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(21) International Application Number: PCT/US91/01740 (22) International Filing Date: 13 March 1991 (13.03.91) (30) Priority data: 9006023.7 16 March 1990 (16.03.90) GB (71) Applicant (for all designated States except US): SMITH-KLINE BEECHAM CORPORATION [US/US]; One Franklin Plaza, P.O. Box 7929, Philadelphia, PA 19101 (US). (72) Inventor; and (75) Inventor/Applicant (for US only) : HENRY, Michael [US/US]; 132 Gulph Hills Road, Radnor, PA 19087 (US). (74) Agents: DUSTMAN, Wayne, J. et al.; Corporate Patents - UW2220, SmithKline Beecham Corporation, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).		(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), SE (European patent), US. Published <i>With international search report.</i>
(54) Title: SYNERGISTIC COMPOSITIONS (57) Abstract Invented are compositions of competitive and uncompetitive inhibitors of steroid 5- α -reductase, pharmaceutical compositions containing the compositions of inhibitors and methods of using these compositions to inhibit steroid 5- α -reductase.		

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SYNERGISTIC COMPOSITIONS

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This invention comprises a composition containing one of certain selected compounds which are competitive inhibitors of steroid 5- α -reductase, combined with one of certain selected compounds which are uncompetitive inhibitors of steroid 5- α -reductase. The composition which contains this new combination of medicinal agents demonstrates improved suppression of plasma dihydrotestosterone (DHT) and improved prostate and seminal vesicle weight reduction.

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Steroid 5- α -reductase is a NADPH-dependent enzyme that mechanistically converts testosterone (T) to DHT. The individual compounds of the claimed compositions produce 5- α -reductase inhibiting activity by binding to a specific enzyme species at a specific stage of the reductive process. Combining inhibitors that bind to a different enzyme species at different stages of the reductive process has been found to lower the level of plasma DHT and to reduce the weight of the prostate and seminal vesicles to an extent beyond which a single inhibitor can achieve alone.

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1 Background of the Invention

 Steroid 5- α -reductase is a NADPH-dependent enzyme that converts testosterone to DHT. Recognition of the importance of elevated DHT levels in various disease states has stimulated many efforts to synthesize inhibitors of this enzyme.

 The first inhibitor described was 4-androsten-3-one-17 β -carboxylic acid by Hsia and Voight in 1973. J. Invest. Dermat. 62:224-227. (4R)-5,10-seco-19-norpregna-4,5-diene-3,10,20-triene was the next inhibitor to be described and also has found utility as an affinity label for 5- α -reductase. Robaire, B., et al., (1977), J. Steroid Biochem. 8:307-310. (5 α ,20-R)-4-diazo-21-hydroxy-20-methylpregnan-3-one has been reported as a potent, time-dependent inhibitor of steroid 5- α -reductase. Blohm, T.R., et al., (1980), Biochem. Biophys. Res. Comm. 95:273-280; United States Patent 4,317,817, March 2, 1982: 17 β -N,N-diethylcarbamoyl-4-methyl-4-aza-5- α -androstan-3-one is exemplary of a group of 4-aza steroid inhibitors of steroid 5- α -reductase described in United States Patent 4,377,584 which issued March 22, 1983, and in Liang, T., et al., (1983), J. Steroid Biochem. 19, 385-390. 17 α -acetoxy-6-methylene-pregn-4-ene-3,20-dione also has been shown to be a time-dependent inactivator of steroid 5- α -reductase. Petrow, V., et al., (1981), Steroids 38:121-140.

 Other steroid 5- α -reductase inhibitors also have been described. United States Patent 4,361,578 which issued June 2, 1986, describes a class of homosteroid enzyme inhibitors. United States Patent 4,191,759 discloses amides of 17 β -carboxy-4-androsten-3-one that are active as steroid 5- α -reductase inhibitors. Japanese Patents J60146855-A and J60116657-A disclose various aniline derivatives having numerous activities including 5- α -reductase inhibiting activity. Japanese Patent I60142941-A discloses phenyl-substituted ketones having

- 1 5- α -reductase inhibiting activity and European Patent
EP173516-A discloses various phenyl-substituted amides
having similar activity. Shiseido referenced terpene
derivatives that are active inhibitors of steroid
5 5- α -reductase. Japanese Patent J59053417-A.

Recently it has been shown that steroid
5- α -reductase follows an ordered kinetic mechanism
(Houston et al., (1987), Steroids 49, 355-369; Metcalf,
B., et al., (1989) Bioorganic Chemistry 17, 372-376) in
10 which the nicotinamide cofactors are the first substrate
binding to the enzyme and the last product released from
the enzyme (Figure 1). Dead-end inhibitors of bisubstrate
enzymes which follow ordered kinetic mechanisms can
associate to one or more existing enzyme species. With
15 steroid 5- α -reductase, enzyme forms to which such a
steroidal-inhibitor might bind would include free enzyme,
E, and enzyme binary complexes with cofactor, E-NADPH and
E-NADP⁺. For example, the 4-aza steroidal inhibitors,
such as N-t-butyl-5- α -androst-1-ene-4-aza-17 β -
20 carboxamide-3-one (compound A) (Liang et al., (1985),
Endocrinology 117, 571-579), inhibit enzyme activity by
forming an enzyme-NADPH-inhibitor dead-end complex
(E-NADPH-1, Figure 1).

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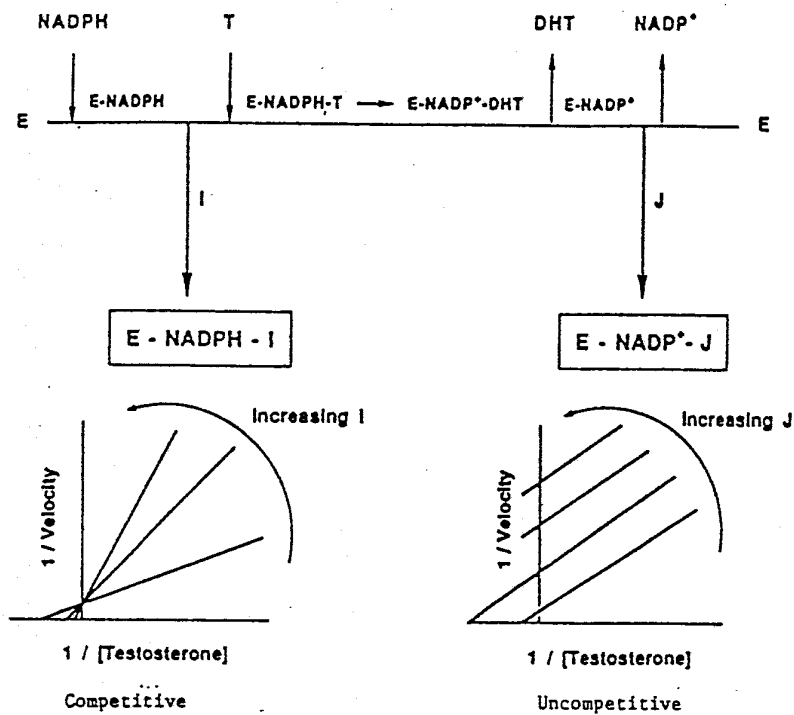
FIGURE 1

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Figure 1: Kinetic Mechanisms of Inhibition of Steroid 5- α -Reductase by competitive and uncompetitive inhibitors. As depicted a competitive inhibitor is designated as I and an uncompetitive inhibitor is designated as J .

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In this context, the binding of inhibitors to each of these enzyme forms would be kinetically distinct as described in Irwin H. Segel, Enzyme Kinetics, Pub: John Wiley & Sons, Inc. (1975). The response of the velocity of enzyme catalysis with varying concentrations of testosterone in the presence of an inhibitor that binds to the $E-NADPH$ complex can be described by equation 1; this model is denoted as competitive inhibition versus the variable substrate, in this case testosterone. Similarly, the model for an inhibitor that preferentially associates to the $E-NADP^+$ complex would be described by equation 2; such a compound is referred to as an uncompetitive

1 inhibitor versus the variable substrate. In equations 1
and 2, v is the observed velocity of product formation,
 V_m is the maximal enzyme velocity at saturating
concentrations of the variable substrate (A), I is the
5 concentration of the inhibitor with apparent inhibition
constants of K_{is} or K_{ii} , and K_a is the apparent
Michaelis constant for the variable substrate.

$$v = V_m A / [K_a (1 + I/K_{is}) + A] \quad (1)$$

10

$$v = V_m A / [K_a + A(1 + I/K_{ii})] \quad (2)$$

Velocities (v) determined at variable concentrations of
substrate (A) and inhibitor (I) are evaluated by
15 non-linear curve fittings with computer programs as
described by Cleland (Cleland, W.W. (1979) Methods in
Enzymology 63, 103-138), to determine the best fit to
equations 1 or 2. The results of these analyses are
typically expressed in double reciprocal plots: 1/velocity
20 versus 1/[testosterone]. The patterns in Figure 1 are
characteristic of a competitive and an uncompetitive
inhibitor versus the second substrate in an ordered
kinetic mechanism, and can be used to distinguish between
the binding of a reversible dead-end inhibitor to E-NADPH
25 or E-NADP⁺, respectively. Since the same uncompetitive
model (equation 2) is used to describe an inhibitor that
binds to either E-NADPH or E-NADP⁺ upon variation of the
first substrate which binds to the surface of an ordered-
enzyme, discrimination between these two mechanisms would
30 not be possible by such an experiment with varying NADPH;
the results from such an experiment can be used, however,
to discriminate from a mechanism of inhibition resulting
from binding to free enzyme (E).

The effects of inhibitors which act by these two
35 differing mechanisms under comparable conditions are shown
in Figure 2; in this example, the concentration of each

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1 inhibitor has been set to be equal to its inhibition
 constant (K_{ii} or K_{is}) and the Michaelis-constant for
 substrate (K_m) has been set at 1 concentration unit.
 Curves B and C represent inhibitors that preferentially
 5 bind to the E-NADPH and E-NADP⁺ complexes while curve A
 represents the uninhibited velocity. Note that curves A
 and B intersect on the ordinate (competitive pattern)
 while curves A and C are parallel (uncompetitive pat-
 tern). From this plot, it can be seen that a competitive
 10 inhibitor (E-NADPH-I) is more efficient at low substrate
 concentration, while the uncompetitive inhibitor
 (E-NADP⁺-J) is more efficient at higher concentrations
 of substrate. The intersection point for these two curves
 occurs at the concentration of substrate that equals its
 15 K_m .

FIGURE 2

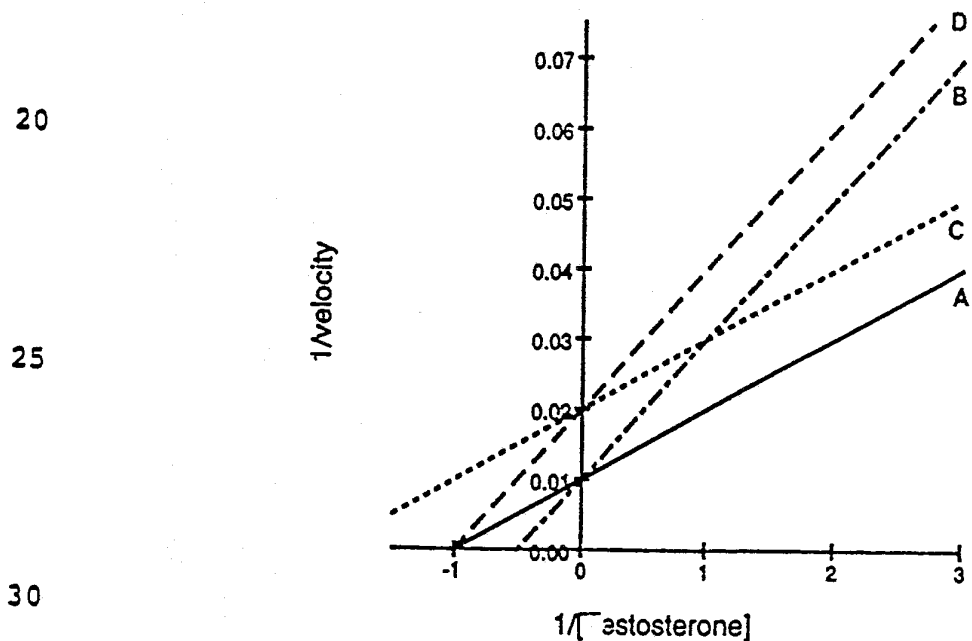


Figure 2. Model for Inhibition of
 Steroid 5- α -Reductase with Compounds
 that Bind to E-NADPH and E-NADP⁺:
 Additive Effects of Inhibitors that
 Bind to Different Enzyme Forms.

1 A single molecule that could bind equally well to
both E-NADPH and E-NADP⁺ would demonstrate the additive
effects in slope and intercept of the two double-
reciprocal plots. This is described by equation 3, and is
5 represented by curve D in Figure 2.

$$v = V_m A / [K_a (1 + I/K_{is}) + A(1 + I/K_{ii})] \quad (3)$$

Inhibition by a molecule that demonstrates such a mixed
10 (noncompetitive) mode of action is more efficient than
either of the single mechanisms alone over the entire
substrate concentration range. This description of a
mixed mode interaction is equally applicable to inhibition
of steroid 5- α -reductase in the presence of two
15 different molecular species which interact independently
with the different enzyme forms, E-NADPH and E-NADP⁺.
Thus, curve D also describes the inhibition by two
compounds, each at concentrations equivalent to their
inhibition constants for the E-NADPH and E-NADP⁺
20 complexes. Again, the result is superior to that of the
single inhibitor throughout the entire concentration range
of substrate.

Such additive inhibitory effects of a competitive
inhibitor (N-t-butyl-5- α -androst-1-ene-4-aza-17 β -
25 carboxamide-3-one, compound A) and an uncompetitive
inhibitor (N,N-diisopropyl-androst-3,5-diene-17 β -
carboxamide-3-carboxylic acid, compound B) have been
demonstrated in vitro with double inhibition experiments;
increasing concentrations of one inhibitor in the presence
30 of the second induces greater enzyme inhibition (Figure
3), while also demonstrating mutually exclusive binding.
For a given concentration of one of the inhibitors, which
binds to E-NADPH (competitive) or E-NADP⁺ (uncompetitive),
supplementation with any amount of the second would
35 increase the observed enzyme inhibition over that of the
reference inhibitor alone.

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FIGURE 3

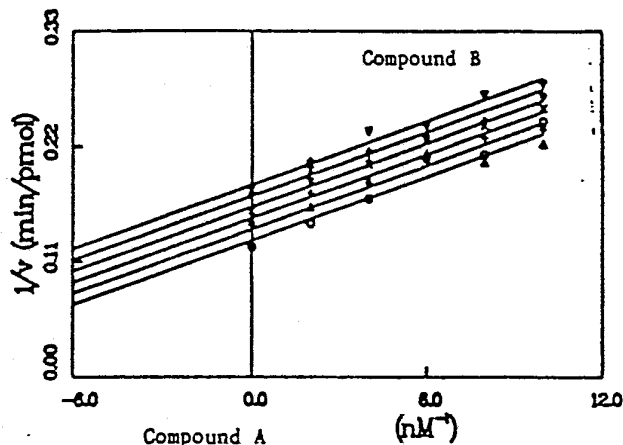


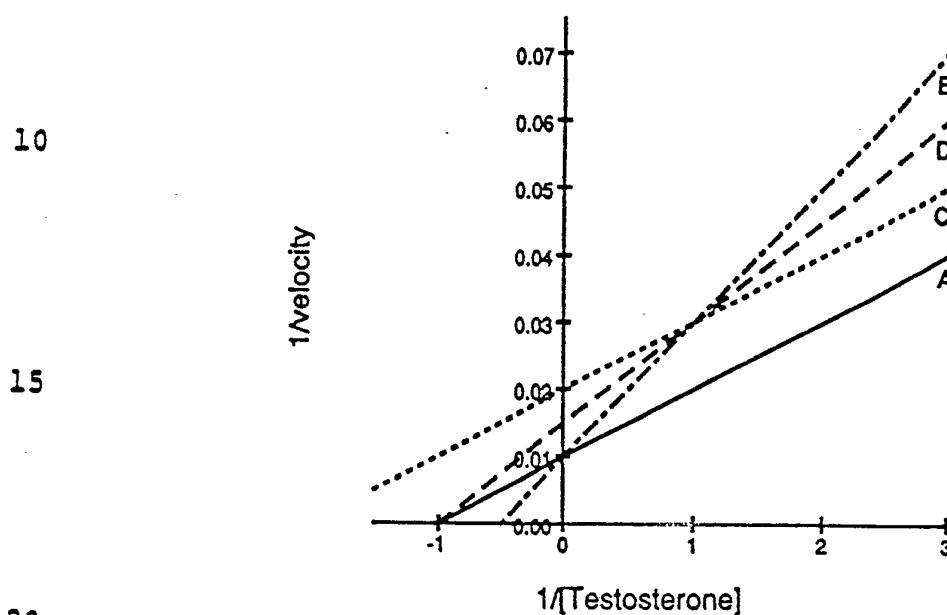
Figure 3. Inhibition of Rat Liver Steroid 5- α -Reductase: Effects of compound A Plus compound B. Rat liver steroid 5- α -reductase was assayed at constant concentrations of T (1.0 μ M) and NADPH (100 μ M) in the presence of a cofactor regenerating system. The concentrations of compound A were varied from 0 to 10 nM in the presence of 0(o), 3(Δ), 5(+), 9(x), 12 ($\diamond$), and 15(∇) nM where $v_i = v_o/[1 + I/K_I + J/K_J + IJ/BK_IK_J]$.

In comparison, Figure 4 represents a model incorporating conservation of drug substance. Here, the calculated curves are based on the total drug substance relative to its respective inhibition constant: total inhibitor = constant = $\Sigma([inhibitor]/K_{i,app})$. Curve A represents the uninhibited velocity, curves B and C represent the presence of only competitive or uncompetitive inhibitors, respectively, while curve D presents conservation of inhibitor substance composed of half competitive and uncompetitive inhibitors. Here, combination of inhibitors which function by the two different mechanisms is superior to the competitive inhibitors if the substrate concentration exceeds its K_m , and is more efficient than the uncompetitive

1 inhibitors at low substrate concentration below K_m . A
similar analysis versus the other substrate, NADPH, would
demonstrate no difference in inhibition efficiency since
both kinetic models are described by equation 2.

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FIGURE 4



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Figure 4. Model for Inhibition of
Steroid 5- α -Reductase with Compounds
that Bind to E-NADPH and E-NADP⁺:
Conservation of Inhibitor Substance.

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No prior art of combining 5- α -reductase
inhibitors of different mechanistic types into a
pharmaceutical composition to achieve superior results is
known to the applicants.

30

Description of the Invention

A combination selected from "competitive" and
"uncompetitive" inhibitors of 5- α -reductase are used in
a pharmaceutical composition to affect the enzymatic
reduction of testosterone to DHT. The "competitive"
35 inhibiting components of this invention are compounds

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1 which form an enzyme-NADPH-inhibitor dead-end complex and
the "uncompetitive" inhibiting components of this
invention are compounds which form an enzyme-NADP⁺-
inhibitor dead-end complex as described above.

5 Also included are derivatives of these compounds
which may either give rise to the parent compounds in vivo
or be useful themselves, such as pharmaceutically
acceptable addition salts. Salts of these compounds
containing a basic group are formed with organic or
10 inorganic acids in the presence of a basic compound by
methods known to the art. For example, the compound is
reacted with an inorganic or organic acid in an aqueous
miscible solvent such as ethanol with isolation of the
salt by removing the solvent or in an aqueous immiscible
15 solvent when the acid is soluble therein, such as ethyl
ether or chloroform, with the desired salt separating
directly or isolated by removing the solvent. Exemplary
of the acid addition salts which are included in this
invention are maleate, fumarate, lactate, oxalate, methane-
20 sulfonate, ethanesulfonate, benzenesulfonate, tartrate,
citrate, hydrochloride, hydrobromide, sulfate, phosphate
and nitrate salts. Pharmaceutically acceptable base
addition salts of compounds of the invention containing an
acidic group are prepared by known methods from organic
25 and inorganic bases include nontoxic alkali metal and
alkaline earth bases, for example, calcium, sodium, and
potassium hydroxide; ammonium hydroxide, and nontoxic
organic bases such as triethylamine, butylamine,
piperazine, and (trihydroxymethyl)methylamine. Prodrug
30 derivatives include O-esters, especially the tri-O-lower
alkanoyl ester having from 2-8 carbon atoms in each
alkanoyl group; O-methyl ethers or sulfate esters.
Separated R and S stereoisomers are also useful.

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1 Compounds that are considered to be competitive
steroid 5- α -reductase inhibitors as described above
include: 3-oxa-4-aza steroidal compounds such as

5 N-t-butyl-5- α -androst-1-ene-4-aza-17 β -carbox-
amide-3-one,

 (20R)-hydroxymethyl-4-methyl-4-aza-5- α -
pregnane-3-one,

 17 β -N,N-diisopropylcarboxamide-5- α -8(14)-
androst-4-methyl-4-aza-3-one, and

10 17 β -N-t-butylcarboxamide-5- α -8(14)-androst-4-
methyl-4-aza-3-one.

Also 3-nitro steroidal compounds have been found to be
competitive inhibitors including:

 17 β -N,N-diisopropylcarboxamide-3-nitro-5- α -
15 androst-3-ene,

 17 β -N-t-butylcarboxamide-3-nitro-5- α -androst-3-
ene, and

 17 β -N,N-diisopropylcarboxamide-3-nitro-5 α -
androst-2-ene.

20 Compounds that are considered to be uncompetitive
steroid 5- α -reductase inhibitors as described above
include:

 N-t-butyl-androst-3,5-diene-17 β -carboxamide-3-
carboxylic acid or a salt thereof,

25 N,N-diisopropyl-androst-3,5-diene-17 β -carboxamide-
3-carboxylic acid or a salt thereof,

 17 β -(N,N-diisopropylcarboxamide)-estra-1,3,5(10)-
triene-3-carboxylic acid or a salt thereof,

 17 β -(N-t-butylcarboxamide)-estra-1,3,5(10)-triene-
30 3-carboxylic acid or a salt thereof,

 17 β -(N,N-diisopropylcarboxamide)-estra-1,3,5(10)-
triene-3-sulfonic acid or a salt thereof,

 20- α -(hydroxymethyl)-A-nor-5- α -pregn-1-ene-2-
carboxylic acid or a salt thereof,

35 17 β -(N-t-butylcarboxamide)-estra-1,3,5(10)-triene-
3-sulfonic acid or a salt thereof,

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- 1 17 β -(N,N-diisopropylcarboxamide)estra-1,3,5(10)-
 triene-3-phosphonic acid or a salt thereof,
 17 β -(N-t-butylcarboxamide)-estra-1,3,5(10)-triene-
 3-phosphonic acid or a salt thereof,
5 17 β -(N,N-diisopropylcarboxamide)estra-1,3,5(10)-
 triene-3-phosphonic acid or a salt thereof,
 17 β -(N-t-butylcarboxamide)-estra-1,3,5(10)-triene-
 3-phosphonic acid or a salt thereof,
 17 β -N-t-butylcarboxamide-androst-3,5-diene-3-
10 phosphonic acid or a salt thereof,
 17 β -N,N-diisopropylcarboxamide-androst-3,5-diene-3-
 phosphonic acid or a salt thereof,
 17 β -N-t-butylcarboxamide-androst-3,5-diene-3-
 phosphonic acid or a salt thereof, and
15 17 β -N,N-diisopropylcarboxamide-androst-3,5-diene-3-
 phosphonic acid or a salt thereof.

 Persons skilled in the art can readily determine
 if an inhibitor is a competitive or an uncompetitive type
 by the method set forth above. All such compounds are
20 included within the scope of this invention.

 Because the claimed combinations inhibit steroid
 5- α -reductase activity, they have therapeutic utility in
 treating diseases and conditions wherein decreases in DHT
 activity produce the desired therapeutic effect. Such
25 diseases and conditions include acne vulgaris, seborrhea,
 female hirsutism, prostate diseases such as benign
 prostatic hypertrophy, and male pattern baldness.

 Certain combinations of the invention were tested
 for their in vivo potency in inhibiting steroid
30 5- α -reductase activity.

 When male rats are castrated and allowed to go
 untreated for 1 month, the ventral prostates involute to
 approximately 10% of its original total cell number. If
 exogenous testosterone is given to these castrated rats
35 to restore the physiological serum level of testosterone,
 the involuted ventral prostate rapidly proliferates

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- 1 restoring its original cell content within 2-3 weeks.
Using the ability to inhibit this testosterone induced
regrowth of the rat ventral prostate, the efficacy and
potency of twice daily oral administering of the
5 "competitive" inhibitor and the "uncompetitive" inhibitor
individually and in combination were compared.

The competitive inhibitor being N-t-butyl-5- α -
androst-1-ene-4-aza-17 β -carboxamide-3-one (Compound A) and
the uncompetitive inhibitor being N-t-butyl-androst-3,5-
10 diene-17 β -carboxamide-3-carboxylic acid (Compound C).

To perform these experiments, a total of 50 male
Sprague Dawley rats (from Harlan Sprague-Dawley, Inc.) of
250-300 gm starting weight were used. Forty-five of these
animals were castrated and allowed to go untreated for 1
15 month to produce maximal ventral prostatic and seminal
vesicle involution. After this 1 month period, 40 of the
45 castrated animals were implanted subcutaneously in the
flank with a 2.5 cm long testosterone filled silastic
capsule. The 50 animals were set up in 10 groups as
20 follows:

- | | | |
|------------|---|--|
| Group 1 | - | intact rats fed twice a day with vehicle alone (intact controls). |
| 25 Group 2 | - | castrated rats fed twice a day with vehicle and not implanted with testosterone capsule (castrate controls). |
| Group 3 | - | castrated rats implanted with a testosterone capsule fed twice a day with vehicle (restoration control). |
| 30 Group 4 | - | castrated rats plus testosterone implant plus uncompetitive inhibitor (BID) 12.5 mg/kg. |
| Group 5 | - | castrated rats plus testosterone implant plus uncompetitive inhibitor (BID) 25 mg/kg. |
| 35 Group 6 | - | castrated rats plus testosterone implant plus uncompetitive inhibitor (BID) 50 mg/kg. |
| Group 7 | - | castrated rats plus testosterone implant plus competitive inhibitor (BID) 12.5 mg/kg. |

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- 1 Group 8 - castrated rats plus testosterone implant
plus competitive inhibitor (BID) 25 mg/kg.
- Group 9 - castrated rats plus testosterone implant
plus competitive inhibitor (BID) 50 mg/kg.
- 5 Group 10 - castrated rats plus testosterone implant
plus uncompetitive inhibitor (BID) 50 mg/kg
plus competitive inhibitor (BID) 50 mg/kg.

The animals were administered the 5- α -reductase inhibiting compounds twice a day (BID) for 10 consecutive
10 days. The test compound was dissolved in propylene glycol and diluted in normal saline. At the end of the treatment period blood was collected from the animals and then they were sacrificed, the ventral prostates were excised and weighed, and DHT levels were measured by the following
15 procedure.

Prostate tissue was excised, trimmed, weighed, minced and washed with phosphate buffer. The tissue then was homogenized in phosphate buffer and extracted by addition of ethyl acetate and mixing on an orbital mixer
20 for forty-five minutes. The ethyl acetate was evaporated, the residue was reconstituted in ethanol, and was centrifuge filtered using 0.45 μ M filter paper. The components then were separated using reverse-phase HPLC collecting the DHT fraction. The fraction was reduced to
25 dryness and reconstituted in standard commercial DHT assay buffer. DHT levels then were measured using standard techniques such as radioimmunoassay.

In the rats treated with increasing amounts of a single inhibitor the realized prostate weight reduction
30 plateaued at significantly higher levels than in the castrated control model, while in the combination-treated rats the prostate weights were reduced to castrate levels. This plateau dose-response effect regarding the competitive inhibitor compound A has also been reported in
35 other studies. (Stoner, et al. (1987). Endocrinology, 120:774). Thus, the administration of the claimed

1 combinations produces prostate weight reduction levels
significantly lower than can be obtained by a single
inhibitor alone.

The claimed combinations are incorporated into
5 convenient dosage forms such as capsules, tablets, or
injectable preparations. Solid or liquid pharmaceutical
carriers are employed. Solid carriers include starch,
lactose, calcium sulfate dihydrate, terra alba, sucrose,
talc, gelatin, agar, pectin, acacia, magnesium stearate,
10 and stearic acid. Liquid carriers include syrup, peanut
oil, olive oil, saline, and water. Similarly, the carrier
or diluent may include any prolonged release material,
such as glyceryl monostearate or glyceryl distearate,
alone or with a wax. The amount of solid carrier varies
15 widely but, preferably, will be from about 25 mg to about
1 g per dosage unit. When a liquid carrier is used, the
preparation will be in the form of a syrup, elixir,
emulsion, soft gelatin capsule, sterile injectable liquid
such as an ampoule, or an aqueous or nonaqueous liquid
20 suspension.

The pharmaceutical preparations are made
following conventional techniques of a pharmaceutical
chemist involving mixing, granulating, and compressing,
when necessary, for tablet forms, or mixing, filling and
25 dissolving the ingredients, as appropriate, to give the
desired oral or parenteral products.

The pharmacokinetic properties of each active
component of the claimed invention must be contemplated
when formulating conventional dosage regimens. To
30 maximize its synergistic effect, the individual compounds
of the claimed combinations can be administered as a
single pharmaceutical composition or consecutively in
separate pharmaceutical compositions. One or more
components can be incorporated into a timed release dosage
35 unit form in which several doses are treated for delayed
or sustained release of the medicament. Such dosage units

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- 1 may comprise sustained release granules, sugar centered
spheres or multilayered tablets in each of which the
availability of the active ingredient is controlled by
coating with a lipid or polymeric material.
- 5 Doses of the present combinations in a
pharmaceutical dosage unit as described above will be an
efficacious, nontoxic quantity selected from the range of
0.1 - 1000 mg/kg of each active compound, preferably 1-100
mg/kg. The selected dose is administered to a human
10 patient in need of steroid 5- α -reductase inhibition from
1-6 times daily, topically, orally, rectally, by
injection, or continuously by infusion. Oral dosage units
for human administration preferably contain from 1 to 500
mg of active compound. Parenteral administration, which
15 uses lower dosages is preferred. Oral administration, at
higher dosages, however, also can be used when safe and
convenient for the patient.

The method of this invention of inhibiting
steroid 5- α -reductase activity in mammals, including
20 humans, comprises administering internally to a subject in
need of such inhibition an effective combination of
steroid 5- α -reductase inhibiting compounds having both
competitive and uncompetitive mechanisms of action.

Following are the results of testing the combina-
25 tions of this invention:

Table I

Effects of various treatments with N-t-butyl-5-
30 α -androst-1-ene-4-aza-17 β -carboxamide-3-one (compound A)
and N-t-butyl-androst-3,5-diene-17 β -carboxamide-3-
carboxylic acid (compound C) on the testosterone induced
regrowth of the involuted ventral prostate and seminal
vesicle of castrated male rats.

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Table I

Group No.	Treatment (N=5 rats/group)	Ventral Prostate weight (mg/gland)	Ventral Prostate DNA (μ g/gland)	Seminal Vesicle weight (mg/gland)	Seminal Vesicle DNA (μ g/gland)
35					
30					
25					
20					
15					
10					
5					
1					
Group 1	None (Intact control)	639 $\pm$ 54	1443 $\pm$ 109	332 $\pm$ 38	1083 $\pm$ 63
Group 2	Castrated (castrate control)	26 $\pm$ 3	165 $\pm$ 21	57 $\pm$ 3	508 $\pm$ 78
Group 3	Castrated + testosterone (restoration control) Castrated + testosterone + compound C	416 $\pm$ 32	817 $\pm$ 97	319 $\pm$ 19	1000 $\pm$ 121
Group 4	BID - 12.5 mg/kg	154 $\pm$ 22	359 $\pm$ 36	158 $\pm$ 15	851 $\pm$ 47
Group 5	BID - 25 mg/kg	143 $\pm$ 17	383 $\pm$ 29	170 $\pm$ 19	890 $\pm$ 58
Group 6	BID - 50 mg/kg	147 $\pm$ 15	316 $\pm$ 52	159 $\pm$ 20	855 $\pm$ 63
Group 7	Castrated + testosterone + compound A				
Group 7	BID - 12.5 mg/kg	71 $\pm$ 11	289 $\pm$ 17	118 $\pm$ 5	692 $\pm$ 51
Group 8	BID - 25 mg/kg	55 $\pm$ 7	290 $\pm$ 25	108 $\pm$ 10	708 $\pm$ 72
Group 9	BID - 50 mg/kg	62 $\pm$ 7	284 $\pm$ 19	108 $\pm$ 7	698 $\pm$ 39
Group 10	Castrated + testosterone + compound C (BID) 50 mg/kg + compound A (BID) 50 mg/kg	33* $\pm$ 7	169* $\pm$ 13	88* $\pm$ 3	540* $\pm$ 42

* Statistically significant

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1 The data in the above table demonstrate the
synergistic effects of the "competitive" and "uncompetitive"
steroid 5- α -reductase inhibitor combination on blocking
testosterone induced regrowth of the involuted rat ventral
5 prostate. Additionally, data was obtained showing that the
testosterone levels were unaffected in all groups except in
the castrate control.

10 The following examples illustrate preparation of
the claimed combinations and pharmaceutical compositions
containing these compounds. The examples are not intended
to limit the scope of the invention as defined hereinabove
and as claimed below.

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EXAMPLE 1

An oral dosage form for administering the claimed
compounds and compositions is produced by screening, mixing
and filling into hard gelatin capsules the ingredients in
20 the proportions shown in Table II below.

Table II

<u>Ingredients</u>	<u>Amounts</u>
25 N-t-butyl-5- α -androst-1-ene- 4-aza-17 β -carboxamide-3-one	100 mg
N-t-butyl-androst-3,5-diene-17 β - carboxamide-3-carboxylic acid	100 mg
30 Magnesium stearate	20 mg
Lactose	300 mg

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EXAMPLE 2

The sucrose, calcium sulfate dihydrate and claimed compounds and compositions shown in Table III
5 below, are mixed and granulated in the proportions shown with a 10% gelatin solution. The wet granules are screened, dried, mixed with the starch, talc and stearic acid, screened and compressed into a tablet.

10

Table III

<u>Ingredients</u>	<u>Amounts</u>
N-t-butyl-5- α -androst-1-ene-	100 mg
15 4-aza-17 β -carboxamide-3-one	
N-t-butyl-androst-3,5-diene-17 β -	100 mg
carboxamide-3-carboxylic acid	
Calcium sulfate dihydrate	300 mg
Sucrose	40 mg
20 Starch	20 mg
Talc	10 mg
Stearic acid	6 mg

EXAMPLE 3

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N-t-butyl-5- α -androst-1-ene-4-aza-17 β -carbox-
amide-3-one, 75 mg and N-t-butyl-androst-3,5-diene-17 β -
carboxamide-3-carboxylic acid, 75 mg, are dispersed in 25
ml of normal saline to prepare an injectable preparation.

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EXAMPLE 4

The following compounds (expressed as base
weight) are mixed together with 250 mg of lactose and 10
35 mg of magnesium stearate then filled into a hard gelatin

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1 capsule. These capsules are administered to a patient in need of steroid 5- α -reductase inhibiting activity from 1-6 times daily.

- 5 A. N-t-butyl-5- α -androst-1-ene-4-aza-17 β -carboxamide-3-one, 100 mg; N-t-butyl-androst-3,5-diene-17 β -carboxamide-3-carboxylic acid, 100 mg.
- 10 B. N-t-butyl-5- α -androst-1-ene-4-aza-17 β -carboxamide-3-one, 100 mg; 17 β -(N-t-butylcarboxamide)-ester-1,3,5(10)-triene-3-carboxylic acid, 100 mg.
- 15 C. 17 β -N-t-butylcarboxamide-3-nitro-5- α -androst-3-ene, 100 mg; N-t-butyl-androst-3,5-diene-17 β -carboxamide-3-carboxylic acid, 100 mg.
- 20 D. 17 β -N-t-butylcarboxamide-3-nitro-5- α -androst-3-ene, 100 mg; 17 β -(N-t-butylcarboxamide)-ester-1,3,5(10)-triene-3-carboxylic acid, 100 mg.

While the preferred embodiments of the invention are illustrated by the above, it is to be understood that the invention is not limited to the precise instructions herein disclosed and that the right to all modifications
25 coming within the scope of the following claims is reserved.

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What is claimed is:

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1. A composition comprising:

(a) an uncompetitive inhibitor of steroid 5- α -reductase, and

(b) a competitive inhibitor of steroid 5- α -reductase.

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2. The composition of claim 1 in which the quantity of the uncompetitive inhibitor is selected from 0.1-1000 mg/kg and the quantity of the competitive inhibitor is selected from 0.1-1000 mg/kg.

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3. The composition of claim 1 in which the competitive inhibitor is:

N-t-butyl-5- α -androst-1-ene-4-aza-17 β -carboxamide-3-one;

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17 β -N,N-diisopropylcarboxamide-3-nitro-5- α -androst-3-ene;

(20R)-hydroxymethyl-4-methyl-4-aza-5- α -pregnane-3-one;

17 β -N-t-butylcarboxamide-3-nitro-5- α -androst-3-ene;

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17 β -N,N-diisopropylcarboxamide-3-nitro-5- α -androst-2-ene;

17 β -N,N-diisopropylcarboxamide-5- α -8(14)-androst-4-methyl-4-aza-3-one; or

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17 β -N-t-butylcarboxamide-5- α -8(14)-androst-4-methyl-4-aza-3-one.

4. The composition of claim 1 in which the uncompetitive inhibitor is:

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N-t-butyl-androst-3,5-diene-17 β -carboxamide-3-carboxylic acid or a salt thereof;

N,N-diisopropyl-androst-3,5-diene-17 β -carboxamide-3-carboxylic acid or a salt thereof;

17 β -(N,N-diisopropylcarboxamide)-estra-1,3,5(10)-triene-3-carboxylic acid or a salt thereof;

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- 1 17 β -(N-t-butylcarboxamide)-estra-1,3,5(10)-triene-
3-carboxylic acid or a salt thereof;
 17 β -(N,N-diisopropylcarboxamide)-estra-
1,3,5(10)-triene-3-sulfonic acid or a salt thereof;
5 17 β -(N-t-butylcarboxamide)-estra-
1,3,5(10)-triene-3-sulfonic acid or a salt thereof;
 17 β -(N,N-diisopropylcarboxamide)estra-1,3,5(10)-
triene-3-phosphonic acid or a salt thereof;
 17 β -(N-t-butylcarboxamide)-estra-1,3,5(10)-triene-
10 3-phosphonic acid or a salt thereof;
 17 β -(N,N-diisopropylcarboxamide)estra-1,3,5(10)-
triene-3-phosphinic acid or a salt thereof;
 20- α -(hydroxymethyl)-A-nor-5- α -pregn-1-ene-2-
carboxylic acid or a salt thereof;
15 17 β -(N-t-butylcarboxamide)-estra-1,3,5(10)-triene-
3-phosphinic acid or a salt thereof;
 17 β -N-t-butylcarboxamide-androst-3,5-diene-3-
phosphinic acid or a salt thereof;
 17 β -N,N-diisopropylcarboxamide-androst-3,5-diene-3-
20 phosphinic acid or a salt thereof;
 17 β -N-t-butylcarboxamide-androst-3,5-diene-3-
phosphonic acid or a salt thereof; or
 17 β -N,N-diisopropylcarboxamide-androst-3,5-diene-3-
25 phosphonic acid or a salt thereof.

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5. A composition according to claim 1 substantially as hereinbefore defined with reference to any one of the Examples.

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6. A pharmaceutical composition comprising a composition according to any one of claims 1 to 5, and a pharmaceutically acceptable carrier.

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7. A composition according to any one of claims 1 to 5 for use as an active therapeutic substance.

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8. A composition according to any one of claims 1 to 5 for use in inhibiting steroid 5- α -reductase activity.

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9. Use of a composition according to any one of claims 1 to 5 in the manufacture of a medicament for use in the inhibition of steroid 5- α -reductase activity.

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10. A method of inhibiting steroid 5- α -reductase activity in mammals, which comprises the administration to mammals in need of such treatment, an effective amount of a composition according to anyone of claims 1 to 5.

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11. The method of inhibiting steroid 5- α -reductase activity in mammals in need thereof which comprises administering to the subject consecutively, inseparate pharmaceutical compositions, a competitive inhibitor of steroid 5- α -reductase and an uncompetitive inhibitor of steroid 5- α -reductase.

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US91/01740

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³		
According to International Patent Classification (IPC) or to both National Classification and IPC IPC(5): A01N 45/00; A01N 33/26 U.S.: 514/170, 169, 150		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁴		
Classification System	Classification Symbols	
U.S	514/170, 169, 150	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁵		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴		
Category ⁶	Citation of Document, ¹⁵ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
X	US, A, 4,684,635 (ORENTREICH ET AL) 04 AUGUST 1987 Column 1, line 19 column 3 line 6.	1,2,5-11
Y	US, A, 4,317,817 (BLOHM ET AL) 02 MARCH 1982 Column 2, line 7 to column 6 line 10.	3 and 4
[*] Special categories of cited documents: ¹⁵ "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search ¹	Date of Mailing of this International Search Report ²	
06 MAY 1991	22 MAY 1991	
International Searching Authority ¹	Signature of Authorized Official ¹⁹ NCOC-BO	
ISA/US	INTERNATIONAL DIVISION FREDERICK E. WADDELL <i>Nguyen</i>	