METHODS OF INHIBITING PRIMARY ENDOMETRIAL HYPERPLASIA

The uterine lining (endometrium) is composed of tissue, blood vessels, and glands that grow when stimulated by the hormone estrogen. In women with normal menstrual cycles, hormonal fluctuations trigger the growth and shedding of the endometrium each month. If conception occurs, the endometrium nourishes the developing embryo.

With continuously elevated estrogen levels, the endometrium remains in its growth phase at all time, leading to an overabundance of endometrial tissue or endometrial hyperplasia. Overgrowth of the endometrium is often a benign condition, but it can also be a precursor of endometrial cancer. Because of this risk, doctors urge women avoid long-term estrogen therapy, which can cause endometrial overgrowth, and to seek prompt treatment for conditions that cause excessive estrogen production.

Continuous estrogen stimulation of the endometrium without the balancing effects of the hormone progesterone leads to endometrial overgrowth and potentially endometrial cancer. This problem can occur if one begins estrogen replacement therapy after menopause without using progesterone as well; if one is markedly overweight, since excess body fat leads to increased estrogen production; or if one has a condition such as polycystic ovary disease that prevents ones body from maintaining the proper hormonal balance.

Most cases of endometrial carcinoma are associated with a precursor lesion termed "endometrial hyperplasia." The classification of endometrial hyperplasia is based on the presence or absence of cytologic atypia, the presence of dysplasia, and the degree of complexity of the architectural pattern. Cytologic atypia is the most predictive criterion for the likelihood of progression to carcinoma.

In simple or cystic hyperplasia with cytologic atypia present there is about an 8% chance of progression to cancer. With complex or adenomatous hyperplasia with cytologic atypia present, there is 29% chance. When no cytologic atypia is present, the progression rate is 1% for simple and 3% for complex hyperplasia.

Gynecologists diagnose the disorder by examining endometrial tissue taken either as a biopsy or in a procedure called a D&C (dilation and curettage), which involves dilating the cervix and using a sharp, curved instrument to scrape out the entire endometrium. Neither of these procedures is performed routinely. Instead, doctors recommend them for people who have symptoms of endometrial overgrowth or who are at risk of developing it, including post-menopausal women on estrogen therapy and women who are obese or who have fertility problems.

Doctors base their treatment decisions or several factors. First, they examine the cells obtained in the biopsy or D&C. If the cells are normal but simply over abundant, future development of cancer is less likely than if the cells are atypical, displaying enlarged nuclei and other unusual features. In some cases, a D&C will show that cancer has already developed.

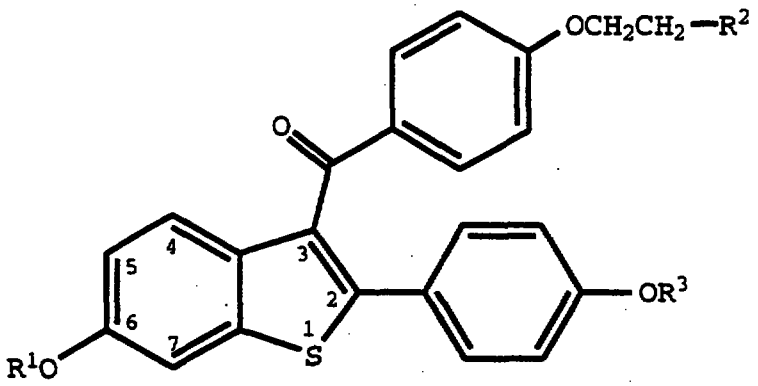
The woman's age and desire to have children are also factors to consider. Some doctors advise post-menopausal women with endometrial overgrowth to undergo hysterectomies (surgery to remove the uterus) even if the endometrial cells are not atypical. Others use more conservative treatments, including a D&C (which can be a therapeutic as well as a diagnostic tool) or hormonal therapy. Doctors rarely recommend hysterectomies for younger women unless cancer is actually present or the endometrial cells are extremely atypical.

Women whose endometrial overgrowth is a result of estrogen therapy should add progesterone to their hormone regimens for the last 12 days of each month. After

6 months of this treatment, the doctor will take another endometrial sample. If hyperplasia is still present, the woman can either stop the estrogen or take both estrogen and progesterone throughout the month. If the overgrowth persists, a hysterectomy may be necessary.

The problems with the current therapies, however, indicate a need for a novel method of inhibiting primary endometrial hyperplasia, and its progression to endometrial cancer.

This invention provides methods of inhibiting primary endometrial hyperplasia comprising administering to a human in need thereof an effective amount of a compound of formula I



(I)

wherein R^1 and R^3 are independently hydrogen, $-CH_3$, $-C-(C_1-C_6\ alkyl)$, or $-C-Ar$, wherein Ar is optionally substituted phenyl;

R^2 is selected from the group consisting of pyrrolidino, hexamethyleneimino, and piperidino; and pharmaceutically acceptable salts and solvates thereof.

The current invention concerns the discovery that a select group of 2-phenyl-3-aroylbenzothiophenes (benzothiophenes), those of formula I, are useful for inhibiting primary endometrial hyperplasia.

The therapeutic and prophylactic treatments provided by this invention are practiced by administering to a human in need thereof a dose of a compound of formula I or a pharmaceutically acceptable salt or solvate thereof, that is effective to inhibit primary endometrial hyperplasia or its symptoms.

The term "inhibit" includes its generally accepted meaning which includes prohibiting, preventing, restraining, and slowing, stopping or reversing progression, severity or a resultant symptom. As such, the present method includes both medical therapeutic and/or prophylactic administration, as appropriate.

Raloxifene is a preferred compound of this invention and it is the hydrochloride salt of a compound of formula 1 wherein R^1 and R^3 are hydrogen and R^2 is 1-piperidinyl.

Generally, at least one compound of formula I is formulated with common excipients, diluents or carriers, and compressed into tablets, or formulated as elixirs or solutions for convenient oral administration, or administered by the intramuscular or intravenous routes. The compounds can be administered transdermally, and may be formulated as sustained release dosage forms and the like.

The compounds used in the methods of the current invention can be made according to established procedures, such as those detailed in U.S. Patent Nos. 4,133,814, 4,418,068, and 4,380,635 all of which are incorporated by reference herein. In general, the process starts with a benzo[b]thiophene having a 6-hydroxyl group and a 2-(4-hydroxyphenyl) group. The starting compound is protected, acylated, and deprotected to form the formula I compounds. Examples of the preparation of such compounds are provided

in the U.S. patents discussed above. The term "optionally substituted phenyl" includes phenyl and phenyl substituted once or twice with C_1-C_6 alkyl, C_1-C_4 alkoxy, hydroxy, nitro, chloro, fluoro, or tri(chloro or fluoro)methyl.

The compounds used in the methods of this invention form pharmaceutically acceptable acid and base addition salts with a wide variety of organic and inorganic acids and bases and include the physiologically acceptable salts which are often used in pharmaceutical chemistry. Such salts are also part of this invention. Typical inorganic acids used to form such salts include hydrochloric, hydrobromic, hydroiodic, nitric, sulfuric, phosphoric, hypophosphoric and the like. Salts derived from organic acids, such as aliphatic mono and dicarboxylic acids, phenyl substituted alkanoic acids, hydroxyalkanoic and hydroxyalkandioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, may also be used. Such pharmaceutically acceptable salts thus include acetate, phenylacetate, trifluoroacetate, acrylate, ascorbate, benzoate, chlorobenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, methylbenzoate, o-acetoxybenzoate, naphthalene-2-benzoate, bromide, isobutyrate, phenylbutyrate, ß-hydroxybutyrate, butyne-1,4-dioate, hexyne-1,4-dioate, caprate, caprylate, chloride, cinnamate, citrate, formate, fumarate, glycollate, heptanoate, hippurate, lactate, malate, maleate, hydroxymaleate, malonate, mandelate, mesylate, nicotinate, isonicotinate, nitrate, oxalate, phthalate, teraphthalate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, propiolate, propionate, phenylpropionate, salicylate, sebacate, succinate, suberate, sulfate, bisulfate, pyrosulfate, sulfite, bisulfite, sulfonate, benzene-sulfonate, p-bromophenylsulfonate, chlorobenzenesulfonate, ethanesulfonate, 2-hydroxyethanesulfonate, methanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, p-toluenesulfonate,

xylenesulfonate, tartarate, and the like. A preferred salt is the hydrochloride salt.

The pharmaceutically acceptable acid addition salts are typically formed by reacting a compound of formula I with an equimolar or excess amount of acid. The reactants are generally combined in a mutual solvent such as diethyl ether or benzene. The salt normally precipitates out of solution within about one hour to 10 days and can be isolated by filtration or the solvent can be stripped off by conventional means.

Bases commonly used for formation of salts include ammonium hydroxide and alkali and alkaline earth metal hydroxides, carbonates, as well as aliphatic and primary, secondary and tertiary amines, aliphatic diamines. Bases especially useful in the preparation of addition salts include ammonium hydroxide, potassium carbonate, methylamine, diethylamine, ethylene diamine and cyclohexylamine.

The pharmaceutically acceptable salts generally have enhanced solubility characteristics compared to the compound from which they are derived, and thus are often more amenable to formulation as liquids or emulsions.

Pharmaceutical formulations can be prepared by procedures known in the art. For example, the compounds can be formulated with common excipients, diluents, or carriers, and formed into tablets, capsules, suspensions, powders, and the like. Examples of excipients, diluents, and carriers that are suitable for such formulations include the following: fillers and extenders such as starch, sugars, mannitol, and silicic derivatives; binding agents such as carboxymethyl cellulose and other cellulose derivatives; alginates, gelatin, and polyvinyl pyrrolidone; moisturizing agents such as glycerol; disintegrating agents such as calcium carbonate and sodium bicarbonate; agents for retarding dissolution such as paraffin; resorption accelerators such as quaternary ammonium compounds; surface

active agents such as cetyl alcohol, glycerol monostearate; adsorptive carriers such as kaolin and bentonite; and lubricants such as talc, calcium and magnesium stearate, and solid polyethyl glycols.

The compounds can also be formulated as elixirs or solutions for convenient oral administration or as solutions appropriate for parenteral administration, for instance by intramuscular, subcutaneous or intravenous routes. Additionally, the compounds are well suited to formulation as sustained release dosage forms and the like. The formulations can be so constituted that they release the active ingredient only or preferably in a particular part of the intestinal tract, possibly over a period of time. The coatings, envelopes, and protective matrices may be made, for example, from polymeric substances or waxes.

The particular dosage of a compound of formula I required to inhibit primary endometrial hyperplasia or its symptoms, according to this invention, will depend upon the severity of the condition, the route of administration, and related factors that will be decided by the attending physician. Generally, accepted and effective daily doses will be from about 0.1 to about 1000 mg/day, and more typically from about 50 to about 200 mg/day. Such dosages will be administered to a subject in need thereof from once to about three times each day, or more often as needed, and for a time to effectively treat or prevent the disease or its symptoms.

It is usually preferred to administer a compound of formula I in the form of an acid addition salt, as is customary in the administration of pharmaceuticals bearing a basic group, such as the piperidino ring. For such purposes the following oral dosage forms are available.

Formulations

In the formulations which follow, "Active ingredient" means a compound of formula I.

Formulation 1: Gelatin Capsules

Hard gelatin capsules are prepared using the following:

Ingredient	Quantity (mg/capsule)
Active ingredient	0.1 - 1000
Starch, NF	0 - 650
Starch flowable powder	0 - 650
Silicone fluid 350 centistokes	0 - 15

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

Examples of specific capsule formulations of raloxifene that have been made include those shown below:

Formulation 2: Raloxifene capsule

Ingredient	Quantity (mg/capsule)
Raloxifene	1
Starch, NF	112
Starch flowable powder	225.3
Silicone fluid 350 centistokes	1.7

Formulation 3: Raloxifene capsule

Ingredient	Quantity (mg/capsule)
Raloxifene	5
Starch, NF	108
Starch flowable powder	225.3
Silicone fluid 350 centistokes	1.7

Formulation 4: Raloxifene capsule

Ingredient	Quantity (mg/capsule)
Raloxifene	10
Starch, NF	103
Starch flowable powder	225.3
Silicone fluid 350 centistokes	1.7

Formulation 5: Raloxifene capsule

Ingredient	Quantity (mg/capsule)
Raloxifene	50
Starch, NF	150
Starch flowable powder	397
Silicone fluid 350 centistokes	3.0

The specific formulations above may be changed in compliance with the reasonable variations provided.

A tablet formulation is prepared using the ingredients below:

Formulation 6: Tablets

Ingredient	Quantity (mg/tablet)
Active ingredient	0.1 - 1000
Cellulose, microcrystalline	0 - 650
Silicon dioxide, fumed	0 - 650
Stearate acid	0 - 15

The components are blended and compressed to form tablets.

Alternatively, tablets each containing 0.1 - 1000 mg of Active ingredient are made up as follows:

Formulation 7: Tablets

Ingredient	Quantity (mg/tablet)
Active ingredient	0.1 - 1000
Starch	45
Cellulose, microcrystalline	35
Polyvinylpyrrolidone (as 10% solution in water)	4
Sodium carboxymethyl cellulose	4.5
Magnesium stearate	0.5
Talc	1

The Active ingredient, starch, and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders which are then passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50°-60° C and passed through a No. 18 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 60 U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets.

Suspensions each containing 0.1 - 1000 mg of Active ingredient per 5 mL dose are made as follows:

Formulation 8: Suspensions

Ingredient	Quantity (mg/5 ml)
Active ingredient	0.1 - 1000 mg
Sodium carboxymethyl cellulose	50 mg
Syrup	1.25 mg
Benzoic acid solution	0.10 mL
Flavor	q.v.
Color	q.v.
Purified water to	5 mL

The Active ingredient is passed through a No. 45 mesh U.S. sieve and mixed with the sodium carboxymethyl cellulose and syrup to form a smooth paste. The benzoic acid solution, flavor, and color are diluted with some of the water and added, with stirring. Sufficient water is then added to produce the required volume.

ASSAYS

ASSAY 1

I. Primary Culture

Tissue from human or animal endometrial lesions are harvested and maintained in vitro as primary cultures. Surgical specimens are pushed through a sterile mesh or sieve or alternately teased or digested apart from surrounding tissue to produce a single cell suspension. Alternately cultures are propagated from tissue explants. Cells are maintained in growth media containing serum and antibiotics.

Rates of growth in the presence and absence of estrogen is determined. In vitro cultures are assessed for their proliferative response following treatment with estrogen, progestins, and compounds of Formula I. Levels of steroid hormone receptors are assessed to determine whether important cell characteristics are maintained in vitro. Tissue from 1-10 patients are utilized. In absence of a proliferative response, estrogen and anti-estrogen effects are assessed by monitoring transcriptional activation of estrogen regulated genes.

II. Continuous Cultures

Continuous cultures of transformed endometrial cells reflective of endometrial hyperplasia and/or carcinoma are maintained in culture. Estrogen receptor containing cells (such as the Ishikawa line) are assessed for proliferation in response to estrogen and compounds of Formula I. Responsiveness is assessed by monitoring

transcription of known estrogen/anti-estrogen regulated genes such as progesterone receptor, PS2 and others.

ASSAY 2

I. Induced Animal Models - Uterine hyperplasia and carcinoma is induced by injections of estrogenic substances such as 17-β-estradiol or DES during neonatal development. The presence of hyperplasia or carcinoma in the adult animal is confirmed by biopsy or affected animals and/or sacrifice and biopsy of syngeneic animals identically treated. After evaluation of the lesions, affected animals are treated with estrogen, compounds of formula I, or progestins for 1-8 weeks. After treatment, lesions of surviving animals are examined for progression, regression or stasis.

II. Xenograft models - Implantation of endometrial lesions from humans or other animals or transformed cultured cells into immunodeficient rodents.

Tissue from endometrial lesions is implanted into the peritoneum or under the skin of sexually mature, castrated female immunocompromised rodents. Exogenous estrogen is supplied to induce growth of the explanted tissue. In some cases the harvested endometrial cells are cultured in vitro prior to implantation. Treatment consisting of estrogens, a compound of formula I, or vehicle will be supplied by gastric lavage on a daily basis for 3-16 weeks. Following treatment, implants are assessed for growth or regression. At the time of sacrifice, the uteri are harvested to assess the status of the intact endometrium.

ASSAY 3

Five to fifty women are selected for the clinical study. The women suffer from primary endometrial hyperplasia, but otherwise are in good general health. The study has a placebo control group, *i.e.*, the women are

divided into two groups, one of which receives a compound of formula 1 as the active agent and the other receives a placebo. Women in the test group receive between 50-200 mg of the drug per day by the oral route. They continue this therapy for 3-12 months. Accurate records are kept as to the number and severity of the symptoms in both groups and at the end of the study these results are compared. The results are compared both between members of each group and also the results for each patient are compared to the symptoms reported by each patient before the study began.

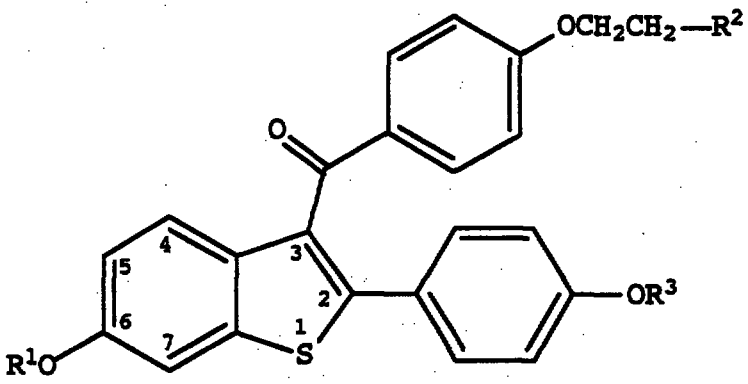
Utility of the compounds of formula I is illustrated by the positive impact they have in at least one of the assays described above.

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* C L M S *

I claim:

1. A method of inhibiting primary endometrial hyperplasia comprising administering to a human in need thereof an effective amount of a compound having the formula



(I)

wherein R^1 and R^3 are independently hydrogen, $-CH_3$, $-\overset{O}{\overset{\|}{C}}-(C_1-C_6\ \text{alkyl})$, or $-\overset{O}{\overset{\|}{C}}-Ar$, wherein Ar is optionally substituted phenyl;

R^2 is selected from the group consisting of pyrrolidine, hexamethyleneimino, and piperidino; or a pharmaceutically acceptable salt of solvate thereof.

2. The method of Claim 1 wherein said compound is the hydrochloride salt thereof.

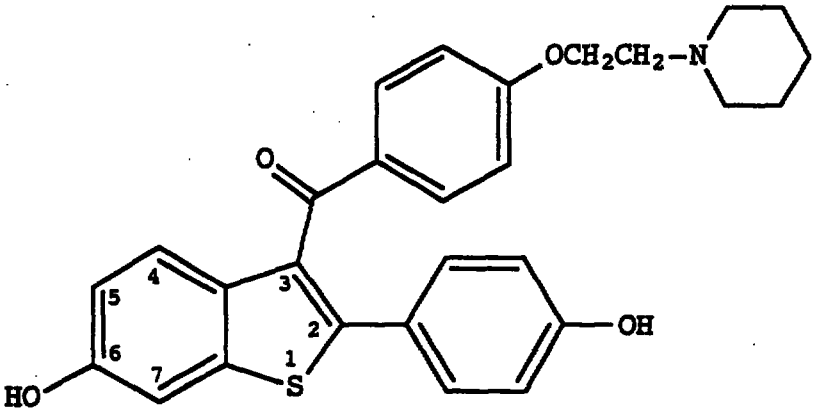
3. The method of Claim 1 wherein said administration is prophylactic.

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4. The method of Claim 1 wherein said compound is



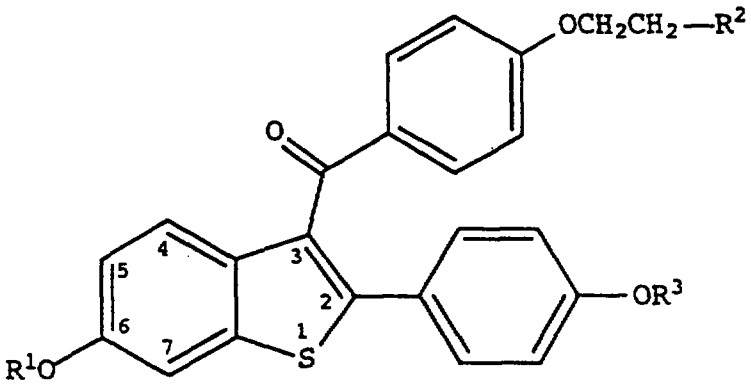
or its hydrochloride salt.

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I claim:

1. A use of an effective amount of a compound having the formula



(I)

wherein R^1 and R^3 are independently hydrogen, $-CH_3$, $-C(=O)-(C_1-C_6\ \text{alkyl})$, or $-C(=O)-Ar$, wherein Ar is optionally substituted phenyl;

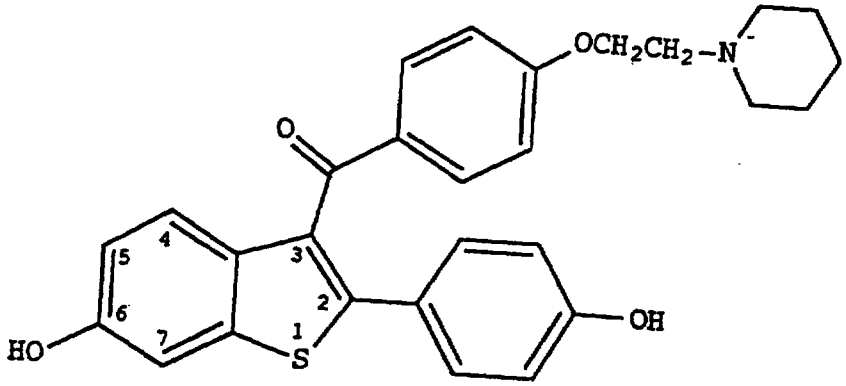
R^2 is selected from the group consisting of pyrrolidine, hexamethyleneimino, and piperidino; or a pharmaceutically acceptable salt of solvate thereof.
in inhibiting primary endometrial hyperplasia in a human in need thereof.

2. The use of Claim 1 wherein said compound is the hydrochloride salt thereof.

3. The use of Claim 1 is prophylactic.

REPLACEMENT SHEET
20 MAR 1998

4. The use of Claim 1 wherein said compound is



or its hydrochloride salt.

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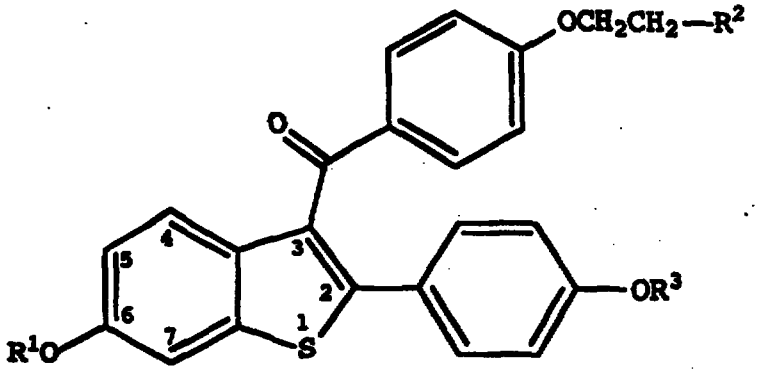
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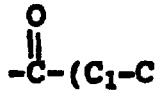
(54) Title: METHODS OF INHIBITING PRIMARY ENDOMETRIAL HYPERPLASIA

(57) Abstract

A method of inhibiting primary endometrial hyperplasia comprising administering to a human in need thereof an effective amount of a compound having formula (I) wherein R^1 and R^3 are independently hydrogen, $-CH_3$, (a) or (b), wherein Ar is optionally substituted phenyl; R^2 is selected from the group consisting of pyrrolidine, hexamethyleneimino, and piperidino; or a pharmaceutically acceptable salt or solvate thereof.



(I)



(a)



(b)