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(71) Applicant: SEPRACOR, INC. [US/US]; 33 Locke Drive, Marlborough, MA 01752 (US).
(72) Inventor: GRAY, Nancy, M.; 261 DeSimone Drive, Marlborough, MA 01752 (US).

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(54) Title: TREATING ESTROGEN-DEPENDENT DISEASES WITH (−)-FADROZOLE

(57) Abstract

Methods and compositions are disclosed utilizing optically pure (−)-fadrozole for the treatment of estrogen-dependent breast cancer, gynecomastia, systemic lupus erythematosus and premature labor while substantially reducing the concomitant liability of adverse effects associated with the racemic mixture of fadrozole. (−)-Fadrozole is an inhibitor of aromatase and is therefore useful in the treatment of other conditions supported by estrogen or caused by elevated estrogen levels.
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TREATING ESTROGEN-DEPENDENT DISEASES WITH (-)-FADROZOLE

BACKGROUND OF THE INVENTION

This invention relates to novel compositions of matter containing optically pure (-)-fadrozole. These compositions possess potent activity in treating estrogen-dependent breast cancer, gynecomastia, systemic lupus erythematosus, premature labor and other diseases including those that would benefit from a selective inhibition of aromatase. Optically pure (-)-fadrozole provides this treatment while substantially reducing adverse effects including, but not limited to, nausea, vomiting, decreased appetite, fatigue, leg cramps, light-headedness, orthostatic hypotension, hot flashes and suppressed serum aldosterone levels, which are associated with the administration of the racemic mixture of fadrozole. Also disclosed are methods for treating the above described conditions in a human while substantially reducing the adverse effects that are associated with the racemic mixture of fadrozole by administering the (-) isomer of fadrozole to said human.

The active compound of these compositions and methods is an optical isomer of fadrozole. The preparation of racemic fadrozole is described in U.S. Patent 4,617,307 and in Browne et al., J. Med. Chem. 34, 725-736 (1991). Chemically, the active compound is the (-) isomer of 4-(5,6,7,8-tetrahydroimidazo[1,5-a]pyridin-5-yl)benzonitrile, hereinafter referred to as fadrozole. The absolute stereochemistry of the (-) isomer is presently
believed to be S, as shown in Formula I [see Furet et al. *J. Med. Chem.* 36, 1393-1400 (1993)].

\[ \text{Formula I} \]

\(-\text{I} \)

\((-\))-Fadrozole, which is the subject of the present invention, is not presently commercially available. All of the clinical results that have been reported have been obtained with the racemic mixture, which is available for research purposes only.

Many organic compounds exist in optically active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L or R and S are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (−) are employed to designate the sign of rotation of plane-polarized light by the compound, with (−) or l meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. There is no correlation between nomenclature for the absolute stereochemistry and for the rotation of an enantiomer. Thus, D-lactic acid is the same as (−) lactic acid, and L-lactic acid is (+). For a given chemical structure, these chiral compounds exist as a pair of enantiomers.
which are identical except that they are non-superimposable mirror images of one another. A specific stereoisomer may also be referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric or racemic mixture.

Stereochemical purity is of importance in the field of pharmaceuticals, where 12 of the 20 most prescribed drugs exhibit chirality. A case in point is provided by the L-form of the beta-adrenergic blocking agent, propranolol, which is known to be 100 times more potent than the D-enantiomer.

Furthermore, optical purity is important since certain isomers may actually be deleterious rather than simply inert. For example, it has been suggested that the D-enantiomer of thalidomide was a safe and effective sedative when prescribed for the control of morning sickness during pregnancy, while the corresponding L-enantiomer has been believed to be a potent teratogen.

The chromatographic separation of racemic fadrozole into (+)-fadrozole and (-)-fadrozole on an analytical scale is described by Browne et al. (op. cit.); however, the enantioselective synthesis of (-)-fadrozole has not been described. The IC\textsubscript{50} of (-)-fadrozole in inhibiting aromatase was reported by Browne to be 4.6 nM in human placental microsomes in vitro.


Estrogen has been implicated in the progression of several diseases, including human breast cancer, where estrogens appear to provide the major hormonal support for cancer cells. The main source of estrogen production in postmenopausal women is the extraglandular conversion of androstenedione to estrone; the adrenal glands per se produce little or no estrogens. Androstenedione is secreted and converted to estrone in peripheral tissues via the multicomponent aromatase enzyme complex. Estrone can be either conjugated into estrone sulfate to form a slowly revolving storage pool with a potential for back conversion to estrone, or reduced to estradiol (E₂), the major active estrogenic steroid. Fat and muscle contain the majority of extraglandular aromatase activity present in postmenopausal women.

It has recently been reported that approximately two thirds of human breast cancers contain measurable aromatase activity. It is generally accepted that estrogen deprivation plays an important role in the endocrine treatment strategy for patients with breast cancer. Inhibitors of the aromatase enzyme system have become of interest during recent years; they are used both to lower systemic estrogen levels and, perhaps more importantly, to inhibit intracellular
conversion of androgens to estrogens by tumor-cell aromatase.

Aromatase occurs widely in tissues such as adipose tissue, brain, testes, and ovaries. The enzyme is a membrane-bound microsomal complex containing NADPH-cytochrome c reductase and cytochrome P-450 units. The mechanism by which androstenedione is converted to estrone has been studied in detail. This conversion can be competitively inhibited by substrate mimics. Aromatase inhibition can also be achieved by compounds which bind directly to the cytochrome P-450 of the enzyme. The inhibition of 20,22-desmolase (lyase), also a cytochrome P-450 containing enzyme, prevents the oxidative side-chain cleavage of 20α,22β-dihydroxycholesterol and results in a blockade of not only estrogen biosynthesis but also the biosynthesis of other important secretory steroids. Since this cleavage is a step common also to cortisol biosynthesis, replacement therapy is necessary when desmolase is inhibited. Inhibition of 21β-hydroxylase, also a P-450 enzyme, leads to suppression of aldosterone. Aromatization is the final step in the biogenesis of estrone and estradiol and is, consequently, the most effective step at which to selectively interfere without affecting the biogenesis of other steroids. Racemic fadrozole has been found to be an effective inhibitor of mammalian aromatase at doses that produce little inhibitory effect on desmolase.

Racemic fadrozole appears to inhibit both aromatase and 21β-hydroxylase, although perhaps with
differing $K_i$'s. Lipton et al. [Cancer 65, 1279-1285 (1990)] reported that there appeared to be inhibition of 21$\beta$-hydroxylase at the 8 and 16 mg/day dosage levels; Santen et al. [J. Clin. Endocrinol. Metab. 68, 99-106 (1989)] similarly reported 21$\beta$-hydroxylase inhibition at 4-16 mg/day.

Racemic fadrozole is absorbed rapidly with $T_{\text{max}}$ values ranging from 1 to 2 hours. The average half life for the racemic compound, estimated from oral studies, was 10.5 hours. While clinical trials have so far been limited to breast cancer, it is believed that as a result of its aromatase inhibitory activity racemic fadrozole may also be useful to treat gynecomastia, systemic lupus erythematosus, premature labor and other conditions.

In human volunteers doses of 1-4 mg p.o. per day for three months or more resulted in a median survival time of 22.6 months and a median response rate of 12 to 34% (Raats, op.cit.). Twenty-eight percent of the patients receiving racemic fadrozole reported hot flashes, 13% reported nausea and vomiting, 8% reported fatigue and 5% reported loss of appetite. In another study (Stein et al., op.cit.), 13 out of 31 patients reported side effects as above and, in addition, headaches and dry mouth. In one study (Dowsett et al., op. cit.), a significant suppression of serum aldosterone was observed at 1 mg b.i.d.

Thus it would be particularly desirable to find a compound with the advantages of the racemic mixture
of fadrozole which would not have the aforementioned disadvantages.

SUMMARY OF THE INVENTION

It has now been discovered that the optically pure (-) isomer of fadrozole is an effective agent for treating estrogen-dependent breast cancer, gynecomastia, systemic lupus erythematosus and premature labor and other conditions including those that would benefit from a selective inhibition of aromatase. The optically pure (-) isomer of fadrozole provides this effective treatment while substantially reducing adverse effects of racemic fadrozole including, but not limited to, nausea, vomiting, decreased appetite, fatigue, leg cramps, light-headedness, orthostatic hypotension, hot flashes and suppressed serum aldosterone levels. The present invention also includes methods for treating the above described conditions in a human while substantially reducing adverse effects that are associated with the racemic mixture of fadrozole by administering the optically pure (-) isomer.

DETAILED DESCRIPTION OF THE INVENTION

The present invention encompasses a method of treating breast cancer, which comprises administering to a human in need of such therapy, an amount of (-)-fadrozole, or a pharmaceutically acceptable salt thereof, substantially free of its (+) stereoisomer, said amount being sufficient to retard the growth of the cancer. The method substantially reduces the concomitant liability of adverse effects associated
with the administration of the racemic compound by providing an amount which is insufficient to cause adverse effects associated with the racemic mixture of fadrozole.

A further aspect of the present invention includes a method of treating a condition supported by estrogen or caused by or contributed to by elevated estrogen levels in a human, which comprises administering to a human in need of such therapy, an amount of (-)-fadrozole, or a pharmaceutically acceptable salt thereof, substantially free of its (+) stereoisomer, sufficient to reduce estrogen levels. The method substantially reduces the concomitant liability of adverse effects associated with the administration of racemic fadrozole by providing an amount which is insufficient to cause adverse effects associated with the administration of racemic fadrozole. Conditions associated with elevated estrogen levels in humans may include, but are not limited to, gynecomastia, systemic lupus erythematosus, and premature labor.

In addition, the invention encompasses a pharmaceutical composition which comprises a therapeutically effective amount of (-)-fadrozole or a pharmaceutically acceptable salt thereof, substantially free of its (+) stereoisomer, and a pharmaceutically accepted carrier.

The racemic mixture of fadrozole (i.e., a 1:1 mixture of the two enantiomers) exhibits anticancer activity through its selective and potent aromatase inhibition, thus providing therapy and a reduction of
symptoms in a variety of conditions and disorders related to the presence of estrogen in disadvantageous amounts. However, this racemic mixture, while offering the expectation of efficacy, causes adverse effects. Utilizing the optically pure or substantially optically pure isomer of (-)-fadrozole results in enhanced efficacy, diminished adverse effects and, accordingly, an improved therapeutic index. It is therefore more desirable to use the (-) isomer of fadrozole than to administer the racemic mixture.

The term "adverse effects" includes, but is not limited to, nausea, vomiting, decreased appetite, fatigue, leg cramps, light-headedness, orthostatic hypotension, hot flashes and suppressed serum aldosterone levels.

The term "substantially free of its (+) stereoisomer" as used herein means that the compositions contain at least 90% by weight of (-)-fadrozole and 10% by weight or less of (+)-fadrozole. In a more preferred embodiment the term "substantially free of the (+) isomer" means that the composition contains at least 99% by weight of (-)-fadrozole, and 1% or less of (+)-fadrozole. In the most preferred embodiment, the term "substantially free of its (+) stereoisomer" as used herein means that the composition contains greater than 99% by weight of (-)-fadrozole. These percentages are based upon the total amount of fadrozole in the composition. The terms "substantially optically pure (-) isomer of fadrozole" or "substantially optically pure
(-)-fadrozole" and "optically pure (-) isomer of fadrozole" and "optically pure (-)-fadrozole" are also encompassed by the above-described amounts.

The term "treating breast cancer" as used herein means treating, alleviating or palliating such condition and suppressing the growth of cancerous tissue, thus providing increased survival time.

The term "treating a condition supported by estrogen or contributed to by elevated levels of estrogen" as used herein means treating, alleviating or palliating such disorders, thus providing relief from the symptoms of the aforementioned conditions or slowing the progression of the disease. Among such conditions are gynecomastia, systemic lupus erythematosus, and premature labor.

The term "therapeutically effective amount" refers to an amount sufficient to retard the growth of a cancer (in a method for treating cancer) or sufficient to reduce estrogen levels (for treating conditions supported by estrogen).

The chemical synthesis of the racemic mixture of fadrozole can be performed by the method described in U.S. Patent 4,617,307 cited above. An improved synthesis is provided by Browne et al. (op.cit.). The (-) isomer of fadrozole may be obtained by resolution of the enantiomers of fadrozole or of precursors thereto using conventional means such as fractional crystallization of diastereomeric salts with chiral acids. Furet et al. (op cit.) has described a resolution of racemic fadrozole by HPLC
on benzoylcellulose. Other standard methods of resolution known to those skilled in the art including, but not limited to, simple crystallization, can also be used. (See for example, E.L. Eliel, *Stereochemistry of Carbon Compounds*, McGraw Hill (1962) and Wilen and Lochmuller, "Tables of Resolving Agents", *Journal of Chromatography* 113, 283-302 (1975)). In addition, the carboxylic acid precursor of Browne et al. [4-(5,6,7,8-tetrahydroimidazo[1,5-a]pyridin-5-yl)benzoic acid] may be resolved by fractional crystallization of diastereomeric salts with chiral amines.

The magnitude of a prophylactic or therapeutic dose of (-)-fadrozole in the acute or chronic management of disease will vary with the severity and nature of the condition to be treated and the route of administration. The dose and perhaps the dose frequency will also vary according to the age, body weight and response of the individual patient. In general, the total daily dose range for (-)-fadrozole for the conditions described herein is from about 0.5 mg to about 8 mg in single or divided doses. Preferably a daily dose range should be from about 1 mg to about 4 mg in single or divided doses, while most preferably a daily dose range should be about 2 mg, in single or divided doses. In managing the patient, the therapy should be initiated at a lower dose, perhaps at about 0.5 mg to about 2 mg, and increased up to about 4 mg or higher depending on the patient's global response. It is further recommended that patients over 65 years and those with impaired renal or hepatic function initially receive low doses and that they be titrated based on individual
response(s) and blood level(s). It may be necessary
to use dosages outside these ranges in some cases, as
will be apparent to those skilled in the art.
Further, it is noted that the clinician or treating
physician will know how and when to interrupt,
adjust, or terminate therapy in conjunction with
individual patient response. The terms "a
therapeutically effective amount", "an amount
sufficient to suppress cancer but insufficient to
cause said adverse effects" and "an amount sufficient
to reduce estrogen levels but insufficient to cause
said adverse effects" are encompassed by the above-
described dosage amounts and dose frequency schedule.

Any suitable route of administration may be
employed for providing the patient with an effective
dosage of (-)-fadrozole. For example, oral, rectal,
parentral (subcutaneous, intramuscular,
intravenous), transdermal, and like forms of
administration may be employed. Dosage forms include
tablets, troches, dispersions, suspensions,
solutions, capsules, patches, and the like.

The pharmaceutical compositions of the present
invention comprise (-)-fadrozole as the active
ingredient, or a pharmaceutically acceptable salt
thereof, and may also contain a pharmaceutically
acceptable carrier, and optionally, other therapeutic
ingredients.

The terms "pharmaceutically acceptable salts" or
"a pharmaceutically acceptable salt thereof" refer to
salts prepared from pharmaceutically acceptable non-
toxic acids. Suitable pharmaceutically acceptable
acid addition salts for the compound of the present
invention include acetic, benzenesulfonic (besylate),
benzoic, camphorsulfonic, citric, ethanesulfonic,
fumaric, gluconic, glutamic, hydrobromic,
hydrochloric, isethionic, lactic, maleic, malic,
mandelic, methanesulfonic, mucic, nitric, pamoic,
pantothenic, phosphoric, succinic, sulfuric,
tartaric, p-toluene sulfonic, and the like.
Hydrochloride salts are particularly preferred, and
the hydrochloride hemihydrate has particular utility.

The compositions of the present invention
include suspensions, solutions, elixirs, or solid
dosage forms. Carriers such as starches, sugars, and
microcrystalline cellulose, diluents, granulating
agents, lubricants, binders, disintegrating agents,
and the like are suitable in the case of oral solid
preparations (such as powders, capsules, and
tables), and oral solid preparations are preferred
over the oral liquid preparations.

Because of their ease of administration, tablets
and capsules represent the most advantageous oral
dosage unit forms, in which case solid pharmaceutical
carriers are employed. If desired, tablets may be
coated by standard aqueous or nonaqueous techniques.

In addition to the common dosage forms set out
above, the compounds of the present invention may
also be administered by controlled release means and
delivery devices such as those described in
U.S. Patent Nos.: 3,845,770; 3,916,899; 3,536,809;
3,598,123; and 4,008,719, the disclosures of which
are hereby incorporated by reference.
Pharmaceutical compositions 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, or as a solution or a suspension in an aqueous liquid, a non-aqueous liquid, an oil-in-water emulsion, or a water-in-oil liquid emulsion. Such compositions may be prepared by any of the methods of pharmacy, but all methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired presentation.

For example, a tablet may be prepared 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 powder or granules, optionally mixed with a binder, lubricant, inert diluent, surface active agent 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. Desirably, each tablet contains from about 0.5 mg to about 8 mg of the active ingredient, and each cachet or capsule contains from about 0.5 mg to about 8 mg of the active ingredient. Most preferably, the tablet, cachet or capsule contains
either one of three dosages, about 1.0 mg, about 2.0 mg or about 4.0 mg of \((-\)-fadrozole for oral administration.

The invention is further defined by reference to the following examples describing in detail the preparation of the compositions of the present invention, as well as their utility. It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced without departing from the purpose and interest of this invention.

The relative activity, potency and specificity of optically pure fadrozole and racemic fadrozole as an inhibitor of aromatase can be determined by a pharmacological study in vitro according to the methods of Browne et al. (op.cit.) or Furet et al. (op. cit.). The assay according to Browne is performed in a total volume of 1 mL at 37° C. Briefly, 120 μg of human placental microsomal protein is incubated with 11 μg of \([4-^{14}C]\)androstene-3,17-dione \([4-^{14}C]A\), 2.4 \(\times 10^4\) M NADPH (tetrasodium salt), and the appropriate concentration of inhibitor. The \([4-^{14}C]A\) is added as a solution in 1.7% ethanol in 0.05M potassium phosphate buffer (pH 7.4) so that the final concentration of ethanol does not exceed 0.02% (v/v). The reaction is started by the addition of enzyme and stopped after 20 min by the addition of ethyl acetate. Following extraction and centrifugation, the aqueous phase is reextracted with ethyl acetate. The extract is evaporated to dryness and dissolved in acetone prior to being chromatographed on silica gel 60 thin-layer plates.
using ethyl acetate/isoctane (140:60) or toluene/chloroform/methanol (70:140:20). The radioactive estradiol and estrone peaks are identified by comparison with authentic standards and quantified with a liquid scintillation counter. The tests provide an estimate of relative activity and potency.

Differential inhibition by racemic fadrozole and its enantiomers of the various enzymes involved in conversion of cholesterol to other steroids can be assessed by the method of Newton et al. [J. Steroid Biochem. Molec. Biol. 39, 723-727 (1991)].

A male guinea pig (Duncan-Hartley, 500-650g) is killed by cervical dislocation and the kidneys and attached adrenal glands are carefully removed. The kidney and fatty tissue are dissected away and the intact adrenal gland is cut in half, length-wise. The two pieces of each adrenal are placed on a cooled Petri dish (4°C) and cut into cubes of about 1 mm³ with a fine blade. The divided tissue is then placed into 10mL Minimal Essential Medium (MEM) containing Earl's salts, 20 mM Hepes buffer, but without L-glutamine (MEM-A) (4°C), previously gassed for 5-10 minutes with 95% O₂/5% CO₂. Fragments of tissue are washed (x2) with fresh media (MEM-A, 4°C) and poured into a small gas chamber (50 mL with magnetic stirring bar). MEM (10 mL) containing 2mg/mL collagenase (MEM-B) is added. The chamber is kept at 37°C C and gassed with 95% O₂/5% CO₂. Cells are allowed to disperse for 10 minutes following which the medium is removed and discarded. A further 10 mL aliquot of MEM-B is added to fragments and dispersion
is carried out for 10 minutes. Medium containing dispersed cells (supernatant 2) is removed and kept on ice. Cell dispersion is carried out for a further 2 cycles and supernatants 2-4 are finally centrifuged at 4°C for 5 minutes at 400 G. A portion of each supernatant is removed and discarded leaving 5 mL above the cell pellet and 1.5 mL MEM containing 2mM ascorbate 0.5% BSA and 8 mM CaCl₂ (MEM-C, made up just prior to addition) is added. Starting with supernatant 3, cells are carefully resuspended (without air bubbles) and suspensions are filtered through 1 μm nylon mesh. The pooled cell suspension is centrifuged at 400 G for 5 minutes, the supernatant removed and 10 mL MEM-C added to resuspend cells. Centrifugation is again performed for 5 minutes at 400 G and the pellet is resuspended in 3 mL MEM-C. Approximately equal aliquots of this suspension are then placed into two chambers of a multi-well plate (24 well) and left for 2 h in an incubation chamber flushed with 95% O₂/5% CO₂.

Following this period, cells are resuspended by gentle flushing with covering medium, removed from the wells and centrifuged for 5 minutes at 400 G. The supernatant is removed and the pelleted cells are resuspended in 2mL MEM containing 4mM ascorbate, 0.5% BSA and 8 mM CaCl₂ (MEM-D). Cell number is determined with a haemocytometer using trypan blue to exclude counting non-viable cells. The number of cells in the preparation is adjusted to give between 2.5 x 10⁶ and 5 x 10⁶ cells/mL by the addition of MEM-D.

To determine the basal production of steroids, portions of the cell suspension are added to wells of microtitre plates followed by 25 μL of a 10% solution
of dimethylsulphoxide (DMSO) in MEM-A containing 0.5% BSA and 8 mM CaCl$_2$ (MEM-E) and 25 µL MEM-A alone. To observe the effect of ACTH on steroid output, 25 µL MEM-E containing 10% DMSO is added to 50 µL cell suspension followed by 25 µL ACTH (200 pg/mL in MEM-A). The effect of (+)-fadrozole, (-) fadrozole and racemic fadrozole on ACTH-stimulated steroidogenesis is determined in wells containing 50 µL cell suspension, 25 µL drug solution in MEM-E and 25 µL ACTH in MEM-A. Incubation is conducted for 90 minutes in an atmosphere of 95% O$_2$/5% CO$_2$.

17-Hydroxyprogesterone and androstenedione (A) are measured using direct radioimmunoassays (RIAs). Medium from each well is diluted 1:5 in steroid stripped human serum prior to assay. Cortisol is measured in the medium covering each of the cell layers by a direct RIA.

The aromatase enzyme is measured by quantifying the amount of tritiated water released from radiolabelled A during aromatization to oestrone. Placental microsomes and NADPH (1mM) are added in 0.1 M sodium phosphate buffer (pH 7.4) to assay tubes containing 0.5 µCi [$\beta^3$H]A and unlabelled aromatase inhibitors ($10^{-10}$-10$^{-6}$M final concentration), to give a final volume of 1 mL. After incubation for 1 hour at 37° C, 0.5 mL of trichloroacetic acid is added followed by 1 mL of activated charcoal suspension (5% v/v). Following incubation for 30 minutes at 37° C, tubes and contents are centrifuged at 1500 G for 15 minutes at 4° C and radioactivity is determined in 1 mL aliquots of each supernatant. In order to determine IC$_{50}$ values, counts observed at each
concentration are calculated as a percentage of those observed in control tubes.

Additional experiments are performed on adult, male ferrets to correlate dosage and a side effect not necessarily related to steroid enzyme inhibition (i.e., emesis). The animals are first adapted to wearing a nylon jacket connected to a stainless-steel cable, which in turn is attached to a brass swivel at the cage top. After habituation to the tether-harness, each animal receives a surgically implanted catheter in its right jugular vein. The catheter is flushed daily with heparinized sodium chloride. The drug studies are conducted 1 week after the surgical procedure. Tethered animals are individually housed.

Eight to eleven animals are used to evaluate each dose of each test compound. Individual animals are weighed weekly and randomly given, at greater than 48 hour intervals, a single i.v. or p.o. dose of control vehicle, racemic fadrozole, (+)-fadrozole or (-)-fadrozole. At least three dose levels of each test compound are evaluated.

Individual animals are observed for 30 min following administration of test substance. The frequency of, and latency to all expulsions, retches and defections are recorded. Data obtained from dose-response curves is tested for statistical significance by chi-square analysis. ED₉₀ values are determined for each compound.
EXAMPLES

Example 1

ORAL FORMULATION

Capsules:

<table>
<thead>
<tr>
<th>Formula</th>
<th>Quantity per capsule in mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>(-)-Fadrozole</td>
<td>0.5</td>
</tr>
<tr>
<td>Lactose</td>
<td>104</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>20.0</td>
</tr>
<tr>
<td>Magnesium Stearate</td>
<td>0.5</td>
</tr>
<tr>
<td>Fill Weight</td>
<td>125</td>
</tr>
</tbody>
</table>

The (-)-fadrozole, lactose and cornstarch are blended until uniform and then the magnesium stearate is blended into the resulting powder, which is sieved and filled into suitably sized, two-piece, hard gelatin capsules using conventional machinery. Other doses may be prepared by altering the fill weight and, if necessary, changing the capsule size to suit.
Example 2

ORAL FORMULATION

<table>
<thead>
<tr>
<th>Tablets:</th>
<th>Formula</th>
<th>Quantity per tablet in mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>(-)-Fadrozole</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Lactose</td>
<td>101.5</td>
<td>101</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Water (per thousand Tablets)*</td>
<td>30 mL</td>
<td>30 mL</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>19.5</td>
<td>19.5</td>
</tr>
<tr>
<td>Magnesium Stearate</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Compression Weight</td>
<td>125</td>
<td>125</td>
</tr>
</tbody>
</table>

*The water evaporates during manufacture

The (-)-fadrozole is blended with the lactose until a uniform blend is formed. The smaller quantity of cornstarch is blended with the water to form the resulting cornstarch paste. This is then mixed with the uniform blend until a uniform wet mass is formed. The remaining cornstarch is added to the resulting wet mass and mixed until uniform granules are obtained. The granules are then screened through a suitable milling machine, using a 1/4 inch stainless steel screen. The milled granules are dried in a suitable drying oven until the desired moisture content is obtained. The dried granules are then milled through a suitable milling machine,
magnesium stearate is blended in, and the resulting mixture is compressed into tablets of the desired shape, thickness, hardness and disintegration. Tablets of other strengths may be prepared by altering the ratio of active ingredient to the excipients or to the final weight of the tablet.
What is claimed is:

1. A method of treating breast cancer in a human which comprises administering to said human a therapeutically effective amount of (-)-fadrozole, or a pharmaceutically acceptable salt thereof, substantially free of its (+) stereoisomer.

2. The method of claim 1 wherein (-)-fadrozole is administered by parenteral, transdermal, or oral administration.

3. The method of claim 2 wherein the amount of (-)-fadrozole or a pharmaceutically acceptable salt thereof administered is from about 0.5 mg to about 8 mg per day.

4. The method of claim 3 wherein the amount administered is from about 1 mg to about 4 mg per day.

5. The method of claim 4 wherein the amount administered is about 2 mg per day.

6. The method of claim 1 wherein the amount of (-)-fadrozole or a pharmaceutically acceptable salt thereof is greater than approximately 90% by weight of the total weight of fadrozole.

7. The method of claim 1 wherein the amount of said (-)-fadrozole or a pharmaceutically acceptable salt thereof, substantially free of its (+) stereoisomer, is administered together with a pharmaceutically acceptable carrier.
8. A method of treating a condition supported by estrogen or caused by elevated estrogen levels in a human which comprises administering to said human a therapeutically effective amount of (-)-fadrozole, or a pharmaceutically acceptable salt thereof, substantially free of its (+) stereoisomer.

9. The method according to claim 8 wherein said condition is chosen from the group consisting of gynecomastia, systemic lupus erythematosus, and premature labor.

10. The method of claim 8 wherein (-)-fadrozole is administered by parenteral, transdermal, or oral administration.

11. The method of claim 10 wherein the amount of (-)-fadrozole or a pharmaceutically acceptable salt thereof administered is from about 0.5 mg to about 8 mg per day.

12. The method of claim 11 wherein the amount administered is from about 1 mg to about 4 mg per day.

13. The method of claim 12 wherein the amount administered is about 2 mg per day.

14. The method of claim 8 wherein the amount of (-)-fadrozole or a pharmaceutically acceptable salt thereof is greater than approximately 90% by weight of the total weight of fadrozole.
15. The method of claim 8 wherein the amount of said (-)-fadrozole or a pharmaceutically acceptable salt thereof, substantially free of its (+) stereoisomer, is administered together with a pharmaceutically acceptable carrier.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 31/44
US CL :514/300
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/300

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>US, A, 4,617,307 (BROWNE) 14 OCTOBER 1986, see entire document.</td>
<td>1-15</td>
</tr>
<tr>
<td>X</td>
<td>J. MED. CHEM., VOL. 36, issued 1993, FURET et al., &quot;Aromatase Inhibitors; Synthesis, Biological Activity, and Binding Mode of Azole-Type Compounds&quot;, pages 1393-1400, see entire document.</td>
<td>1-15</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C.

See patent family annex.

Date of the actual completion of the international search
09 JUNE 1995

Date of mailing of the international search report
31 JUL 1995

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer: VERNE D. GOLDBERG
Telephone No. (703) 308-1235

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