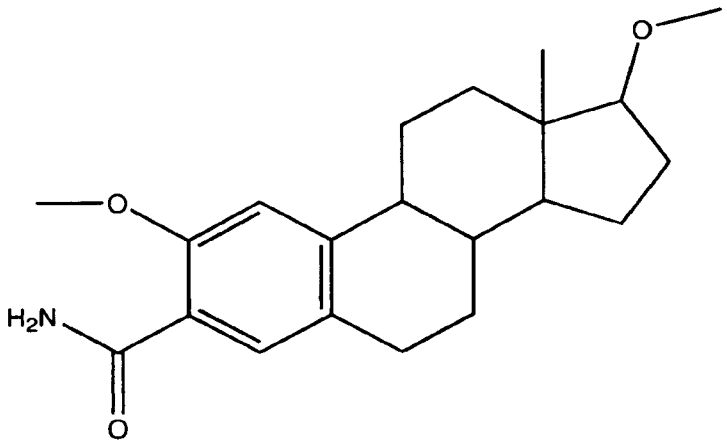
14. The method of Claim 7, wherein the compound is administered in the form of a tablet, a capsule, a lozenge, a cachet, a solution, a suspension, an emulsion, a powder, an aerosol, a suppository, a spray, a pastille, an ointment, a cream, a paste, a foam, a gel, a tampon, a pessary, a granule, a bolus, a mouthwash, or a transdermal patch.

15. The method of Claim 7, wherein the angiogenesis is associated with diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, neovascular glaucoma, retrolental fibroplasias, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, Sjögren's syndrome, acne rosacea, phylectenulosis, syphilis, *Mycobacteria* infections, lipid degeneration, chemical burns, bacterial ulcers, fungal ulcers, *Herpes simplex* infections, *Herpes zoster* infections, protozoan infections, Kaposi's sarcoma, Mooren's ulcer, Terrien's marginal degeneration, marginal keratolysis, trauma, arthritis, rheumatoid arthritis, polyarteritis, systemic lupus, Wegener's sarcoidosis, scleritis, Stevens-Johnson

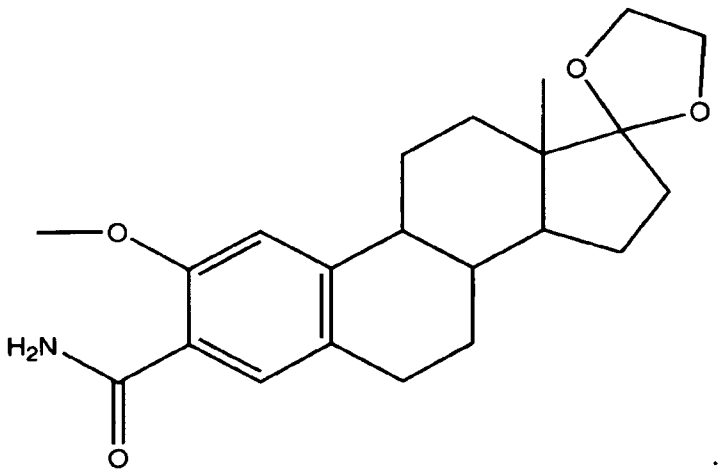
disease, radial keratotomy, macular degeneration, sickle cell anemia, sarcoid, pseudoxanthoma elasticum, Paget's disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis, chronic vitritis, Lyme's disease, Eales' disease, Behcet's disease, myopia, optic pits, Stargardt's disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, post-laser complications, abnormal proliferation of fibrovascular or fibrous tissue, hemangiomas, Osler-Weber-Rendu disease, solid tumors, blood-borne tumors, acquired immune deficiency syndrome, ocular neovascular disease, age-related macular degeneration, osteoarthritis, diseases caused by chronic inflammation, Crohn's disease, ulcerative colitis, tumors of rhabdomyosarcoma, tumors of retinoblastoma, Ewing's sarcoma, neuroblastoma, tumors of osteosarcoma, leukemia, psoriasis, atherosclerosis, pemphigoid, infections causing retinitis, infections causing choroiditis, presumed ocular histoplasmosis, Best's disease, proliferative vitreoretinopathy, Bartonellosis, acoustic neuromas, neurofibroma, trachoma, pyogenic granulomas, vascular malfunctions, abnormal wound healing, gout or gouty arthritis, angiogenesis-dependent cancer, hereditary hemorrhagic telangiectasia, post-menopausal symptoms, osteoporosis, cardiovascular disease, myocardial angiogenesis, plaque neovascularization, hemophiliac joints, angiofibroma, wound granulation, intestinal adhesions, scleroderma, keloids, endometriosis.

16. The method of Claim 7, wherein the angiogenesis is associated with angiogenesis-dependent cancers selected from breast cancer, prostrate cancer, renal cell cancer, brain cancer, ovarian cancer, colon cancer, bladder cancer, pancreatic cancer, stomach cancer, esophageal cancer, cutaneous melanoma, liver cancer, lung cancer, testicular cancer, kidney cancer, bladder cancer, cervical cancer, lymphoma, parathyroid cancer, penile cancer, rectal cancer, small intestine cancer, thyroid cancer, uterine cancer, Hodgkin's lymphoma, lip and oral cancer, skin cancer, leukemia or multiple myeloma.

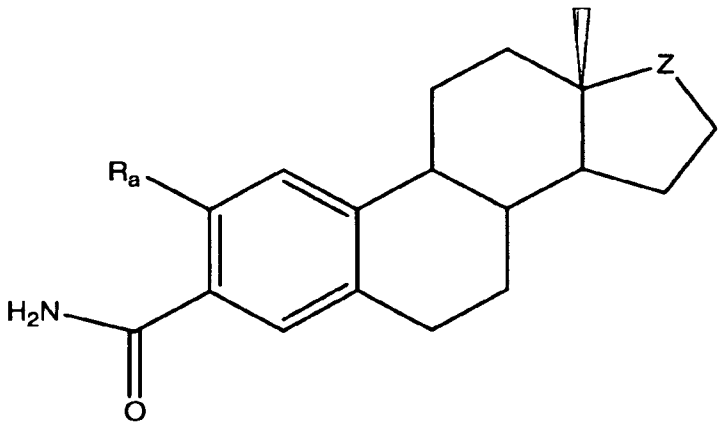
17. The method of Claim 7, wherein the compound is



18. The method of Claim 7, wherein the compound is



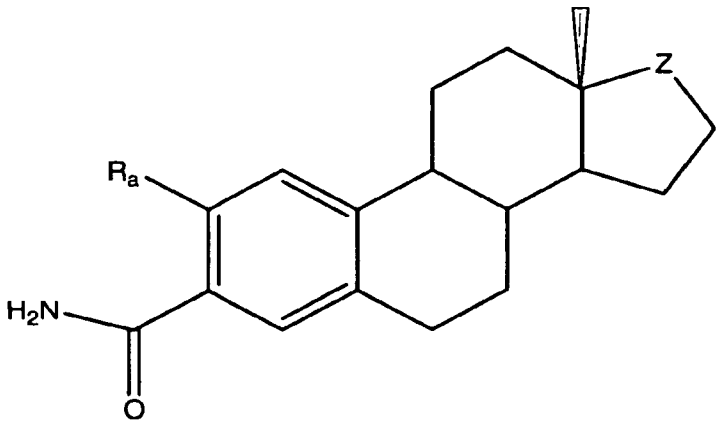
19. A method of treating angiogenesis-dependent cancer in a human or animal comprising administering to the human or animal having the angiogenesis-dependent cancer an effective angiogenesis-dependent cancer treating amount of a compound having the formula



wherein R_a is selected from $-OCH_3$, $-OCH_2CH_3$ or $-CCCH_3$; and Z is selected from >C(H)-OH, >C(H)-O-alkyl, >C(H)-O-sulfamate, or >C(H)-dioxolane, where alkyl is a linear, branched and/or cyclic hydrocarbon chain comprising 1 to 10 carbons.

20. The method of Claim 19, wherein said angiogenesis-dependent cancer is breast cancer, prostrate cancer, renal cell cancer, brain cancer, ovarian cancer, colon cancer, bladder cancer, pancreatic cancer, stomach cancer, esophageal cancer, cutaneous melanoma, liver cancer, lung cancer, testicular cancer, kidney cancer, bladder cancer, cervical cancer, lymphoma, parathyroid cancer, penile cancer, rectal cancer, small intestine cancer, thyroid cancer, uterine cancer, Hodgkin's lymphoma, lip cancer, oral cancer, skin cancer, leukemia or multiple myeloma.

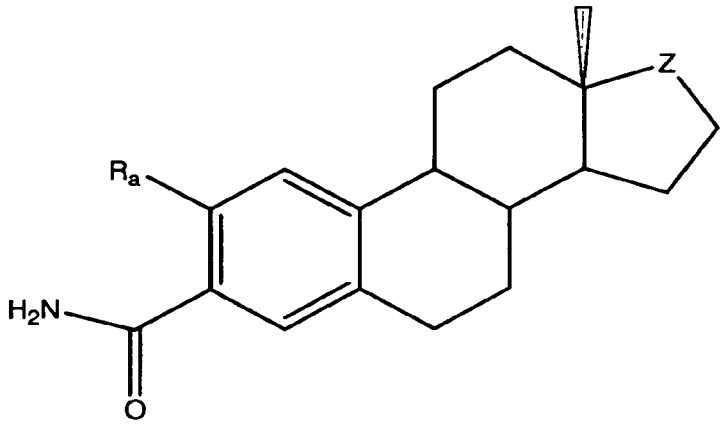
21. A method of treating an eye condition in a human or an animal comprising administering to the human or animal an effective eye treating amount of a compound having the formula



wherein R_a is selected from $-OCH_3$, $-OCH_2CH_3$ or $-CCCH_3$; and Z is selected from >C(H)-OH, >C(H)-O-alkyl, >C(H)-O-sulfamate, or >C(H)-dioxolane, where alkyl is a linear, branched and/or cyclic hydrocarbon chain comprising 1 to 10 carbons.

22. The method of Claim 21, wherein the eye condition is ocular neovascular disease, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasias, epidemic keratoconjunctivitis, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, myopia, chronic retinal detachment, optic pits, Terrien's marginal degeneration, hyperviscosity syndromes, chronic uveitis, chronic vitritis, presumed ocular histoplasmosis, retinitis, choroiditis, proliferative vitreoretinopathy, scleritis, Eales' disease, Best's disease, trachoma, or due to post-laser complications.

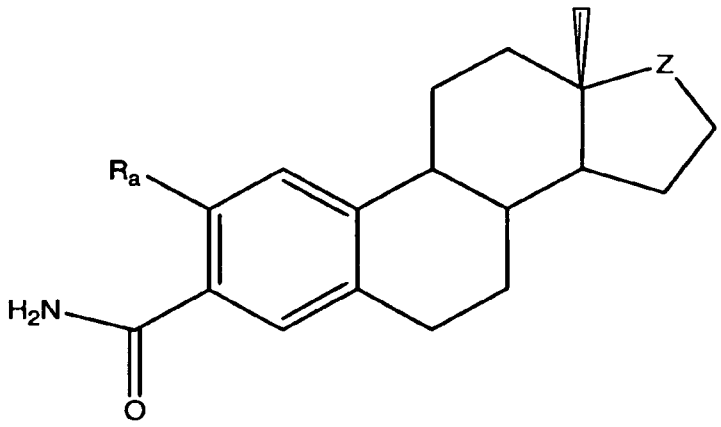
23. A method of treating inflammatory or immune mediated diseases in a human or an animal comprising administering to the human or animal an effective inflammatory or immune mediated disease treating amount of a compound having the formula



wherein R_a is selected from $-OCH_3$, $-OCH_2CH_3$ or $-CCCH_3$; and Z is selected from >C(H)-OH, >C(H)-O-alkyl, >C(H)-O-sulfamate, or >C(H)-dioxolane, where alkyl is a linear, branched and/or cyclic hydrocarbon chain comprising 1 to 10 carbons.

24. The method of Claim 23, wherein the inflammatory or immune mediated disease is rheumatoid arthritis, osteoarthritis, ulcerative colitis, Crohn's disease, Mooren's ulcer, arthritis, sarcoidosis, inflammatory or immune mediated bowel disease, systemic lupus, Wegener's syndrome, Stevens-Johnson disease, Behcet's disease, pemphigoid, Lyme's disease, asthma or acquired immune deficiency syndrome.

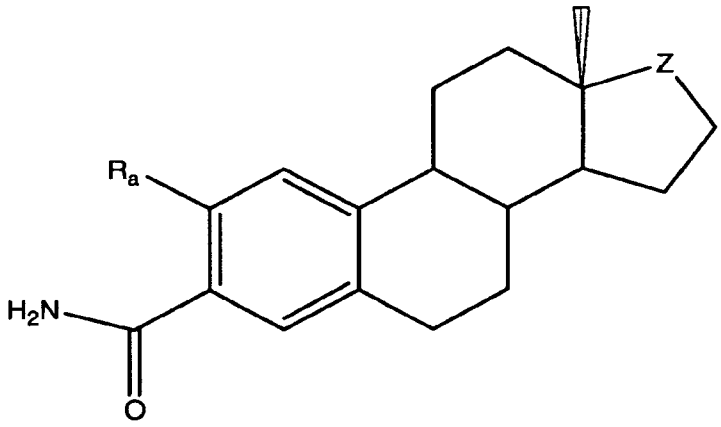
25. A method of treating an infectious disease in a human or an animal comprising administering to the human or animal an effective infectious disease treating amount of a compound having the formula



wherein R_a is selected from $-OCH_3$, $-OCH_2CH_3$ or $-CCCH_3$; and Z is selected from >C(H)-OH, >C(H)-O-alkyl, >C(H)-O-sulfamate, or >C(H)-dioxolane, where alkyl is a linear, branched and/or cyclic hydrocarbon chain comprising 1 to 10 carbons.

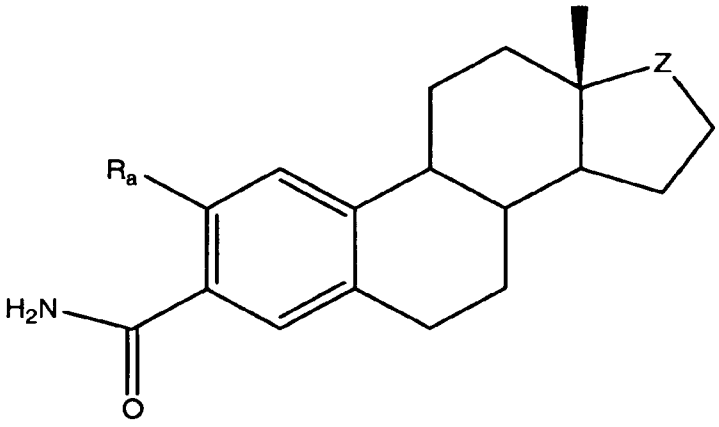
26. The method of Claim 25, wherein the infectious disease is syphilis, a bacterial infection, a *Mycobacterial* infection, a bacterial ulcer, a fungal ulcer, a *Herpes simplex* infection, a *Herpes zoster* infection, a protozoan infection, a Bartonellosis infection, or toxoplasmosis.

27. A method of treating a cancerous disease in a human or an animal comprising administering to the human or animal an effective cancerous disease treating–amount of a compound having the formula



wherein R_a wherein R_a is selected from $-OCH_3$, $-OCH_2CH_3$ or $-CCCH_3$; and Z is selected from >C(H)-OH, >C(H)-O-alkyl, >C(H)-O-sulfamate, or >C(H)-dioxolane, where alkyl is a linear, branched and/or cyclic hydrocarbon chain comprising 1 to 10 carbons, and wherein said cancerous disease is rhabdomyosarcoma, retinoblastoma, Ewing sarcoma, neuroblastoma, osteosarcoma, acoustic neuroma, neurofibromas, or hemangioma.

28. A method of treating a blood or blood vessel disease or condition in a human or an animal comprising administering to the human or animal an effective blood or blood vessel disease treating amount of a compound having the formula



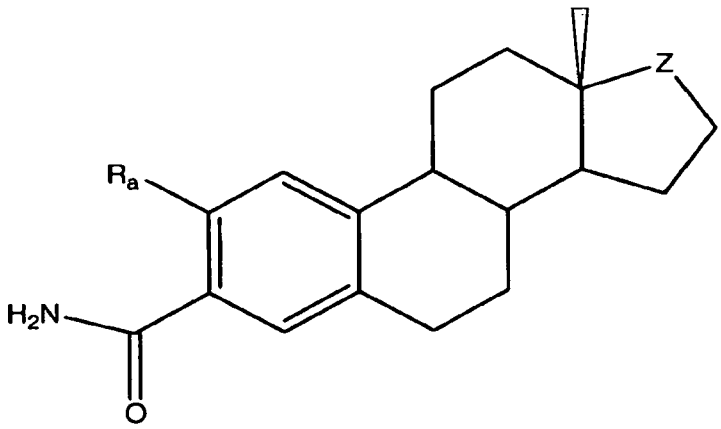
wherein R_a is selected from $-OCH_3$, $-OCH_2CH_3$ or $-CCCH_3$; and Z is selected from >C(H)-OH, >C(H)-O-alkyl, >C(H)-O-sulfamate, or >C(H)-dioxolane, where alkyl is a linear, branched and/or cyclic hydrocarbon chain comprising 1 to 10 carbons.

29. The method of Claim 28, wherein the blood or blood vessel disease or condition is vein occlusion, artery occlusion, carotid obstructive disease, polyarteritis, atherosclerosis, Osler-Weber-Rendu disease, sickle cell anemia, leukemia, acute or chronic neoplastic disease of the bone marrow, hemangiomas, hereditary hemorrhagic telangiectasia, disease of the bone marrow, anemia, restenosis, impaired blood clotting or enlargement of the lymph nodes, liver, or spleen.

30. The method of Claim 29, wherein the acute or chronic neoplastic disease of the bone marrow is multiple myeloma.

31. The method of Claim 29, wherein the acute or chronic neoplastic disease of the bone marrow is myelo dysplastic syndrome.

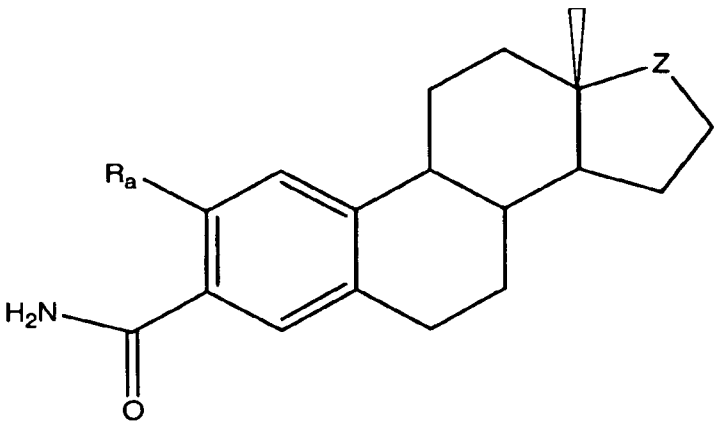
32. A method of treating a skin condition in a human or an animal comprising administering to the human or animal an effective skin condition treating amount of a compound having the formula



wherein R_a is selected from $-OCH_3$, $-OCH_2CH_3$ or $-CCCH_3$; and Z is selected from >C(H)-OH, >C(H)-O-alkyl, >C(H)-O-sulfamate, or >C(H)-dioxolane, where alkyl is a linear, branched and/or cyclic hydrocarbon chain comprising 1 to 10 carbons.

33. The method of Claim 32, wherein the skin condition is abnormal wound healing, acne rosacea, chemical burns of the skin, or psoriasis.

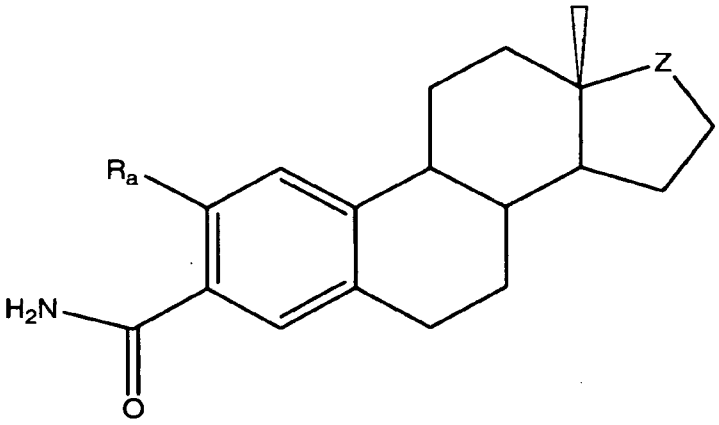
34. A method of treating an angiogenesis dependent tumor in a human or an animal comprising administering to the human or animal an effective angiogenesis dependent tumor treating amount of a compound having the formula



wherein R_a is selected from $-OCH_3$, $-OCH_2CH_3$ or $-CCCH_3$; and Z is selected from >C(H)-OH, >C(H)-O-alkyl, >C(H)-O-sulfamate, or >C(H)-dioxolane, where alkyl is a linear, branched and/or cyclic hydrocarbon chain comprising 1 to 10 carbons.

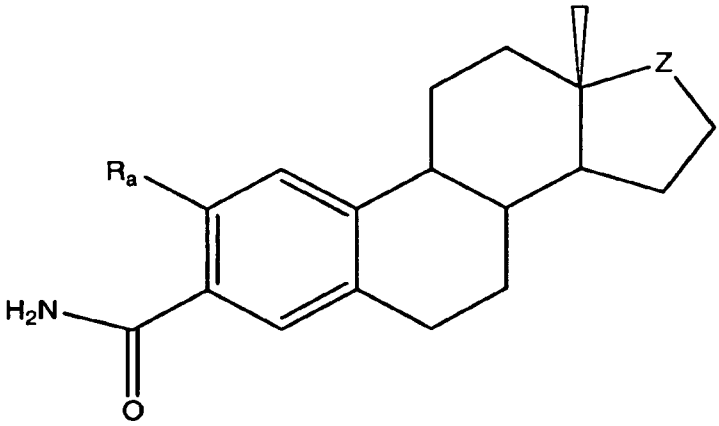
35. The method of Claim 34, wherein the tumor is a blood-borne tumor, a solid tumor, a benign tumor, or a cancerous tumor.

36. A method of treating endometriosis in a human or an animal comprising administering to the human or animal an effective endometriosis treating amount of a compound having the formula



wherein R_a is selected from $-OCH_3$, $-OCH_2CH_3$ or $-CCCH_3$; and Z is selected from >C(H)-OH, >C(H)-O-alkyl, >C(H)-O-sulfamate, or >C(H)-dioxolane, where alkyl is a linear, branched and/or cyclic hydrocarbon chain comprising 1 to 10 carbons.

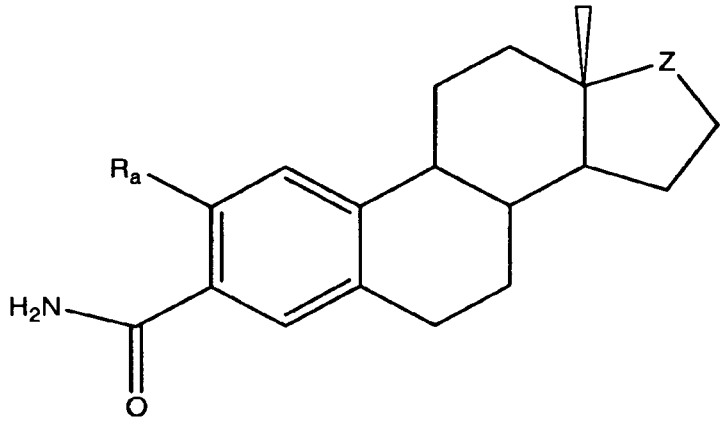
37. A method comprising administering a compound to a female human or animal, wherein said compound has the formula



wherein R_a is selected from $-OCH_3$, $-OCH_2CH_3$ or $-CCCH_3$; and Z is selected from >C(H)-OH, >C(H)-O-alkyl, >C(H)-O-sulfamate, or >C(H)-dioxolane, where alkyl is a linear, branched and/or cyclic hydrocarbon chain comprising 1 to 10 carbons, and wherein said compound is administered in an amount effective to block ovulation, block implantation of a blastula or block menstruation (induce amenorrhea).

38. A pharmaceutical preparation comprising:

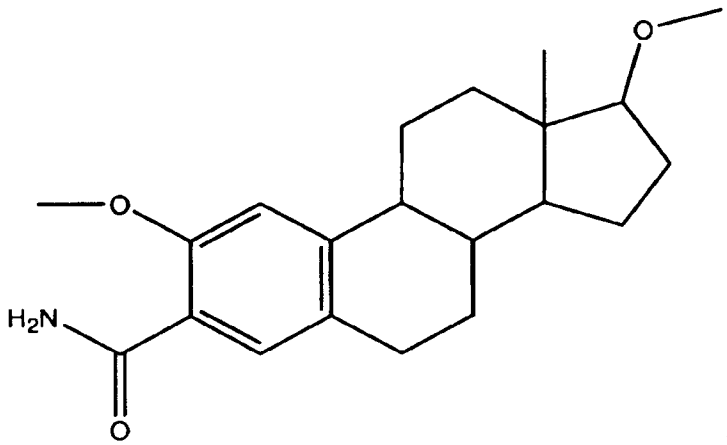
(a)



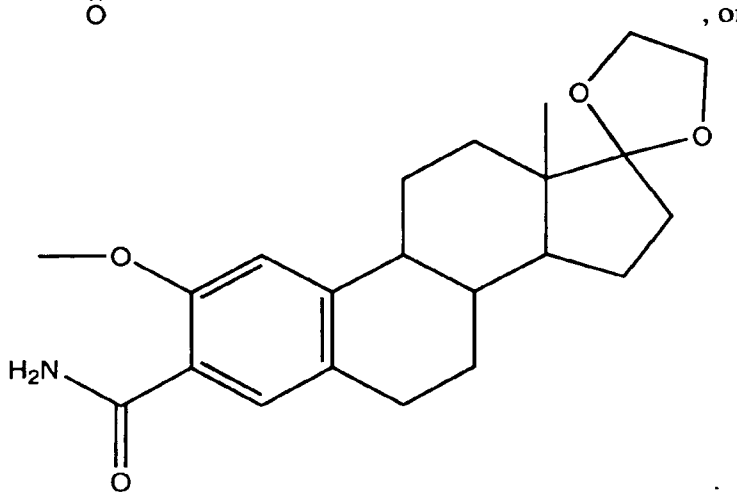
wherein R_a is selected from $-OCH_3$, $-OCH_2CH_3$ or $-CCCH_3$; and Z is selected from >C(H)-OH, >C(H)-O-alkyl, >C(H)-O-sulfamate, or >C(H)-dioxolane, where alkyl is a linear, branched and/or cyclic hydrocarbon chain comprising 1 to 10 carbons; and

(b) a pharmaceutically acceptable carrier, excipient or diluent.

39. The pharmaceutical preparation of Claim 38, wherein said compound is



, or



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