(72) LABRÉE, FERNAND, CA
(72) BELANGER, ALAIN, CA
(72) GAUTHIER, SYLVAIN, CA
(72) LUU-THE, VAN, CA
(72) MERAND, YVES, CA
(72) POIRIER, DONALD, CA
(72) PROVENCHER, LOUIS, CA
(72) SINGH, SHANKAR MOHAN, CA
(71) ENDORECHEURUE, INC., CA
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(54) INHIBITEURS DE LA 17β-HYDROXYSTEROIDE
DESHYDROGENASE DU TYPE 5 ET DU TYPE 3 ET
METHODES D'UTILISATION ASSOCIÉES
(54) INHIBITORS OF TYPE 5 AND TYPE 3 17β-HYDROXYSTEROID
DEHYDROGENASE AND METHODS FOR THEIR USE

(57) L'invention concerne de nouvelles méthodes de
traitement médical et/ou d'inhibition du développement
de maladies, lesquelles méthodes concernent des
maladies sensibles à l'activité androgène et oestrogène.
Lesdits traitements font appel à des inhibiteurs de la 17β-
hydroxystéroïde déshydrogénase du type 5 et/ou du type
3. L'invention concerne également de nouveaux
inhibiteurs de la 17β-hydroxystéroïde déshydrogénase
du type 5 et de nouveaux inhibiteurs de la 17β-
hydroxystéroïde déshydrogénase du type 3.

(57) Novel methods of medical treatment and/or
inhibition of development of diseases are disclosed for
diseases that are sensitive to androgenic or estrogenic
activity. The treatments utilize inhibitors of type 5
and/or type 3 17β-hydroxysteroid dehydrogenase. Novel
inhibitors of type 5 17β-hydroxysteroid dehydrogenase
are also disclosed, as are novel inhibitors of type 3 17β-
hydroxysteroid dehydrogenase.
INHIBITORS OF TYPE 5 AND TYPE 3 17β-HYDROXysteroid Dehydrogenase AND METHODS FOR THEIR USE

Abstract

Novel methods of medical treatment and/or inhibition of development of diseases are disclosed for diseases that are sensitive to androgenic or estrogenic activity. The treatments utilize inhibitors of type 5 and/or type 3 17β-hydroxysteroid dehydrogenase. Novel inhibitors of type 5 17β-hydroxysteroid dehydrogenase are also disclosed, as are novel inhibitors of type 3 17β-hydroxysteroid dehydrogenase.
DEMANDES OU BREVETS VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME _1_ DE _2_

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JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME _1_ OF _2_

NOTE: For additional volumes please contact the Canadian Patent Office
INHIBITORS OF TYPE 5 AND TYPE 3 17β-HYDROXYSTEROID DEHYDROGENASE AND METHODS FOR THEIR USE

FIELD OF THE INVENTION

The present invention relates to inhibitors of enzymes involved in the biosynthesis of sex steroids from natural precursors and to their use in the treatment of sex steroid dependent diseases. In particular, inhibitors are disclosed which suppress the activity of type 3 and type 5 17β-hydroxysteroid dehydrogenase, thus diminishing the production of androgens catalyzed by these two enzymes. Pharmaceutical use of the inhibitors may reduce the natural production of androgens such as testosterone and dihydrotestosterone, and thereby beneficially treat diseases whose onset or progress is aided by androgenic activity. Because androgens formed by reactions catalyzed by type 3 or type 5 enzyme are precursors to estrogens, the invention also has applicability to diseases whose onset or progress is aided by estrogenic activity.
BACKGROUND OF THE RELATED ART

Many androgen-sensitive diseases, i.e. diseases whose onset or progress is aided by androgenic activity, are known. They include but are not limited to prostate cancer, benign prostatic hyperplasia, acne, seborrhea, hirsutism, androgenic alopecia, precocious puberty, adrenal hyperplasia, and polycystic ovarian syndrome. Estrogen sensitive diseases, i.e. diseases whose onset or progress is aided by estrogenic activity are also known. They include but are not limited to breast cancer, endometriosis, leiomyoma, and precocious puberty.

Precocious puberty is usually associated with an excess of androgen secretion, usually of adrenal origin. The current treatments include blockade of adrenal secretion by glucocorticoids with its associated side effects. Blockade of type 5 17β-HSD would be an advantage, thus desirably reducing the dose or avoiding the use of glucocorticoids. Another treatment is the use of LHRH agonists to cause medical castration. A better controlled inhibition of androgen formation could be achieved with type 5 and 3 17β-HSD inhibitors.

Polycystic ovarian syndrome is associated with an excess of androgen secretion by the ovaries. LHRH agonists are used among other, as treatment, to cause medical castration. The use of an inhibitor of 17β-HSD would be advantageous.

Estrogen sensitive diseases may vary in their responses to androgens. They may, for example, respond favorably, unfavorably or not at all to androgens. Likewise, androgen-sensitive diseases may respond differently to estrogens. Thus, the treatment of sex steroid sensitive
diseases may involve increasing or decreasing androgenic activity depending on whether the disease in question responds favorably or unfavorably to androgenic activity. Treatment may also involve increasing or decreasing estrogenic activity depending on whether the disease in question responds favorably or unfavorably to estrogenic activity. Breast cancer, for example, is known to respond favorably to androgenic activity and negatively to estrogenic activity. Benign prostatic hyperplasia is believed to respond negatively to both androgenic and estrogenic activity.

Androgenic and estrogenic activity may be suppressed by administering androgen receptor antagonists ("antiandrogens") or estrogen receptor antagonists ("antiestrogens"), respectively. See e.g. WO 94/26767 and WO 96/26201. Androgenic and estrogenic activity may also be reduced by suppressing androgen or estrogen biosynthesis using inhibitors of enzymes that catalyze one or more steps of such biosynthesis or by suppressing ovarian or testicular secretions by known methods. See e.g. WO 90/10462, WO 91/00731, WO 91/00733, and WO 86/01105. Type 5 17β-hydroxysteroid dehydrogenase is described in WO 97/11162.

Effective inhibitors of type 5 17β-hydroxysteroid dehydrogenase enzyme are provided by the present invention. The prior art is not believed to have provided compounds sufficiently effective at simultaneously (1) inhibiting type 5 or type 3 17β-hydroxysteroid dehydrogenase while (2) desirably failing to substantially inhibit other 17β-hydroxysteroid dehydrogenases or other catalysts of sex steroid degradation. Medroxyprogesterone acetate, megestrol acetate, and chlormadinone acetate which the prior art as used as pharmaceutical agents for other
purposes are not believed to have been disclosed as inhibitors of type 5 17β-hydroxysteroid dehydrogenase, although applicants' research has now shown them to inhibit that type 5 enzyme.

SUMMARY OF THE INVENTION

It is accordingly an object of the present invention to more selectively and effectively inhibit type 3 and/or type 5 17β-hydroxysteroid dehydrogenase while preferably avoiding inhibition of other 17β-hydroxysteroid dehydrogenases, type 1 or 2 3α-hydroxysteroid dehydrogenases, or other androgen degradation enzymes.

It is another object to provide novel inhibitors of types 3 and 5 17β-hydroxysteroid dehydrogenase and pharmaceutical compositions thereof.

It is another object to provide treatment and prevention regimens for androgen and estrogen sensitive diseases which regimens include inhibiting activity of type 3 or type 5 17β-hydroxysteroid dehydrogenase.
In one embodiment, the invention provides a method of inhibiting activity of type 5 17β-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

![Molecule Structure](image)

wherein the dotted line is optional pi bond;

wherein A is selected from the group consisting of straight or branched C₁-C₄ alkyl, -OR⁺ (R⁺ being a C₁-C₆ alkyl radical), and -N(R⁺⁺)R⁺⁺⁺ (R⁺⁺ and R⁺⁺⁺ being independently hydrogen or C₁-C₆ alkyl or aryl), and unsaturated analogs of any of the foregoing definitions for substituent A;

wherein R¹ is selected from the group consisting of hydrogen and methyl;

wherein R⁶ is selected from the group consisting of hydrogen, and halogen, and C₁-C₃ alkyl;

wherein R⁸ is selected from the group consisting of straight or branched C₁-C₃ alkylene, C₃-C₇ cycloalkylene; and

R⁸⁺ is selected from the group consisting of hydrogen, substituted or unsubstituted phenyl, C₂-C₁₀ acyl, C₂-C₁₀ acyloxy, C₂-C₁₀ alkoxycarbonyl, and halogen;

provided that when A is methyl, R⁺ and R⁻ together have at least two carbon atoms, and R¹ is methyl.
It is preferred that the optional pi bond at 6 is present that R⁶ is methyl, that R⁴ is C₁-C₆ alkylene or that A is either methyl or -N(R⁴)R⁴.

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It is also preferred when wherein A is -N(R⁴)R⁴ that R⁴ is methyl or that R⁴ is C₁-C₆ alkyl or C₇-C₁₂ phenyl alkyl.

In another embodiment, the invention provides a method of inhibiting activity of type 5 17β-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

![Chemical Structure](image)

wherein the dotted line is an optional pi bond;

wherein R¹⁶₈ is selected from the group consisting of hydrogen, fluoro, chloro, C₁-C₃ alkyl, C₁-C₄ haloalkyl, a moiety which together with R¹⁶₄ is C₄-C₇ spirocycloalkyl, C₄-C₇ halospirocycloalkyl, or \( \text{R}^{16} \) (\( \text{R}^{16} \) being C₁-C₃ alkyl) and unsaturated analogs of any of the foregoing definitions of R¹⁶₈;

wherein R¹⁶₄ is selected from the group consisting of hydrogen, C₁-C₃ alkyl, C₁-C₄ haloalkyl, a moiety which together with R¹⁶₈ forms
C₄-C₇ spirocycloalkyl, C₄-C₇ halospirocycloalkyl, or \( \text{R}^{16} \) (\( \text{R}^{16} \) being C₁-C₃ alkyl) and unsaturated analogs of any of the foregoing;

wherein \( \text{R}^{15a} \) is selected from the group consisting of hydrogen, C₁-C₄ alkyl, C₁-C₄ alkenyl and C₁-C₄ alkynyl;

wherein \( \text{R}^{19} \) is either \( \cdot \text{H} \) or \( \cdot \text{CH}_3 \); and

wherein \( \text{R}^{6} \) is selected from the group consisting of \( \cdot \text{H} \), \( \cdot \text{CH}_3 \), and halo;

Provided that \( \text{R}^{16a} \), \( \text{R}^{16b} \), and \( \text{R}^{15a} \) are not simultaneously hydrogen.

It is preferred that \( \text{R}^{16a} \) is a larger substituent than \( \text{R}^{16b} \), that \( \text{R}^{6} \) is hydrogen, that the optional pi bond at position 1 is not present or that \( \text{R}^{16a} \) is a C₃-C₅ alkyl chain.

In another embodiment, the invention provides a method of inhibiting activity of type 5 17β-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

![Molecular structure diagram]

wherein the dotted line is optional pi bond

wherein X is C₁-C₃ alkyl;

wherein Y is hydrogen or an acyloxy moiety;
wherein $R^6$ is $\text{H}$ or $\text{CH}_3$;
wherein $R^{16}$ is $\text{H}$ or halo;
wherein $R^1$ is $\text{H}$ or $\text{CH}_3$.

5

It is preferred that $R^6$ is methyl that the optional pi bond at position 1 is present, that $Y$ is a C$_3$-C$_6$ fluoroacyloxy or that $X$ is methyl.

In another embodiment, the invention provides a method of inhibiting activity of type 5 17β-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

![Molecular Structure](image)

15

wherein $n$ is an integer from 1-2;
wherein the dotted lines are optional double bonds;
wherein $X$ and $Y$ are independently selected from the group consisting of $\text{H}$, (C$_1$-C$_3$)alkyl, (C$_2$-C$_3$) alkenyl, and methoxycarbonyl;
wherein $Z$ is selected from the group consisting of $\text{H}$ and (C$_1$-C$_3$)alkyl;
wherein $R^3$ is selected from the group consisting of hydrogen, acyl, carboxyl, alkoxy carbonyl, substituted or unsubstituted carboxamide,
cyano, alkoxy, alkoxyalkoxy, alkythioalkoxy, acyloxy; hydroxy, halo, -O-SO₂R⁸ (R⁸ being selected from the group consisting of C₁-C₅ alkyl and C₆-C₁₀ aryl), and a moiety which, together with R₂, is a 5-6 member ring containing at least one oxygen and one nitrogen atom;

wherein R² is selected from the group consisting of hydrogen, amido, acyloxy, carboxyl, carboxamide, alkoxy carbonyl, cyano, halo, nitro, C₁-C₅ alkyl, and CF₃ and a moiety which, together with R₃, is a 5-6 member ring containing at least one oxygen and one nitrogen atom;

wherein R⁴ is hydrogen or halo;

wherein R⁶ is selected from the group consisting of hydrogen and oxo;

wherein R⁹ is H or -OH;

provided that X, Y, and Z are not all hydrogens when R³ is methoxy.

It is preferred that R³ is alkoxyalkoxy, carboxamide, carboxyl or alkoxy, that at least one of X, Y or Z is methyl, that both X and Y are methyl, that n is 1 or that R⁶ is oxo.
In another embodiment, the invention provides a method of inhibiting activity of type 5 17β-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

![Molecular structure diagram]

wherein the dotted lines are optional pi bonds;
wherein \( n = 1 \) or \( 2 \); and
wherein \( a \) is either \( -H \) or \( -CH_3 \);
wherein \( b \) and \( c \) are independently hydrogen or methyl;
wherein \( Z \) is oxygen or sulfur.

It is preferred that \( n \) is 1, that at least one of \( b \) or \( c \) is methyl, both \( b \) and \( c \) are methyl or that \( Z \) is oxygen.
In another embodiment, the invention provides a method of inhibiting activity of type 5 17\textbeta\)-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 17\textbeta\)-hydroxysteroid dehydrogenase selected from the group consisting of:

![EM-1291](image)

![EM-1195-CS](image)

![CS-243](image)
EM-1165

and

EM-1122-CS

It is preferred that the type 5 inhibitor is selected from the group consisting of:

EM-1404
In another embodiment, the invention provides a method of inhibiting activity of type 5 17β-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase selected from the group consisting of:

- Medroxyprogesterone acetate
- Megestrol acetate
- Chlormadinone acetate
- 1-dehydromegestrol acetate
and compounds having the molecular structure:

wherein the dotted lines are optional pi bonds;

wherein A is selected from the group consisting of straight or branched C₁-C₄ alkyl, -OR⁵ (R⁵ being a C₁-C₄ alkyl radical), and -N(R⁶)R⁷ (R⁶ and R⁷ being independently hydrogen or C₁-C₈ alkyl or aryl), and unsaturated analogs of any of the foregoing definitions for substituent A;

wherein R¹ is selected from the group consisting of hydrogens, and methyl;
wherein R⁶ is selected from the group consisting of hydrogen, halogen, and C₁-C₅ alkyl;

wherein R₁₆ is selected from the group consisting of H, H and CH₂;

wherein R₆ is selected from the group consisting of straight or branched C₁-C₅ alkylene, C₃-C₇ cycloalkylene; and R₅ is selected from the group consisting of hydrogen, substituted or unsubstituted phenyl, C₂-C₁₀ acyl, C₂-C₁₀ acyloxy, C₂-C₁₀ alkoxy carbonyl, and halogen.

In another embodiment, the invention provides a method of inhibiting activity of type 5 17β-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

![Chemical Structure](image)

wherein the dotted line are optional pi bonds;

wherein R₁⁶ is selected from the group consisting of hydrogen, fluoro, chloro, C₁-C₅ alkyl, C₁-C₅ haloalkyl, a moiety which together with R₈ forms C₄-C₇ spirocycloalkyl, C₄-C₇ halospirocycloalkyl, or \( R^{16} \) (R¹₆ being C₁-C₅ alkyl) and unsaturated analogs of any of the foregoing definitions of R₁⁶.
wherein $R^{16a}$ is selected from the group consisting of hydrogen, $C_1$-$C_8$ alkyl, $C_1$-$C_8$ haloalkyl, a moiety which together with $R^{16b}$ forms $C_4$-$C_7$ spirocycloalkyl $C_4$-$C_7$ halospirocycloalkyl, or $R^{16}$ (being $C_1$-$C_3$ alkyl) and unsaturated analogs of any of the foregoing;

wherein $R^{15a}$ is selected from the group consisting of hydrogen, $C_1$-$C_4$ alkyl, $C_1$-$C_4$ alkenyl and $C_1$-$C_4$ alkynyl;

wherein $R^{19}$ is either $\cdot H$ or $\cdot CH_3$; and

wherein $R^6$ is selected from the group consisting of $\cdot H$, $\cdot CH_3$, and halo;

provided that $R^{16b}$, $R^{16a}$, and $R^{15a}$ are not simultaneously hydrogen.

In another embodiment, the invention provides a method of inhibiting activity of type 5 17β-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

\[
\begin{align*}
\text{wherein } n &= 1 \text{ or } 2; \\
\text{wherein the dotted lines are optional pi bonds;} 
\end{align*}
\]
wherein X and Y are independently selected from the group consisting of \( \cdot H, (\text{C}_1-\text{C}_3)\text{alkyl}, (\text{C}_2-\text{C}_3)\text{alkenyl}, \) and methoxycarbonyl;

wherein Z is selected from the group consisting of \( \cdot H \) and \( (\text{C}_1-\text{C}_3)\text{alkyl}; \)

wherein \( R^3 \) is selected from the group consisting of acyl, carboxyl, alkoxy carbonyl, substituted or unsubstituted carboxamide, cyano, alkoxy, alkoxyalkoxy, alkythioalkoxy, acyloxy; hydroxy, halo, \(-\text{O-SO}_2\text{R}^4\) \( (R^4 \text{ being selected from the group consisting of } \text{C}_1-\text{C}_5 \text{ alkyl and } \text{C}_6-\text{C}_{10} \text{ aryl}), \) and a moiety which, together with \( R_2 \), is a 5-6 member ring containing at least one oxygen and one nitrogen atom;

wherein \( R^2 \) is selected from the group consisting of amido, acyloxy, carboxyl, carboxamide, alkoxy carbonyl, cyano, halo, nitro, \( \text{C}_1-\text{C}_5 \text{ alkyl and CF}_3 \) and a moiety which, together with \( R_3 \), is a 5-6 member ring containing at least one oxygen and one nitrogen atom;

wherein \( R^4 \) is hydrogen or halo;

wherein \( R^6 \) is selected from the group consisting of hydrogen and oxo;

wherein \( R^9 \) is \( \cdot H \) or \( \cdot \text{OH}; \)

provided that \( X, Y, \) and \( Z \) are not hydrogens when \( R^3 \) is methoxy.

It is preferred that \( R^3 \) is alkoxyalkoxy, carboxamide, carboxyl or alkoxy, that at least one of \( X, Y \) or \( Z \) is methyl, that both \( X \) and \( Y \) are methyl, that \( n \) is 1 or that \( R^6 \) is oxo.
In another embodiment, the invention provides a method of inhibiting activity of type 5 17β-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

\[
\begin{align*}
\text{wherein the dotted lines are optional pi bonds;} \\
\text{wherein } n = 1 \text{ or } 2; \text{ and} \\
\text{wherein } a \text{ is either } \cdot \text{H or } \cdot \text{CH}_3; \\
\text{wherein } b \text{ and } c \text{ are independently hydrogen or methyl;} \\
\text{wherein } Z \text{ is oxygen or sulfur.}
\end{align*}
\]
In another embodiment, the invention provides a method of inhibiting activity of type 5 17β-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

![Chemical Structure](image)

wherein the dotted line is an optional pi bond;

wherein A is selected from the group consisting of straight or branched C₁-C₄ alkyl, -OR (R being a C₁-C₅ alkyl radical), and -N(R⁴)R⁵ (R⁴ and R⁵ being independently hydrogen or C₁-C₅ alkyl or aryl), and unsaturated analogs of any of the foregoing definitions for substituent A;

wherein R¹ is selected from the group consisting of hydrogen, methyl, and ethyl;

wherein R⁶ is selected from the group consisting of hydrogen, halogen, and C₁-C₅ alkyl;

wherein R⁸ is selected from the group consisting of straight or branched C₁-C₅ alkylene, C₃-C₇ cycloalkylene; and

R⁹ is selected from the group consisting of hydrogen, substituted or unsubstituted phenyl, C₂-C₁₀ acyl, C₂-C₁₀ acyloxy, C₂-C₁₀ alkoxycarbonyl, and halogen;

provided that when A is methyl, R¹ is methyl.
In another embodiment, the invention provides a method of inhibiting activity of type 5 17β-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

![Molecular Structure Image]

wherein the dotted lines are optional π bonds
wherein X is C1-C3 alkyl;
wherein Y is hydrogen or an acyloxy moiety;
wherein R^6 is ·H or ·CH₃;
wherein R^16 is ·H or halo;
wherein R^1 is ·H or ·CH₃.
In another embodiment, the invention provides a pharmaceutical composition comprising a pharmaceutically acceptable excipient, diluent or carrier and a therapeutically acceptable amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

\[
\begin{align*}
\text{A} & \quad \text{O} \\
\text{R} & \quad \text{R}^1 \\
\text{R}^a & \quad \text{R}^b
\end{align*}
\]

wherein the dotted lines are optional \(\pi\) bonds;

wherein \(A\) is selected from the group consisting of straight or branched \(C_1-C_4\) alkyl, \(-OR^c\) (\(R^c\) being a \(C_1-C_8\) alkyl radical), and \(-N(R^d)R^e\) (\(R^d\) and \(R^e\) being independently hydrogen or \(C_1-C_8\) alkyl or aryl), and unsaturated analogs of any of the foregoing definitions for substituent \(A\);

wherein \(R^1\) is selected from the group consisting of hydrogen and methyl;

wherein \(R^a\) is selected from the group consisting of hydrogen, halogen, and \(C_1-C_8\) alkyl;

wherein \(R^b\) is selected from the group consisting of straight or branched \(C_1-C_8\) alkylene, \(C_3-C_7\) cycloalkylene; and

\(R^b\) is selected from the group consisting of hydrogen, substituted or unsubstituted phenyl, \(C_2-C_{10}\) acyl, \(C_2-C_{10}\) acyloxy, \(C_2-C_{10}\) alkoxy carbonyl, and halogen;

provided that when \(A\) is methyl, \(R^a\) and \(R^b\) together have at least two carbon atoms, and \(R^1\) is methyl.
It is preferred that the optional pi bond at 6 is present, that R⁶ is methyl, that R⁵ is C₁-C₆ alkylene or that A is either methyl or -N(R⁴)R⁶.

It is also preferred when A is -N(R⁴)R⁶, that R⁴ is methyl or that R⁶ is C₁-C₆ alkyl or phenyl C₁-C₆ alkyl.

In another embodiment, the invention provides a pharmaceutical composition comprising a pharmaceutically acceptable excipient, diluent or carrier and a therapeutically acceptable amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

![Chemical Structure](image)

wherein the dotted line is an optional pi bond;

wherein R¹⁶β is selected from the group consisting of hydrogen, fluoro, chloro, C₁-C₃ alkyl, C₁-C₃ haloalkyl, a moiety which together with R¹⁶α is C₄-C₇ spirocycloalkyl, C₄-C₇ halospirocycloalkyl, or ———R¹⁶ (R¹⁶ being C₁-C₃ alkyl) and unsaturated analogs of any of the foregoing definitions of R¹⁶β;

wherein R¹⁶α is selected from the group consisting of hydrogen, C₁-C₃ alkyl, C₁-C₃ haloalkyl, a moiety which together with R¹⁶β forms C₄-C₇ spirocycloalkyl, C₄-C₇ halospirocycloalkyl, or ———R¹⁶ (R¹⁶ being C₁-C₃ alkyl) and unsaturated analogs of any of the foregoings.
wherein $R^{15a}$ is selected from the group consisting of hydrogen, $C_1$-$C_4$ alkyl, $C_1$-$C_4$ alkenyl and $C_1$-$C_4$ alkynyl;

wherein $R^{19}$ is either $\cdot$H or $\cdot$CH$_3$; and

wherein $R^6$ is selected from the group consisting of $\cdot$H, $\cdot$CH$_3$, and halo;

provided that $R^{16b}$, $R^{16a}$, and $R^{15a}$ are not simultaneously hydrogen.

It is preferred that $R^{16a}$ is a larger substituent than $R^{16b}$ that $R^6$ is hydrogen, that the optional pi bond at position 1 is not present or that $R^{16a}$ is a $C_3$-$C_5$ alkyl chain.

In another embodiment, the invention provides a pharmaceutical composition comprising a pharmaceutically acceptable excipient, diluent or carrier and a therapeutically acceptable amount of an inhibitor of type 5 17$\beta$-hydroxysteroid dehydrogenase having the molecular structure:

![Molecular Structure]

wherein the dotted line is an optional pi bond
wherein $X$ is $C_1$-$C_3$ alkyl;

wherein $Y$ is hydrogen or an acyloxy moiety;

wherein $R^6$ is $\cdot$H or $\cdot$CH$_3$;

wherein $R^{16}$ is $\cdot$H or halo;

wherein $R^1$ is $\cdot$H or $\cdot$CH$_3$. 
It is preferred that \( R^6 \) is methyl, that the optional pi bond at position 1 is present, that \( Y \) is a \( C_5-C_6 \) fluoroacyloxy or that \( X \) is methyl.

In another embodiment, the invention provides a pharmaceutical composition comprising a pharmaceutically acceptable excipient, diluent or carrier and a therapeutically acceptable amount of an inhibitor of type 5 17\( \beta \)-hydroxysteroid dehydrogenase having the molecular structure:

\[
\begin{align*}
\text{wherein } n & \text{ is an integer from 1-2;} \\
\text{wherein the dotted lines are optional pi bonds;} \\
\text{wherein } X \text{ and } Y & \text{ are independently selected from the group consisting of } -H, (C_1-C_3)\text{alkyl}, (C_2-C_3)\text{ alkenyl}, \text{ and methoxycarbonyl;} \\
\text{wherein } Z & \text{ is selected from the group consisting of } -H \text{ and } (C_1-C_3)\text{alkyl;} \\
\text{wherein } R^3 & \text{ is selected from the group consisting of hydrogen, acyl, carboxyl, alkoxy carbonyl, substituted or unsubstituted carboxamide, cyano, alkoxy, alkoxyalkoxy, alkylthioalkoxy, acyloxy; hydroxy, halo, } -O-SO_2R^* \text{ (} R^* \text{ being selected from the group consisting of } C_1-C_6 \text{ alkyl and } C_6-C_{10} \text{ aryl), and a moiety which, together with } R_2, \text{ is a 5-6 member ring containing at least one oxygen and one nitrogen atom;}
\end{align*}
\]
wherein R² is selected from the group consisting of hydrogen, amido, acyloxy, carboxyl, carboxamide, alkoxy carbonyl, cyano, halo, nitro, C₁-C₅ alkyl, and CF₃ and a moiety which, together with R₃, is a 5-6 member ring containing at least one oxygen and one nitrogen atom;

wherein R⁴ is hydrogen or halo;

wherein R⁶ is selected from the group consisting of hydrogen and oxo;

wherein R⁸ is -H or -OH,

provided that X, Y, and Z are not all hydrogen when R³ is methoxy.

It is preferred that R³ is alkoxyalkoxy, carboxamide, carboxyl or alkoxyl, that at least one of X, Y or Z is methyl, that both X and Y are methyl, that n is 1 or that R⁶ is oxo.

In another embodiment, the invention provides a pharmaceutical composition comprising a pharmaceutically acceptable excipient, diluent or carrier and a therapeutically acceptable amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:
wherein the dotted lines are optional pi bonds;
wherein \( n = 1 \) or \( 2 \); and
wherein \( a \) is either \( \cdot \text{H} \) or \( \cdot \text{CH}_3 \);
wherein \( b \) and \( c \) are independently hydrogen or methyl;
wherein \( Z \) is oxygen or sulfur.

It is preferred that \( n \) is 1, that at least one of \( b \) or \( c \) is methyl, that both \( b \) and \( c \) are methyl or that \( Z \) is oxygen.

In another embodiment, the invention provides a pharmaceutical composition comprising a pharmaceutically acceptable excipient, diluent or carrier and a therapeutically acceptable amount of an inhibitor of type 5 17\( \beta \)-hydroxysteroid dehydrogenase having a molecular structure selected from the group consisting of:

\[
\begin{align*}
\text{EM-1291} \\
\text{EM-1195-CS}
\end{align*}
\]
EM-1159

EM-1165

and

EM-1122-CS
It is preferred that the type 5 inhibitor is selected from the group consisting of:

EM-1404

and

EM-1394

In another embodiment, the invention provides a pharmaceutical composition comprising a pharmaceutically acceptable excipient, diluent or carrier and a therapeutically acceptable amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:
wherein the dotted line is an optional pi bond;

wherein A is selected from the group consisting of straight or branched C₃-C₄ alkyl, -OR (R being a C₁-C₅ alkyl radical), and -N(R⁴)R⁶ (R⁴ and R⁶ being independently hydrogen or C₁-C₅ alkyl or aryl), and unsaturated analogs of any of the foregoing definitions for substituent A;

wherein R¹ is selected from the group consisting of hydrogen and methyl;

wherein R⁶ is selected from the group consisting of hydrogen, and halogen, and C₁-C₅ alkyl;

wherein R⁴ is selected from the group consisting of straight or branched C₁-C₅ alkylene, C₃-C₇ cycloalkylene; and

R⁵ is selected from the group consisting of hydrogen, substituted or unsubstituted phenyl, C₂-C₁₀ acyl, C₂-C₁₀ acyloxy, C₂-C₁₀ alkoxy carbonyl, and halogen;

provided that when A is methyl, R¹ is methyl or ethyl.

In another embodiment, the invention provides a pharmaceutical composition comprising a pharmaceutically acceptable excipient, diluent or carrier and a therapeutically acceptable amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:
wherein the dotted lines are optional pi bonds

wherein X is C₁-C₃ alkyl;

wherein Y is hydrogen or an acyloxy moiety;

wherein R⁶ is -H or -CH₃;

wherein R¹⁶ is -H or halo;

wherein R¹ is -H or -CH₃.

In another embodiment, the invention provides an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

![Molecular Structure Image]

wherein the dotted line is optional pi bond;

wherein A is selected from the group consisting of straight or branched C₁-C₄ alkyl, -OR⁸ (R⁸ being a C₁-C₃ alkyl radical), and -N(R⁶)R⁸ (R⁶ and R⁸ being independently hydrogen or C₁-C₃ alkyl or aryl), and unsaturated analogs of any of the foregoing definitions for substituent A;

wherein R¹ is selected from the group consisting of hydrogen and methyl;

wherein R⁶ is selected from the group consisting of hydrogen, and halogen, and C₁-C₃ alkyl;

wherein R⁸ is selected from the group consisting of straight or branched C₁-C₃ alkylene, C₅-C₇ cycloalkylene; and
R\textsuperscript{b} is selected from the group consisting of hydrogen, substituted or unsubstituted phenyl, C\textsubscript{2}-C\textsubscript{10} acyl, C\textsubscript{2}-C\textsubscript{10} acyloxy, C\textsubscript{2}-C\textsubscript{10} alkoxycarbonyl, and halogen;

provided that when A is methyl, R\textsuperscript{a} and R\textsuperscript{b} together have at least two carbon atoms, and R\textsuperscript{3} is methyl.

It is preferred that the optional pi bond at 6 is present, that R\textsuperscript{a} is methyl; or that R\textsuperscript{a} is C\textsubscript{1}-C\textsubscript{6} alkyne.

It is preferred that A is either methyl or -N(R\textsuperscript{d})R\textsuperscript{e}.

It is also preferred when A is N(R\textsuperscript{d})R\textsuperscript{e}, that R\textsuperscript{d} is methyl or that R\textsuperscript{e} is C\textsubscript{1}-C\textsubscript{6} alkyl or phenyl C\textsubscript{1}-C\textsubscript{6} alkyl.

In another embodiment, the invention provides an inhibitor of type 5 17\textbeta-hydroxysteroid dehydrogenase having the molecular structure:

wherein the dotted line is an optional pi bond;

wherein R\textsuperscript{16\textbeta} is selected from the group consisting of hydrogen, fluoro, chloro, C\textsubscript{1}-C\textsubscript{4} alkyl, C\textsubscript{1}-C\textsubscript{4} haloalkyl, a moiety which together with R\textsuperscript{16\alpha} is C\textsubscript{4}-C\textsubscript{7} spirocycloalkyl, C\textsubscript{4}-C\textsubscript{7} halo spirocycloalkyl, or \(=\)—R\textsuperscript{16} (R\textsuperscript{16} being C\textsubscript{1}-C\textsubscript{3} alkyl) and unsaturated analogs of any of the foregoing definitions of R\textsuperscript{16\textbeta}.
wherein $R^{16a}$ is selected from the group consisting of hydrogen, $C_1$-$C_3$ alkyl, $C_1$-$C_3$ haloalkyl and a moiety which together with $R^{16a}$ forms $C_4$-$C_7$ spirocycloalkyl, $C_4$-$C_7$ halospirocycloalkyl, or $R^{16}$ (being $C_1$-$C_3$ alkyl) and unsaturated analogs of any of the foregoings;

wherein $R^{15a}$ is selected from the group consisting of hydrogen, $C_1$-$C_4$ alkyl, $C_1$-$C_4$ alkenyl and $C_1$-$C_4$ alkynyl;

wherein $R^{18}$ is either $-\text{H}$ or $-\text{CH}_3$; and

wherein $R^6$ is selected from the group consisting of $-\text{H}$, $-\text{CH}_3$, and halo;

provided that $R^{16b}$, $R^{16a}$, and $R^{15a}$ are not simultaneously hydrogen.

It is preferred that $R^{16a}$ is a larger substituent than $R^{16b}$, that $R^6$ is hydrogen, that the optional pi bond at position 1 is not present or that $R^{16a}$ is a $C_3$-$C_5$ alkyl chain.

In another embodiment, the invention provides an inhibitor of type 5 $17\beta$-hydroxysteroid dehydrogenase having the molecular structure:

wherein the dotted line is optional pi bond

wherein $X$ is $C_1$-$C_3$ alkyl;

wherein $Y$ is hydrogen or an acyloxy moiety;

wherein $R^6$ is $-\text{H}$ or $-\text{CH}_3$;
wherein \( R^{16} \) is \( \cdot \text{H} \) or halo;

wherein \( R^1 \) is \( \cdot \text{H} \) or \( \cdot \text{CH}_3 \).

It is preferred that \( R^5 \) is methyl that the optional pi bond at position 1 is present, that \( Y \) is a \( C_3-C_6 \) fluoroacyloxy or that \( X \) is methyl.

In another embodiment, the invention provides an inhibitor of type 5 17\( \beta \)-hydroxysteroid dehydrogenase having the molecular structure:

\[
\begin{align*}
\text{(Diagram showing molecular structure)}
\end{align*}
\]

wherein \( n \) is an integer from 1-2;

wherein the dotted lines are optional pi bonds;

wherein \( X \) and \( Y \) are independently selected from the group consisting of \( \cdot \text{H}, (C_1-C_3)\text{alkyl}, (C_2-C_3)\text{ alkenyl}, \) and methoxycarbonyl;

wherein \( Z \) is selected from the group consisting of \( \cdot \text{H} \) and \( (C_1-C_3)\text{alkyl} \);

wherein \( R^3 \) is selected from the group consisting of hydrogen, acyl, carboxyl, alkoxycarbonyl, substituted or unsubstituted carboxamide, cyano, alkoxy, alkoxyalkoxy, alkythioalkoxy, acyloxy; hydroxy, halo, \(-\text{O-SO}_2 R^*\) (\( R^* \) being selected from the group consisting of \( C_1-C_6 \) alkyl and \( C_6-C_{10} \) aryl), and a moiety which, together with \( R_2 \), is a 5-6 member ring containing at least one oxygen and one nitrogen atom;
wherein $R^2$ is selected from the group consisting of hydrogen, amido, acyloxy, carboxyl, carboxamide, alkoxycarbonyl, cyano, halo, nitro, C$_1$-C$_8$ alkyl, and CF$_3$ and a moiety which, together with $R_3$, is a 5-6 member ring containing at least one oxygen and one nitrogen atom;

wherein $R^4$ is hydrogen or halo;
wherein $R^6$ is selected from the group consisting of hydrogen and oxo;

wherein $R^9$ is -H or -OH,
provided that $X$, $Y$, and $Z$ are not hydrogen when $R^3$ is methoxy.

It is preferred that $R^3$ is alkoxyalkoxy; carboxamide, carboxyl or alkoxy, that at least one of $X$, $Y$ or $Z$ is methyl, that $X$ and $Y$ are methyl, that $n$ is 1 or $R^6$ is oxo.

In another embodiment, the invention provides an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

![Chemical Structure]

wherein the dotted lines are optional pi bonds;
wherein $n = 1$ or 2; and
wherein $a$ is either -H or -CH$_3$;
wherein $b$ and $c$ are independently hydrogen or methyl;
wherein $Z$ is oxygen or sulfur.
It is preferred that \( n \) is 1, that at least one of \( b \) or \( c \) is methyl, that both \( b \) and \( c \) are methyl or that \( Z \) is oxygen.

In another embodiment, the invention provides an inhibitor of type 5 17\( \beta \)-hydroxysteroid dehydrogenase having a molecular structure selected from the group consisting of:

5

EM-1291

10

EM-1195-CS

CS-243
It is preferred that the type 5 inhibitor is selected from the group consisting of:
In another embodiment, the invention provides a method of inhibiting type 3 17β-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 3 17β-hydroxysteroid dehydrogenase having the molecular structure:

wherein R is selected from the group consisting of alkoxy, alkylthio, alkoxyalkoxy, alkoxyalkylthio, alkylthioalkoxy, and alkylthioalkylthio,
wherein \( n \) is an integer from 1 to 4.

It is preferred that the said type 3 inhibitor is selected from the group consisting of:

- EM-1071
- EM-1065
- EM-1066
- EM-1064
- EM-1074
- EM-1073
In another embodiment, the invention provides a pharmaceutical composition comprising a pharmaceutically acceptable excipient, diluent or carrier and a therapeutically acceptable amount of an inhibitor of type 3 17β-hydroxysteroid dehydrogenase having the molecular structure:

wherein R is selected from the group consisting of alkoxy, alkylthio, alkoxyalkoxy, alkoxyalkylthio, alkylthioalkoxy, and alkylthioalkylthio,
wherein n is an integer from 1 to 4.

It is preferred that the said type 3 inhibitor is selected from the group consisting of:

- **EM-1071**: $\text{H}_2\text{O}[(\text{CH}_2)_n\text{C}_6\text{H}_5]$  
- **EM-1065**: $\text{3CS(}\text{H}_2\text{C})_2\text{S}$  
- **EM-1066**: $\text{H}_3\text{COSH}_2\text{CO}$  
- **EM-1064**: $\text{3COH}_{2}\text{CS}$  
- **EM-1074**: $\text{3C(}\text{H}_2\text{C})_3\text{O}$  
- **EM-1073**: $\text{3CO(}\text{H}_2\text{C})_2\text{O}$
In another embodiment, the invention provides an inhibitor of type 3 17β-hydroxysteroid dehydrogenase having the molecular structure:

wherein R is selected from the group consisting of alkoxyethoxy, alkoxyalkythio, alkylthioalkoxy, and alkylthioalkylthio,
wherein n is an integer from 1 to 4.

It is preferred that the said type 3 inhibitor is selected from the group consisting of:

- EM-1071
- EM-1065
- EM-1066
- EM-1064
A patient in need of treatment or reducing the risk of onset of a given disease is one who has either been diagnosed with such disease or one who is particularly susceptible to acquiring such disease, for example, one at higher risk of acquiring the disease than the general population.

Except where otherwise stated, the preferred dosage of the active compounds of the invention is identical for both therapeutic and prophylactic purposes. The dosage for each active component discussed herein is the same regardless of the disease being treated (or prevented).
As used in the methods of medical treatment or methods of reduction of risk of onset of disease herein, an "inhibitor of type 5 or 3 17β-hydroxysteroid dehydrogenase" means a compound whose IC_{50} of inhibition for the enzyme in question (computed in the same manner as described in connection with Table 1 herein) is no higher than 500 nM. It is preferred that such inhibitor be no higher than 20 nM, most preferably lower than 10 nM. In some embodiments of the type 5 or type 3 inhibitor, it is also preferred that IC_{50} of undesirable inhibition of type 2 17β-hydroxysteroid dehydrogenase be more than 5 times that for inhibition of type 5, preferably more than 10 times, and most preferably more than 25 times. In some embodiments, it is preferred that the percentage of inhibition of the binding of [3H]R1881 on the androgen receptor (as described in D-Androgen Receptor (AR) assays, supra), by the inhibitor of type 5 or 3 17β-hydroxysteroid dehydrogenase at the concentration of 10^{-6} M be less than 30% most preferably less than 20%.

Where two or more different active agents are discussed as part of a combination therapy herein (e.g. an enzyme inhibitor and an antiandrogen), a plurality of different compounds are administered rather than a single compound having multiple activities.

Except where otherwise noted or where apparent from context, dosages herein refer to weight of active compounds unaffected by pharmaceutical excipients, diluents, carriers or other ingredients, although such additional ingredients are desirably included, as shown in the examples herein. Any dosage form (capsule, tablet, injection or the like) commonly used in the pharmaceutical industry, is appropriate for use herein, and the terms "ingredient", "diluent" or "carrier" include such non active
ingredients as are typically, included together with active ingredients, used in such dosage form in the industry.

**BRIEF DESCRIPTION OF THE DRAWINGS**

Figures 1a to 1d show autoradiographs of ³H-labelled type 5 17β-HSD antisense and sense riboprobes (35 base pairs) hybridized in situ to human benign prostatic hyperplasia (BPH) tissue.

- Figure 1a shows semithin Epon section (0.7 µm-thick) hybridized with the antisense probe. Epithelial cells lining the tube-alveoli as well as some of the surrounding stromal cells are labelled. In the epithelium, the dashed line approximately indicates the boundary between the basal and the luminal cells. The basal cells are intensively labelled in comparison with the luminal cells where only a few grains are seen. (X 1000)

- Figure 1b shows similar area from the same prostate hybridized with the sense probe as a control. Only scattered silver grains can be detected. (X 1000)

- Figure 1c shows the antisense probe generates strong radioautographic signals in the wall of blood vessels (arrows). (X 600)

- Figure 1d shows similar blood vessels hybridized with the sense probe as a control. No significant labeling could be seen. (X 600)

Figure 2 shows the specificity of the antiserum used in the immunostaining of type 5 17β-HSD. Transfected human kidney cells (293) with human type 5 17β-HSD, types 1 and 3 3α-HSD and types 1 and 2 5α-reductase, were used for immunoblot analysis. The results indicate that the antiserum specifically reacts with type 5 17β-HSD. Types 1 and 3 3α-HSD which share 84 and 86% amino acid identity with type 5 17β-HSD,
respectively, and types 1 and 2 5α-reductase, two other androgen-synthesizing enzymes present at high concentration in prostatic tissue were used as controls.

Figure 3a to 3e show paraffin sections of normal human prostate and cultured epithelial cells (PrEC 5500-1) immunostained with antibody to type 5 17β-HSD (X 800).

- Figure 3a shows the staining reaction is observed in basal cells (arrows) and in most of the stromal cells situated below. The luminal cells of the epithelium (above the basal cells) are not reactive.

- Figure 3b shows all epithelial cells are immunoreactive.

- Figure 3c shows the basal cells are not very well seen (thick arrows) but they are labelled as well as some luminal cells (thin arrows) of the tube-alveoli.

- Figure 3d shows an immunostained paraffin section of normal human prostate epithelial cells (PrEC 5500-1). The staining reaction can be seen in the cytoplasm of most of the cells.

- Figure 3e shows the same cultured cells as in figure 3d, but the antiserum was incubated with an excess of antigen. No staining reaction can be seen.

Figures 4a to g show paraffin sections of BPH tissue immunostained with antibody to type 5 17β-HSD.

- Figure 4a shows all of the basal cells show a strong positive staining reaction (arrowheads). Note the absence of reaction in the luminal cells (L) while the fibroblasts of the stroma are stained (arrows) (X 500).

- Figure 4b shows some of the luminal cells are immunoreactive (arrows) as well as basal cells (arrowheads) (X500).
- Figure 4c shows a section consecutive to that shown in fig. 4a. Immunoabsorption of the antiserum with an excess of antigen completely prevents immunostaining (X500).

- Figure 4d shows, in the stroma, smooth muscle cells (arrows) are not labeled while the surrounding fibroblasts are stained (X800).

- Figure 4e shows the positive staining in the wall of a large vein (V). The endothelial cells of small blood vessels are well-labeled (arrows) (X 800).

- Figure 4f shows the low magnification of an artery showing the labelling of endothelial cells (arrows) and of fibroblasts of the tunica adventitia (arrowheads) while smooth muscle cells of tunica media (m) are weakly labeled (X 200).

- Figure 4g shows the high magnification of an artery which clearly demonstrates the labelling in the cytoplasm of endothelial cells (arrows) as well as of fibroblasts (arrow heads) while smooth muscle cells of the tunica media (m) are not labeled (X 800).

Figures 5a to e show paraffin sections of BPH tissue immunostained with antibodies for type 5 17β-HSD (a), 3β-HSD (b) and androgen receptor (c, d and e) (X800).

- Figures 5a and b show consecutive sections immunostained for type 5 17β-HSD (5a) and 3β-HSD (5b). Although the reaction for 3β-HSD is somewhat weaker than that obtained for type 5 17β-HSD, the distribution of the two enzymes in basal (arrowheads) and luminal cells (L) is similar.

- Figure 5c shows an androgen receptor immunoreactivity was found to be exclusively localized in the nuclei. In the epithelium, the reaction can be seen in the majority of the luminal cells (L) nuclei, but not in the nuclei of most basal cells (arrowheads).
- Figure 5d shows, in the fibromuscular stroma, most of the nuclei are labeled. Some smooth muscle cell nuclei are labeled (arrows) while others show not detectable reaction (arrowheads).

- Figure 5e shows the wall of the blood vessels shows labeled and unlabeled nuclei. Some of the nuclei of the endothelial cells lining the lumen of an arteriole are labeled (arrows), while others show no staining (arrowheads).

Figure 6 shows human skin hybridized with type 5 17β-HSD cRNA probe.

Figure 7 shows monkey ovary hybridized with type 5 17β-HSD.

Figure 8 is a schematic diagram showing the biosynthetic pathway of active androgens in human prostatic tissue.

**DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS**

There are several types of 17β-hydroxysteroid dehydrogenases. Of the various types of 17β-hydroxysteroid dehydrogenases, type 1 catalyzes conversion of estrone to estradiol. Types 2 and 4 catalyze the reverse reaction. Type 1 also catalyzes conversion of DHEA to 5-diol while type 4 catalyzes the reverse reaction. Types 3 and 5 catalyze conversion of 4-dione to testosterone while type 2 catalyzes the reverse reaction. Types 3 and 5, the primary concern of the present application, catalyze in vivo the biosynthesis of androgenic compounds, such as the biosynthesis of testosterone from androst-4-ene-dione ("4-dione") or the conversion of androstanedione (A-dione) to DHT. The reaction catalyzed by type 3 or
type 5 17β-hydroxysteroid dehydrogenase is a reversible reaction, and the reverse reaction can be catalyzed by type 2 17β-hydroxysteroid dehydrogenase, whose activity is preferably not inhibited. It is also desirable to avoid inhibition, for example, of type 4 17β-hydroxysteroid dehydrogenase which desirably (for androgen sensitive diseases) converts a different testosterone precursor to another compound.

For purposes of treating diseases which respond unfavorably to androgenic activity, it is desirable to inhibit androgen formation without hindering androgen degradation. Thus, in one aspect, the present invention endeavors to reduce the level of androgens by selectively inhibiting the type 3 and type 5 17β-hydroxysteroid dehydrogenases while desirably allowing the type 2 and type 4 enzymes to remain free of inhibition. Thus, the equilibrium of the reversible androgen (or androgen precursor) synthesis reactions is beneficially affected by inhibiting the forward androgen synthesis reactions while permitting any androgens or precursors that are synthesized (either locally or systemically) to be reconverted to their precursors by action of the type 2 and/or type 4 17β-hydroxysteroid dehydrogenases.

The type 5 enzyme is better inhibited by certain compounds discussed in more detail infra, while the type 3 enzyme is better inhibited by other compounds (also discussed infra). It is therefore possible to provide best inhibition of androgen synthesis by a combination therapy which includes administering both a type 5 inhibitor and a type 3 inhibitor.

Type 5 enzyme tends to predominate in liver, adrenal glands, prostate, and the peripheral tissues whereas type 3 enzyme tends to predominate
in the testes. In the prior art, testicular androgens have been suppressed by either surgical or chemical castration, frequently by administration of an LHRH agonist. The present invention provides inhibitors of type 3 17β-hydroxysteroid dehydrogenase which, like the LHRH agonist of the prior art, reduce testicular androgen production. Thus, in some embodiments of the invention, the type 3 inhibitors of the invention may be used to inhibit testicular androgen secretion, either alone or in combination with prior art chemical or surgical castration. In some embodiments, antiandrogen and/or 5α-reductase inhibitor is also provided. In some embodiments, the inhibitors of the type 3 enzyme are used in addition to chemical castration by LHRH agonist. An undesirable initial upward spike of androgenic production and secretion by the testes occurs during the very early portion of treatment with LHRH agonist, prior to the beginning of the beneficial effect of androgen reduction caused by LHRH agonist. Using type 3 17β-hydroxysteroid dehydrogenase inhibitor in conjunction with LHRH agonist is expected to reduce that initial undesirable androgen elevation.

Both androgen-related and estrogen-related diseases may be treated with the 17β-hydroxysteroid dehydrogenase inhibitors of the present invention. Androgen-sensitive diseases are those whose onset or progress is aided by androgen activation of androgen receptors, and should respond favorably to treatment with the present invention because of the reduction of androgen biosynthesis that is achieved thereby. Estrogen-sensitive diseases (diseases whose onset or progress is aided by activation of the estrogen receptor) should also benefit because many androgens whose biosynthesis is suppressed by the present invention are precursors to estrogens, and the present invention may therefore reduce
estrogen biosynthesis as well. Androgen-sensitive diseases include but are not limited to prostatic cancer, benign prostatic hyperplasia, acne, seborrhea, hirsutism, androgenic alopecia, and polycystic ovarian syndrome. Estrogen-sensitive diseases include but are not limited to breast cancer, endometrial cancer, endometriosis, and endometrial leiomyoma.

For the treatment of androgen-sensitive diseases, it is of course preferred that the inhibitors of 17β-hydroxysteroid dehydrogenase that are administered (whether type 5 or type 3 or both) do not possess inherent androgenic activity. However, breast cancer (and some other estrogen-sensitive diseases, e.g. ovarian cancer, uterine cancer, and endometrial cancer) respond favorably to androgens. Therefore, a compound which inhibits type 5 or type 3 17β-hydroxysteroid dehydrogenase, and which is also androgenic, can be especially useful for the treatment of breast cancer and other diseases which respond negatively to estrogen and positively to androgen.

Type 5 and/or type 3 inhibitors of 17β-hydroxysteroid dehydrogenase may, in accordance with the invention, be utilized as part of a combination therapy with other strategies (listed below) which, attack androgen- or estrogen-sensitive diseases through other mechanisms, thus providing synergistic combinations. These combination therapies include in addition to type 3 or type 5 inhibitors of 17β-hydroxysteroid dehydrogenase one or more of the following strategies:

**Strategy 1:** Suppression of testicular or ovarian hormonal secretion by chemical or surgical castration. This is useful in treatment of diseases
which respond adversely to androgen or estrogen, respectively. When surgical or chemical castration is utilized, chemical castration is preferred utilizing either an LHRH-agonist, an LHRH antagonist or inhibitor of type 3 17β-hydroxysteroid dehydrogenase (as discussed herein). Suitable LHRH agonists are reported in US Patent 4,659,695, but any LHRH agonist showing the ability to induce chemical castration can be used since they all act through the same mechanisms as described (Labrie et al., J. Androl. 1: 209-228, 1980). Dosages are known in the art. Some suitable LHRH antagonists are reported in U.S. Patent 4,666,885 but any LHRH antagonist is acceptable, if used according to the recommendation of the manufacturer.

**Strategy 2:** Utilizing androgen or estrogen receptor antagonists ("antiandrogens" or "antiestrogens") to prevent activation of androgen or estrogen receptors by androgens or estrogens. Strategy 2 is useful against diseases that respond adversely to androgenic or estrogenic activity, respectively. Antiandrogens, and dosages therefor, are known in the art (e.g. Flutamide (N-[4-nitro-3-(trifluoromethyl)phenyl]-2-methyl propanamide) at a dosage of 250 mg, 2 or 3 times a day, Nilutamide at a dosage of 150 mg/day, Casodex at a dosage of 50 to 500 mg/day or EM-250 of the following structure:
Synthesized and used as reported in International Publication WO94/26767.

When antiestrogens are used in accordance with the invention, either alone or as part of one of the combination therapies described herein, the attending clinician should, at least initially, use the dosages recommended by the manufacturer. However, the attending clinician should monitor individual patient response and metabolism and adjust patient dosage accordingly. Indeed, that will be true of all of the strategies discussed herein. One preferred antiestrogen is EM-800 reported in PCT/CA96/00097 (WO 96/26201) The molecular structure of EM-800 is:

![Molecular structure of EM-800](image)

Other suitable antiestrogens include but are not limited to Tamoxifen \((Z)-2-[4-(1,2-diphenyl-1-butenyl)]-N,N\text{-dimethylethanamine}\) and ICI 182780 (available from Zeneca, UK), Toremifene (available from Orion-Farmos Pharmaceuticals, Finland), Droluxifene (Pfizer Inc., USA), Raloxifene (Eli Lilly and Co., USA), LY 335563 and LY 353381 (Eli Lilly and Co., USA), Iodoxifene (SmithKline Beecham, USA), Levormeloxifene (Novo Nordisk, A/S, Denmark). Any antiestrogen used as required for efficacy, as recommended by the manufacturer, can be used. Appropriate dosages are known in the art. Any other antiestrogen commercially available can be used according to the invention.
Strategy 3: Suppression of sex steroid biosynthesis by inhibition of 3β-hydroxysteroid dehydrogenase [e.g. Trilostane (2α-cyano-4α,5α-epoxy-17β-hydroxyandrostan-3-one), Sterling-Winthrop Research Institute, Rensselaer, New York, USA] by administering such inhibitor at a dosage of 1 to 500 mg/day). Desirable serum levels of 3β-hydroxysteroid dehydrogenase inhibitor range from 5 ng/ml to 500 ng/ml, preferably 10 ng/ml to 100 ng/ml. Strategy 3 is useful against both (1) diseases that respond adversely to androgenic activity and (2) some diseases that respond adversely to estrogenic activity.

Strategy 4: Suppression of conversion of the androgen testosterone to the more potent androgen dihydrotestosterone (DHT) by inhibiting the activity of testosterone 5α-reductase (e.g. by administering Proscar, available from Merck Sharp and Dohme Canada, at the recommended dosage). Any other potent 5α-reductase inhibitor can be used. The dosage used can be 2 to 20 mg daily orally. The dosage should be the one recommended by the manufacturer. Strategy 4 is useful against diseases that respond adversely to androgenic activity.

Strategy 5: Utilizing an aromatase inhibitor to reduce estrogen production. Strategy 5 is useful against diseases that respond adversely to estrogenic activity or estrogen receptor-mediated exacerbation of the type of androgen-sensitive diseases that are also estrogen-sensitive diseases (e.g. benign prostatic hyperplasia). When aromatase inhibitors are used in accordance with the invention, either alone or as part of one of the combination therapies described herein, the attending clinician should initially use the dosage recommended by the manufacturer. When administered orally, the dosage which is usually effective to provide the
desired serum levels is between 1.0 mg and 20 mg of active ingredient per day per 50 kg of body weight. For example, Arimidex (Zeneca) is taken at the oral dose of 1 mg daily. However, the attending clinician should monitor individual patient response and metabolism and adjust patient dosage accordingly. Aromatase inhibitors include those set forth in US patent 5,227,375. Aromatase inhibition may also be achieved, for example, by administering Arimidex (2,2'-[5-(1H-1,2,4-triazol-1-ylmethyl)-1,3-phenylene bis (2-methylpropiononitrile)) available from Zeneca, UK, at a dosage of 1 mg/day. Any other aromatase inhibitor can be used according to the recommendations of the manufacturer.

Strategy 6: Administering an androgenic compound. Strategy 6 is useful against a disease which responds favorably to androgenic activity. Preferred androgens include but are not limited to medroxyprogesterone acetate, and megestrol acetate at a dosage of 5-200 mg/day. Desirable low dose and sustained release formulations of such androgens are described in US Patent 5,434,146.

Any of the above strategies useful against diseases that respond negatively to estrogenic activity may also be used to help alleviate estrogenic side effects that may result from treatment of purely androgen-sensitive diseases in accordance with the invention. For example, many androgen precursors are also estrogen precursors. Strategies which suppress formation of certain androgens may increase levels of precursors thereto which are also estrogen precursors thus undesirably increasing estrogen formation. For example, administering inhibitors of testosterone 5α-reductase to suppress conversion of testosterone to dihydrotestosterone may increase the conversion of testosterone to estradiol. In that case, strategy 5 may desirably reduce
that unwanted estrogen production. Strategy 2 (estrogen antagonist aspect) and strategy 3 may also be helpful in that regard.

In general, for both androgen-sensitive diseases and estrogen-sensitive diseases, simultaneous treatment with inhibitors of sex steroid biosynthesis (inhibitors of enzymes which catalyze one or more steps of estrogen or androgen biosynthesis), and with estrogen receptor antagonists and/or androgen receptor antagonists, are believed to have additive rather than redundant effect because they are acting in a beneficial manner by a different mechanism. Likewise, the activity of two different enzyme inhibitors (enzymes which catalyze one or more different steps of sex steroid biosynthesis) are believed to provide additive effect, especially where the inhibitors affect more than one synthetic pathway. Such an approach permits to achieve a more complete effect.

Different sex steroid-dependent diseases respond differently to both androgen receptor activation and estrogen receptor activation. For example, breast cancer responds unfavorably to estrogen receptor activation, but favorably to androgen receptor activation. On the other hand, benign prostatic hyperplasia responds unfavorably to activation of either the estrogen or androgen receptor. The type 5 and/or type 3 inhibitors of the invention may be used in any combination with any of the strategies 1-6 above whose effect (increasing or decreasing androgenic or estrogenic activity) is consistent with desirable effect on the disease in question. With that in mind, set forth below are a list of representative diseases which may be treated, or risk of which may be reduced, in accordance with the present invention. Beneath each disease, is several preferred combination therapies for treatment, or risk reduction, of that
particular disease. However, these combinations may be supplemented using one or more of the six strategies listed above, limited only by whether a particular disease responds favorably or adversely to estrogenic activity or to androgenic activity.

5  A) Prostate cancer (responds adversely to androgenic activity, favorably to estrogenic activity)
   1. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase + LHRH-agonist (or antagonist)
   2. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase + inhibitor of type 3 17β-hydroxysteroid dehydrogenase.
   3. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase + inhibitor of type 3 17β-hydroxysteroid dehydrogenase + LHRH agonist (or antagonist)
   4. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase + LHRH-agonist (or antagonist) + antiandrogen
   5. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase + inhibitor of type 3 17β-hydroxysteroid dehydrogenase + antiandrogen
   6. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase + inhibitor of type 3 17β-hydroxysteroid dehydrogenase + LHRH agonist (or antagonist) + antiandrogen
   7. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase + antiandrogen + 5α-reductase inhibitor + LHRH agonist (or antagonist)
   8. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase + LHRH agonist + 5α-reductase inhibitor
9. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase + inhibitor of type 3 17β-hydroxysteroid dehydrogenase + 5α-reductase inhibitor

10. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase + inhibitor of type 3 17β-hydroxysteroid dehydrogenase + antiandrogen + 5α-reductase inhibitor

11. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase + inhibitor of type 3 17β-hydroxysteroid dehydrogenase + LHRH agonist (or antagonist) + antiandrogen + 5α-reductase inhibitor

B) Benign prostatic hyperplasia (responds adversely to both androgenic activity and estrogenic activity)

1. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase

2. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase + antiestrogen or aromatase inhibitor

3. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase + antiandrogen

4. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase + antiandrogen + 5α-reductase inhibitor + antiestrogen or aromatase inhibitor

5. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase + 5α-reductase inhibitor

6. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase + antiandrogen + 5α-reductase inhibitor

7. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase + 5α-reductase inhibitor + antiestrogen or aromatase inhibitor
C) Acne, seborrhea, hirsutism, androgenic alopecia (responds adversely to androgenic activity)
   1. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase
   2. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase + antiandrogen
   3. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase + 5α-reductase inhibitor
   4. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase + 5α-reductase inhibitor + antiandrogen

D) Polycystic ovarian syndrome (responds adversely to androgenic stimulation)
   1. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase
   2. Inhibitor of type 3 17β-hydroxysteroid dehydrogenase + Inhibitor of type 5 17β-hydroxysteroid dehydrogenase
   3. Inhibitor of type 3 17β-hydroxysteroid dehydrogenase
   4. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase + antiandrogen
   5. Inhibitor of type 3 17β-hydroxysteroid dehydrogenase + Inhibitor of type 5 17β-hydroxysteroid dehydrogenase + antiandrogen
   6. Inhibitor of type 3 17β-hydroxysteroid dehydrogenase + antiandrogen

E) Breast cancer (responds favorably to androgenic activity, adversely to estrogenic activity)
   1. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase + antiestrogen
2. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase +
antiestrogen + androgenic compound

3. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase +
antiestrogen + LHRH agonist (or antagonist)

4. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase +
antiestrogen + LHRH-agonist (or antagonist) + androgenic
compound

F) Endometriosis, leiomyoma (responds adversely to estrogenic
activity)

1. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase

2. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase +
antiestrogen or inhibitor of aromatase

3. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase +
LHRH-agonist (or antagonist)

4. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase +
LHRH-agonist (or antagonist) + antiestrogen or aromatase
inhibitor

G) Precocious puberty (male and female) (responding adversely to
androgenic activity)

1. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase

2. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase +
LHRH agonist (or antagonist) (male only)

3. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase +
antiandrogen

4. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase +
antiandrogen + LHRH agonist (or antagonist) (male only)
5. Inhibitor of type 5 17β-hydroxysteroid dehydrogenase +
inhibitor of type 3 17β-hydroxysteroid dehydrogenase (male only)

6. Inhibitor of type 3 17β-hydroxysteroid dehydrogenase

7. Inhibitor of type 3 17β-hydroxysteroid dehydrogenase +
LHRH agonist (or antagonist) (male only)

8. Inhibitor of type 3 17β-hydroxysteroid dehydrogenase +
antiandrogen

9. Inhibitor of type 3 17β-hydroxysteroid dehydrogenase +
antiandrogen + LHRH agonist (or antagonist) (male only)

When type 5 17β-hydroxysteroid inhibitors are used in accordance with
the invention, either alone or as part of one of the combination therapies
described herein, the attending clinician desirably will target patient
serum concentration of the type 5 inhibitor between 0.5 ng/ml and 100
ng/ml, preferably between 1 ng/ml and 20 ng/ml, and most preferably
between 1 ng/ml and 10 ng/ml. Serum concentration may be measured
by LC/MS. When administered orally, the dosage which is usually
effective to provide the desired serum levels is between 1.0 mg and 1,000
mg of active ingredient per day per 50 kg of body weight, preferably
between 10 mg and 500 mg and most preferably between 10 mg and 100
mg. However, dosage should vary with the bioavailability of the chosen
inhibitor and with individual patient response. For example, when
EM-1394, or EM-1404 are chosen, oral dosage is preferably between 5 mg
and 500 mg per day per 50 kg body weight, more preferably between 10
mg/day and 300 mg/day, for example between 20 mg/day and 100
mg/day. The attending clinician should monitor individual patient
response and metabolism and adjust patient dosage accordingly. When administered by injection, a lesser dosage is usually appropriate, e.g. 10 mg to 100 mg per day per 50 kg of body weight.

When type 3 17β-hydroxysteroid inhibitors are used in accordance with the invention, either alone or as part of one of the combination therapies described herein, the attending clinician desirably will target patient serum concentration of the type 3 inhibitor between 0.5 ng/ml and 100 ng/ml, preferably between 1 ng/ml and 20 ng/ml and most preferably between 1 ng/ml and 10 ng/ml. When administered orally, the dosage is preferably between 1.0 mg and 1,000 mg of active ingredient per day per 50 kg of body weight, preferably between 5 mg and 500 mg and most preferably between 10 mg and 100 mg. However, the attending clinician should monitor individual patient response and metabolism and adjust patient dosage accordingly.

All of the active ingredients used in any of the therapies discussed herein may be formulated in pharmaceutical compositions which include one or more of the other active ingredients. Alternatively, they may each be administered separately but sufficiently simultaneous in time so that a patient eventually has elevated blood levels or otherwise enjoys the benefits of each of the active ingredients (or strategies) simultaneously. In some preferred embodiments of the invention, for example one or more active ingredients are to be formulated in a single pharmaceutical composition. In other embodiments of the invention, a kit is provided which includes at least two separate containers wherein, the contents of at least one container differs in whole or in part from the contents of at least one other container with respect to active ingredients contained therein. Two or more different containers are used in these combination therapies
of the invention. Combination therapies discussed herein also include use of one active ingredient of the combination in the manufacture of a medicament for the treatment (or prevention) of the disease in question where the treatment or prevention further includes the other active ingredient(s) or strategy of the combination. Some embodiments of the methods of treating or preventing disease discussed herein, utilize the type 5 and/or type 3 inhibitors of 17β-hydroxysteroid dehydrogenase discussed herein (i.e. the molecular structures discussed herein).

It is believed that, in some situations, the desirability of using an inhibitor of type 5 17β-hydroxysteroid dehydrogenase implies the desirability of also using an inhibitor of type 3 17β-hydroxysteroid dehydrogenase and vice versa, depending upon the expression level of both types in implicated tissues. Type 3 17β-hydroxysteroid dehydrogenase is mainly expressed in the testis while type 5 17β-hydroxysteroid dehydrogenase is mainly expressed in liver, adrenal glands, prostate, ovary, skin, mammary gland, testis, and in a series of peripheral tissues. LHRH agonists and LHRH antagonists may be used interchangeably to suppress either testicular or ovarian hormonal secretions by known techniques, except where preferences are otherwise stated herein.

In other embodiment, pharmaceutical composition for combination therapy may contain one type 5 17β-hydroxysteroid dehydrogenase inhibitor and at least one of other active ingredients selected from the group consisting of type 3 17β-hydroxysteroid dehydrogenase inhibitor, antiestrogen, antiandrogen, aromatase inhibitor and 5α-reductase inhibitor.
In another embodiment, pharmaceutical composition for combination therapy contains one type 3 17β-hydroxysteroid dehydrogenase inhibitor and at least one of the other active ingredients selected from the group consisting of type 5 17β-hydroxysteroid dehydrogenase inhibitor, antiestrogen, antiandrogen, aromatase inhibitor and 5α-reductase inhibitor.

In another embodiment, kits for combination therapy may contain in two or more containers, one type 5 17β-hydroxysteroid dehydrogenase inhibitors and at least one of other active ingredients selected from the group consisting of type 3 17β-hydroxysteroid dehydrogenase inhibitor, antiestrogen, antiandrogen, aromatase inhibitor, 5α-reductase inhibitor, and an LHRH agonist or antagonist.

In another embodiment, kits for combination therapy may contain in two or more containers one type 3 17β-hydroxysteroid dehydrogenase inhibitors and at least one of other active ingredients selected from the group consisting of type 5 17β-hydroxysteroid dehydrogenase inhibitor, antiestrogen, antiandrogen, aromatase inhibitor, 5α-reductase inhibitor, and an LHRH agonist or antagonist.

It is desired that activation of glucocorticoid receptors be minimized when administering the active ingredients of the invention. In preferred embodiments, active ingredients have no more effect on glucocorticoid receptors than is provided by megestrol acetate.

**Type 5 17β-HSD, 3β-HSD and AR in human prostate.** In order to obtain precise information on the cellular distribution of type 5 17β-HSD and
gain a better knowledge of the role of this enzyme in the human prostate, we performed in situ hybridization and immunocytochemical localization studies in human hyperplastic prostatic tissue (BPH). Normal human prostate tissue and the epithelial prostate cell line (PrEC) were also investigated by immunostaining. In the same series of experiments, the immunocytochemical localization of 3β-HSD was examined to compare the distribution of the two enzymes which are both involved in the biosynthesis of androgens from DHEA. In order to determine the site(s) of action of the locally produced androgens, we have also identified the immunocytochemical localization of the androgen receptor.

MATERIALS AND METHODS

Tissue preparation. Adult prostatic tissue was obtained from 12 patients with symptomatic benign prostatic hyperplasia (BPH) undergoing transurethral prostatectomy. The specimens were fixed by immersion in 2% glutaraldehyde, 4% formaldehyde and 3% dextran in 0.05 M phosphate buffer (pH 7.4). After 4 h, the specimens were processed and embedded in paraffin or frozen at -70°C. Four paraffin blocks of normal human prostate, fixed in 4% formaldehyde (age of patients between 37 and 73) were kindly provided by Dr. Bernard Tetu, Dep. of Pathology, Hotel-Dieu de Quebec.

Cultured cells. Normal prostate epithelial cells PrEC 5500-1 were cultured in PrBGM medium (Clonetics) and harvested after the third passage using a rubber policeman. The cells were then fixed in 2% glutaraldehyde, 4% formaldehyde and 3% dextran in 0.05M phosphate buffer for 20 min and centrifuged at 700 rpm for 5 min. After removing the supernatant, 2% agarose in 0.05 M phosphate buffer was added to the pellet at 55°C (the volume of agarose was twice the volume of the pellet).
After mixing the cells with agarose, the pellet was solidified at 4 °C and immersed in the same fixative for two hours, then washed, processed and paraffin embedded.

**In situ hybridization.** Two different procedures were used for *in situ* hybridization of BPH tissue. In the first one, 10 µm sections were cut from frozen tissue with a cryostat and processed. The second procedure will be described in detail elsewhere (El-Alfy et al, unpublished data). In brief, thick paraffin sections (20 µm) were cut and the unmounted sections were deparaffinized in toluene. The sections were subsequently rehydrated, postfixed in 2% glutaraldehyde, 4% formaldehyde and 3% dextran in 0.05M phosphate buffer and washed in the same buffer containing 7.5% glycine. Hybridization of the floating sections was performed overnight at 40°C with a $^3$H-UTP riboprobe. Following hybridization, they were postfixed in osmium tetroxide, flat-embedded in Epon and cut at 0.7 µm with an ultramicrotome. Both frozen (10 µm) and semithin (0.7 µm) sections were coated with liquid photographic emulsion (Kodak NTB-2) and processed after 14 (semithin sections) or 28 days (frozen sections) of exposure.

Sense and antisense riboprobes were generated by *in vitro* transcription from the p-Bluescript phagemid containing a cDNA insert of 35 nucleotides of the human type 5 17β-HSD. [$^{35}$S]- and [$^{3}$H]-UTP riboprobes were used for hybridization with the frozen and floating deparaffinized sections, respectively.

**Immunocytochemistry.** Twelve paraffin-embedded BPH samples, four normal prostate specimens and PrEC cells in paraffin blocks were serially cut at 4 µm. Sections were incubated overnight at 4°C with the human
type 5 17β-HSD antiserum diluted at 1:1000 in Tris-saline, pH 7.6. The
sections were then washed and incubated at room temperature for 4h
with peroxidase-labelled goat anti-rabbit γ-globulin (Hyclone, Logon, UT)
diluted 1:500. Endogenous peroxidase activity was eliminated by
preincubation with 3% H$_2$O$_2$ for 30 min and peroxidase was then revealed
during incubation with 10 mg of 3,3-diaminobenzidine in 100 ml of Tris-
saline buffer containing 0.03% H$_2$O$_2$. The intensity of staining was
controlled under the microscope. The sections were then counterstained
with hematoxylin. On other sections, immunostaining was performed
using a commercial kit (Vectastain ABC Kit; Vector Laboratories,
Burlingame, CA) and diaminobenzidine was used as the chromogen to
visualize the biotin streptavidin-peroxidase complex. A microwave
retrieval technique was applied for the androgen receptor staining
Control experiments were performed on adjacent sections by substituting
non-immunized rabbit serum (1:1000). In the case of type 5 17β-HSD
antiserum (diluted 1:1000), immunoabsorption with an excess (10$^{-6}$ M) of
the synthetic peptide used to raise the antibodies was also performed. The
number of stained cells (type 5 17β-HSD) and nuclei (AR) were counted
from colored photographs and their number presented in Table 1. The
intensity of staining was compared and evaluated between the different
stained cell types of the prostate on the same section. Similarly, the
density of silver grains was compared between the labeled cells on the
same section. The intensity of immunostaining and in situ hybridization
reaction was presented in Table 2. Paraffin sections of cultured cells were
immunostained using type 5 17β-HSD antiserum as mentioned above and
the number of immunostained cells presented as a percentage of stained
cells.
Antibody preparation

Type 5 17β-HSD. The type 5 17β-HSD peptide sequence N-GLDRNLHYFNSDSFASHPNYPYS located at amino acid position 297 to 320 of the human type 5 17β-HSD was synthesized by "Le Service de Séquence de Peptides de l'Est du Québec" (SSPEQ) (CHUL Research Center, Quebec, Canada) and purified by HPLC. New Zealand rabbits (2.5 Kg) received a subcutaneous injection of 100 µg of the peptide solubilized in 1 ml phosphate saline buffer containing 50% of complete Freund's adjuvant. The animals received at one-month intervals two successive booster injections with 50 µg of the peptide in 1 ml of incomplete Freund's adjuvant. Two weeks after the last injection, the rabbits were killed and the blood collected. The antiserum was obtained by decantation and separation by centrifugation, then affinity purified and stored at -80°C.

Specificity of the antiserum was examined by immunoblot analysis. In brief, human embryonal kidney cells (293) were transfected with CMV-neo vectors expressing human type 5 17β-HSD, types 1 and 3 3α-HSD and types 1 and 2 5α-reductase, respectively. Stable transfectants were selected by their resistance to 10⁻⁷M G-418. Positive clones were confirmed by their ability to efficiently transform the appropriate substrate (Luu-The, et al. unpublished data). Forty micrograms of protein of the homogenate of each cell line were electrophoresed on a 5-15% sodium dodecyl sulfate (SDS)-polyacrylamide gel, before being transferred to the nitrocellulose filter using a Bio-Rad apparatus for 4h at 60V. The blot was treated 3 times with 5% fat-free milk in PBS containing 0.1% Nonidet P-40 for 30 min. The antiserum developed against the type 5 17β-HSD peptide was diluted to 1/1000 and the blot was then incubated
at 4°C for 18 h in the diluted antiserum. The blot was then washed three times with PBS containing 5% fat-free milk and 0.1% Nonidet P-40. After incubation with horseradish peroxidase-conjugated anti-rabbit IgG in solution for 2h, the membrane was washed and bound antibodies were detected with ECL detection reagents (Amersham) and finally the membrane was exposed to Hyperfilm.

\textbf{3\beta-HSD}. The antiserum used for immunocytochemical studies was raised by immunizing rabbits with purified human placental 3\beta-HSD. This antiserum has been widely used to localize the enzyme in tissues of several species including the human.

\textbf{Androgen Receptor (AR)}. AR rabbit antiserum was generated against a synthetic peptide corresponding to the first 20 amino acid residues of the N-terminal domain of the human and rat AR. The antiserum was purified by immunoprecipitation and did not show any crossreactivity with estrogen or progesterone receptors. This antiserum was kindly provided by Dr. Théo H. van der Kwast, Dept. of Pathology, Erasmus University Rotterdam, The Netherlands.

\textbf{RESULTS}

\textbf{Type 5 17\beta-HSD}

\textit{In situ hybridization}. In the prostate specimens hybridized with the [PH]-labeled type 5 17\beta-HSD probe, all basal cells are intensively labeled while the luminal secretory cells are poorly labeled (Fig. 1a). In the fibromuscular stroma, none or only few silver grains are located over smooth muscle cells, while the fibroblasts dispersed throughout the stroma or in association with the wall of blood vessels are well labeled (Fig. 1c). Interestingly, the endothelial cells lining blood vessels are
strongly labeled. The smooth muscle cells and fibroblasts of the tunica media and adventitia show variable labeling intensity. When the hybridization was performed using $^3$H-labelled sense riboprobe as control, only a few scattered silver grains were detected over the epithelium (Fig. 1b) and blood vessels (Fig. 1d). The results obtained using [$^{35}$S]-UTP riboprobes (not shown) were found to be similar to the above mentioned results.

**Immunostaining**

**Type 5 17β-HSD Distribution.** As illustrated in Fig. 2, immunoblot analysis indicates that the antiserum specifically reacts with type 5 17β-HSD. In fact, no crossreactivity was detected, either with types 1 or 3 3α-HSD which share 84% and 86% identity with type 5 17β-HSD, respectively, or with types 1 and 2 5α-reductase, two enzymes which are abundant in prostatic tissue.

When immunostained paraffin sections of BPH specimens and normal prostatic tissues were examined, similar results were found (Figs 3a-c, 4a,b). Some variation in the distribution and intensity of immunostaining was observed between the twelve BPH specimens examined. A similar degree of variation was found between the normal prostate specimens, and the overall pattern was similar between normal and BPH prostatic tissues. The variation in staining of the epithelium lining the tube-alveoli was observed not only between the different specimens but also between the tube-alveoli of the same specimens. A constant finding was the positive reaction detected in the stromal fibroblasts while the smooth muscle cells were not stained (Figs. 3a-c, Figs. 4a, b, d). Strong staining was consistently found in the basal cells of the epithelium (Figs. 3a, 4a,
5a). In contrast, the luminal secretory cells exhibited highly variable and usually low immunoreactivity. In fact, in most alveoli (about 85%), no luminal cells were labelled (Figs. 3a, 4a) while about 10% of alveoli contained a low detectable but positive reaction of all luminal cells (Fig. 3b), the basal cells being always strongly labelled. In some alveoli (about 5%), labeling of few luminal cells and all basal cells (Table 1) was found (Figs. 3c, 4b).

When the antiserum was immunoabsorbed with the antigen or when non-immunized rabbit serum was used, no staining could be detected (Fig. 4c). The endothelial cells of small (Fig. 4e) and large (Figs. 4e-g) blood vessels were strongly immunoreactive. The staining reaction was variable in the smooth muscle cells of the tunica media, while fibroblasts of the tunica adventitia were intensively stained. Veins appeared strongly labelled because of the large number of fibroblasts in their walls (Fig. 4e). In arteries, the tunica media was lightly stained (Figs. 4f, g), while the tunica adventitia was well stained (Fig. 4f).

When the paraffin sections of cultured epithelial cells were examined after immunostaining using type 5 17β-HSD antibody, 58% of these cells were found to be positively stained (Fig. 3d).

3β-HSD Distribution. The results obtained following immunostaining with antibody to 3β-HSD were found to be very similar to those generated with the type 5 17β-HSD antiserum (Figs. 5a, b). Although the staining reaction was generally weaker for 3β-HSD, the cellular distribution of the enzyme corresponds very well to that described above for type 5 17β-HSD. In the glandular epithelium of the prostate, all the
basal cells were generally labelled while, in the luminal cells, the staining was variable, being intense in some cells and weak or absent in most others. In the stroma, the staining was restricted to the cytoplasm of fibroblasts. As observed for type 5 17β-HSD, specific immunolabelling was found in the endothelial cells and fibroblasts of blood vessel walls including arteries, veins and capillaries. In all the 3β-HSD-containing cells, the staining was restricted to the cytoplasm, no significant nuclear staining being detected.

Androgen receptor distribution. The AR appears exclusively localized in the nuclei of prostate cells in all the specimens examined. In the epithelium, immunostaining is detected in almost all the nuclei of the luminal cells, while most of the basal cell nuclei do not exhibit positive staining (Fig. 5c and Table 1). In the stroma, the majority of nuclei of the fibromuscular cells are labelled, but unstained nuclei of smooth muscle cells are also observed (Fig. 5d and Table 1). In the blood vessels, several nuclei of the endothelial cells lining the lumen are positive, but some display no reaction (Fig. 5e). In the tunica media of the arteries, most of the nuclei of the smooth muscle cells are stained while some remain negative (not shown). Comparable results were obtained for the nuclei of fibrocytes of the tunica adventitia.

DISCUSSION
In the present study, we have used two complementary approaches, namely in situ hybridization (using BPH specimens) and immunocytochemistry (using BPH, normal prostate tissues and cultured epithelial cells), to identify the cells which express type 5 17β-HSD in the human prostate. This enzyme was found mainly in the basal cells of the tube-alveoli, the fibroblasts of the stroma and blood vessels as well as in
the endothelial cells of the blood vessels (Table 1, 2). This double approach permits to identify not only type 5 17β-HSD mRNA but also the enzyme itself. The present data are in agreement with results from this laboratory, which indicated the presence of androgenic 17β-HSD activity in human and rhesus monkey prostates.

The stratified epithelium lining the tube-alveoli is divided into two layers, namely the basal layer made of low cuboidal cells and a layer of columnar secretory cells (luminal cells). It is generally believed that prostatic stem cells are located in the basal cell layer. As revealed by both in situ hybridization and immunocytochemistry, the basal cells are expressing type 5 17β-HSD at a much higher level than the luminal cells. In fact, while many luminal cells exhibited some detectable hybridization signal, they have shown a high degree of variation and usually low level of immunostaining (Figs. 3c, 4b). On the other hand, the majority of alveoli contained only strongly labeled basal cells (Figs. 3a, 4a). However, in a few alveoli, staining was detectable either in some luminal cells (Figs. 3c, 4b) or in all of them (Fig. 3b). This variable staining in luminal cells might be explained by variations in the biosynthetic activity among alveoli or among different luminal cells in the same alveolus. It is quite possible that the low level of the protein in an unknown proportion of luminal cells cannot be detected by immunocytochemistry. It is noteworthy to mention that very similar results were obtained with the antibody against 3β-HSD.

The cultured epithelial cells PrEC 5500-1 have shown approximately the same pattern of expression of type 5 17β-HSD as the epithelial cells of BPH and normal prostate tissues. We assumed that the cultured epithelial
cells are a mixture of basal and luminal cells. Therefore, it is not surprising to find that only 58% of these cells expressed the enzyme.

It has been reported that types 1 and 2 5α-reductases are produced by both epithelial and stromal cells in the prostate. Using immunocytochemistry, it has been shown that staining for type 2 5α-reductase could be seen in both basal and luminal epithelial cells. On the basis of studies performed with human prostatic in vitro models, it has been suggested that the basal cells exert a stem cell role. On the other hand, in vivo studies performed in the rat prostate during maturation have established that both basal and secretory luminal cells are self-replicating cell types. The presence of type 5 17β-HSD, 3β-HSD and 5α-reductase isoenzymes in the basal cells suggests that this cell type is actively involved in androgen production and cannot be considered as being only a precursor of the luminal secretory cells.

Using cells transfected with the cDNAs of different types of 17β-HSD, Luu-The et al have shown that types 1 and 3 17β-HSD catalyze the reduction of estrone to estradiol and 4-androstenedione to testosterone, respectively. They have also shown that these enzymes are substrate and orientation selective. In fact, type 3 and 5 17β-HSDs have the same selective function, but type 3 was detected only in the testis and was not found in the human prostate. Therefore, in the prostate, the reduction of 4-Dione to testosterone is probably due to type 5 17β-HSD. Since type 5 17β-HSD and 3β-HSD are both highly expressed in basal cells while the androgen receptor is mainly present in luminal cells (Fig. 5c and Table 1), it is tempting to suggest that testosterone synthesized in the basal cells reaches the luminal cells in a paracrine fashion to be ultimately
transformed into DHT in the luminal cells where the androgenic action is exerted and AR is highly expressed. DHT made in the luminal cells by the action of 5α-reductase would then exert its action in the luminal cells themselves, thus meeting the definition of intracrine activity. The involvement of two cell types in the biosynthesis of steroids has already been shown to occur in the ovary. In fact, in the ovary, C19 steroids (androstenedione and testosterone) synthesized by theca interna cells are transferred to granulosa cells where they are aromatized into estrogens. The present data suggest the possibility of a similar two-cell mechanism of androgen formation in the human prostate: testosterone is first synthesized in the basal cells before diffusing into the luminal cells where transformation into DHT occurs.

In the present study, the fibroblasts present in the stroma as well as those associated with blood vessels are shown to contain type 5 17β-HSD mRNA as well as the immunoreactive type 5 17β-HSD and 3β-HSD enzymes. The two types of 5α-reductase have also been detected in this cell type by various techniques. The role of the steroidogenic enzymes in fibroblasts remains to be established but since androgen receptors are present in the nuclei of most stromal cells (Table 1), it is likely that DHT could act in the fibroblasts themselves (intracrine action) to modulate the activity of these cells.

Previous study has shown that in normal prostate, basal cells contained the mRNA for AR but lacked an immunodetectable receptor while in the luminal cells both mRNA and immunodetectable receptor were present. These authors have also stated that AR localization in BPH was identical to that observed in normal prostate. Similarly, it has been found that the
nuclei of the luminal and the majority of stromal cells were positive to androgen receptor antibody in hyperplastic as well as normal prostatic glands. It has also been found that primary, as well as metastatic, prostate carcinomas show nuclear staining for AR and that the proportion and the intensity of immunostained human prostate tumor cells decreased in the more aggressive tumors. The basal cells also express nuclear AR in normal and hyperplastic tissue. However, the receptor was most frequently expressed at lower levels in the basal cells when compared with the staining intensity detected in secretory luminal cells. It has been found that AR immunostaining was localized to the nuclei of luminal cells but was weak or absent in basal cells and of variable intensity in the stromal cells. In the present study, while 94% of luminal cells expressed nuclear AR, only 37% of basal cells were stained (Table 1) and their staining intensity was lower than that of luminal cells (Table 2). The majority (66%) of fibro-muscular stromal cells also expressed AR. The findings of the present study are thus in agreement with previous studies performed in human, rat and mouse tissue. Since the stroma/epithelium cell ratio is higher in the hyperplastic prostate, it can be hypothesized that androgens synthesized intracellularly by fibroblasts can influence the production of collagen and elastic fibers in the stroma.

An unexpected finding was the localization of type 5 17β-HSD and 3β-HSD in blood vessel walls, including the endothelial cells. This observation, however, correlates well with recent findings from this laboratory indicating the presence of types 1 and 2 5α-reductase mRNA in blood vessel walls in human prostate and skin. Recently, we have also observed that immunoreactive type 5 17β-HSD is present in the blood vessel walls in other tissues such as skin, breast, uterus and ovary. The
role of the steroidogenic intracrine enzymes in these vascular structures is unknown.

Previously, it has been shown that androgen receptors were present in vascular smooth muscle and endothelial cells of human skin. It has been found that nuclear androgen receptors were present in the muscular layer of almost all arteries within the rat testis. It has been suggested that testicular blood vessels could be a target organ for androgens and may mediate some of the effects of androgens on testicular microcirculation. Furthermore, in the developing human prostate, AR was positive in the nuclei of vascular smooth muscle and endothelial cells. Since androgen receptors are present in the endothelial cells, smooth muscle cells and fibroblasts of blood vessels (Table 1), it may be speculated that locally biosynthesized androgens are exerting a paracrine and/or intracrine action in blood vessels. It is also possible that these androgens are, up to an unknown extent, released into the blood circulation to reach some target tissues, although their global impact is likely to be minimal. Interestingly, it has been shown that in the rat prostate, testosterone could induce a rapid response of the vasculature which precedes growth of the glandular epithelium. It might well be that cells of the blood vessels are stimulated by locally made androgens to produce paracrine growth factors, which could promote the growth of the secretory epithelium. Further studies are required to elucidate the role of the steroids synthesized by cells of the blood vessel walls. The present data clearly indicate new mechanisms of androgen formation, which may play an important role, not only in normal human prostate physiology, but also in the pathogenesis of benign prostatic hyperplasia and possibly prostate cancer.
Table 1. Percentage of immunostained cells of BPH and normal human prostate tissues.

<table>
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<tr>
<th></th>
<th>Type 5 17β-HSD (%)</th>
<th>Androgen receptor (%)</th>
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<tr>
<td><strong>Epithelium</strong></td>
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<td></td>
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<tr>
<td>Basal cells</td>
<td>100</td>
<td>37</td>
</tr>
<tr>
<td>Luminal cells</td>
<td>22 (*)</td>
<td>94</td>
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<tr>
<td><strong>Fibro-muscular stromal cells</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrocytes</td>
<td>100</td>
<td>66 (o)</td>
</tr>
<tr>
<td>Smooth muscle cells</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Blood vessels</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelial cells of tunica intima</td>
<td>100</td>
<td>75</td>
</tr>
<tr>
<td>Smooth muscle cells of tunica media</td>
<td>35 (*)</td>
<td>82</td>
</tr>
<tr>
<td>Fibrocytes of tunica adventitia</td>
<td>100</td>
<td>56</td>
</tr>
</tbody>
</table>

(*) This percentage represents the number of stained luminal cells only in about 5% of the alveoli, as shown in figures 3c and 4b. The vast majority of alveoli (about 85%) did not show stained luminal cells while all luminal cells were stained in about 10% of them (Fig. 3b).

(o) The number represents the percentage of stained nuclei of fibrocytes and smooth muscle cells together.

(·) The staining intensity is low in the stained smooth muscle cells of tunica media (as seen in figure 4f) as compared with other stained cells.
Table 2. Intensity of the *in situ* hybridization and immunostaining reactions in the different cell types of BPH and normal human prostatic tissue.

<table>
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<th>Immunostaining</th>
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<td>3β-HSD</td>
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<tr>
<td>Epithelium</td>
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<td></td>
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<tr>
<td>Basal cells</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Luminal cells</td>
<td>+</td>
<td>+/−</td>
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<tr>
<td>Fibro-muscular stromal cells</td>
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<td></td>
</tr>
<tr>
<td>Fibrocytes</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Smooth muscle cells</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Blood vessels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelial cells of tunica intima</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Smooth muscle cells of tunica media</td>
<td>+/−</td>
<td>+/−</td>
</tr>
<tr>
<td>Fibrocytes of tunica adventitia</td>
<td>+/−</td>
<td>++</td>
</tr>
</tbody>
</table>

The presence of silver grains or positive immunostaining reaction is indicated by (+), graded from 1 to 3. The number of (+) thus corresponding to the intensity of the reaction and takes into account the percentage of labelled cells.

The absence of reaction is indicated by (−). The possibility of being positively or negatively labeled is indicated by (+/−).
Human skin hybridized with type 5 17β-HSD cRNA probe. In a preliminary experiment of in situ hybridization, human skin has been hybridized with type 5 17β-HSD antisense and sense riboprobes the results show that the epidermis (except stratum corneum) (Fig. 6a,b), the wall of blood vessels (Fig. 6c,d), hair follicles as well as most of the fibrocytes of the dermis are labeled.

When paraffin sections immunostained with a specific antibody to type 5 17β-HSD were examined, the obtained results were in agreement with the in situ hybridization results. In the epidermis, few cells of the stratum granulosum were found to be heavily immunostained than all the other stained cells.

Localization of type 5 17β-HSD in human mammary gland. Immunostaining of tissue sections from several normal human mammary gland show that the epithelial cells lining the ducts and the alveoli are not stained while the connective tissue surrounding cells are stained. In the only one examined case of mammary gland tumor, the tumor cells themselves were not labeled but the epithelial cells lining the ducts were found to be heavily labeled (Fig. 7a, b).

Localization of type 5 17β-HSD in monkey ovary. In a preliminary study of in situ hybridization, one of the growing follicles was examined. Theca cells, granulosa cells and the oocyte were found to be labeled.
PREFERRED INHIBITORS OF TYPE 5
17β-HYDROXysteroid DEHYDROGENASE

Set forth in the tables below are lists of compounds which we have found to be useful as inhibitors of type 5 17β-hydroxysteroid dehydrogenase. The tables also include in many instances further tests of a particular compound on other important parameters such as androgenic and antiandrogenic activity and the effect of a compound on androgen receptors, androgen-sensitive cells, and other effects more fully explained below. In each of tables 1-5 and 1'-5' below that do not include a "prime" (') in its table number, details of molecular structure of preferred inhibitors are set forth. The corresponding table with a "prime" (') in its table number shows certain information about the functional efficacy of each tested compound. The numbers in the column headings correspond to a description at the end of all of the tables regarding what information is reported in each column and how it is determined. Entries left blank are not yet determined.
Table 1

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<th>Rₜ₂</th>
<th>Δ₁</th>
<th>Δ₆</th>
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**SUBSTITUTE SHEET (RULE 26)**
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Table 1 (Cont'd)

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SUBSTITUTE SHEET (RULE 26)
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| EM-1044| 5.31  | 0.88  | 0.49  | 0.35  | 0.27  | 0.20  | 0.15  | 0.12  | 0.17  | 0.19  | 0.21  | 0.23  | 0.25  | 0.28  | 0.30  |
| CS-209 | ND    | 10.542.7 | [62] |       |       |       |       |       |       |       |       |       |       |       |       |
| CS-256 | 320.7 | 521   |       |       |       |       |       |       |       |       |       |       |       |       |       |
| EM-1022| ND    | 340.6 | [62] |       |       |       |       |       |       |       |       |       |       |       |       |
| EM-1049| 831.16| 921   | [62] |       |       |       |       |       |       |       |       |       |       |       |       |
| EM-1107| ND    | 1713  |       |       |       |       |       |       |       |       |       |       |       |       |       |
| EM-192 | ND    | 17.425.1 | [114]|       |       |       |       |       |       |       |       |       |       |       |       |
| Megestrol Acetate | 9217 | 17.425.8 |       |       |       |       |       |       |       |       |       |       |       |       |       |
| EM-995 | ND    | 21.348.7 |       |       |       |       |       |       |       |       |       |       |       |       |       |
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**SUBSTITUTE SHEET (RULE 26)**
| Name | Def Recombinability | AGE 68 | Inhibition 1 Type 5 | % Inh. 77 | Inhibition 2 Type 4 | % Inh. 55 | Inhibition 3 Type 4 | % Inh. 54 | Inhibition 4 Type 2 | % Inh. 50 | Inhibition 5 Type 2 | % Inh. 50 | Inhibition 6 Type 2 | % Inh. 50 | 20-25 Activity | % Inhibition Androgen | % Inhibition Androgen Resistant | % Inhibition Androgen Resistant Resistant |
|------|---------------------|-------|-------------------|-----------|-------------------|-----------|-------------------|-----------|-------------------|-----------|-------------------|-----------|-------------------|-----------|-------------------|----------------------|----------------------|
| EM-1216 | (33 77) | -14  | -56               | -22       | -60               | -22       | -60               | -22       | -60               | -22       | -60               | -22       | -60               | -22       | -60               | -22       | -60               | -22       | -60               |
| EM-1240 | (67 89) | -10  | -33               | -4        | -56               | -4        | -56               | -4        | -56               | -4        | -56               | -4        | -56               | -4        | -56               | -4        | -56               | -4        | -56               |
| EM-1241 | (1 53)  | -4   | -41               | -3        | -56               | -3        | -56               | -3        | -56               | -3        | -56               | -3        | -56               | -3        | -56               | -3        | -56               | -3        | -56               |
| EM-1242 | (19 78) | -6   | -41               | -12       | -53               | -12       | -53               | -12       | -53               | -12       | -53               | -12       | -53               | -12       | -53               | -12       | -53               | -12       | -53               |
| 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
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**Table 1' (Cont'd)**

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**Note:**

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SUBSTITUTE SHEET (RULE 26)
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LEGENDS TO TABLES

In column 1, the oral bioavailability of preferred type 5 inhibitors, expressed in ng/mL/h, was determined as described below in "Other Tests, A- In Vivo Assays of Bioavailability of Human Type 5 17β-HSD Inhibitors". Higher number are desirable. ND means that a determination was not done.

In column 2, the inhibition of human type 5 17β-hydroxysteroid dehydrogenase activity expressed by the concentration which produce 50% of inhibition (IC₅₀ in nM) is reported (centered numbers). The manner in which IC₅₀ was determined is described in "Efficacy of the Preferred Inhibitors". Lower numbers for IC₅₀ are desirable. When IC₅₀ was not determined, the percentage of inhibition is reported in parentheses at 3.10⁻⁷M (left number) and 3.10⁻⁴M (right number).

In column 3, the reversibility of the inhibition of type 5 17β-hydroxysteroid dehydrogenase activity expressed in percentage of control is reported. The manner in which the reversibility is determined is described in "Efficacy of Preferred Inhibitors: V. Reversibility of Human Type 5 17β-HSD Inhibitory Activity". Lower number is desirable. Blank means that a determination was not done.

In column 4, the inhibition of human type 1 17β-hydroxysteroid dehydrogenase activity expressed by the concentration which produce 50% of inhibition of enzymatic activity (IC₅₀ in nM) is reported. The manner in which IC₅₀ was determined is described in "Efficacy of Preferred Inhibitors: VI. Enzymatic assay for types 1, 2, and 3 17β-HSD and types 1 and 3 3α-HSD". Higher numbers of IC₅₀ are desirable. Blank means that a determination was not done. The % of inhibition at the concentration of 3.10⁻⁶M determined in preliminary screening test is reported in square brackets.

In column 5, the inhibition of human type 2 17β-hydroxysteroid dehydrogenase activity expressed by the concentration which produce 50% of inhibition of enzymatic activity (IC₅₀ in nM) is reported. The manner in which IC₅₀ was determined is described in "Efficacy of Preferred Inhibitors: VI. Enzymatic assay for types 1, 2, and 3 17β-HSD and types 1 and 3 3α-HSD". Higher numbers of IC₅₀ are desirable. Blank means that a determination was not done. The % of inhibition at the concentration of 3.10⁻⁴M determined in preliminary screening test is reported in square brackets.
In column 6, the inhibition of human type 3 17β-hydroxysteroid dehydrogenase activity expressed by the concentration which produce 50% of inhibition of enzymatic activity (IC₅₀ in nM) is reported. The manner in which IC₅₀ was determined is described in "Efficacy of Preferred Inhibitors: VI. Enzymatic assay for types 1, 2, and 3 17β-HSD and types 1 and 3 3α-HSD". Lower numbers of IC₅₀ are desirable. Blank means that a determination was not done. The % of inhibition at the concentration of 3.10⁻⁶M determined in preliminary screening test is reported in square brackets.

In column 7, the inhibition of human type 1 3α-hydroxysteroid dehydrogenase activity expressed by the concentration which produce 50% of inhibition of enzymatic activity (IC₅₀ in nM) is reported. The manner in which IC₅₀ was determined is described in "Efficacy of Preferred Inhibitors: VI. Enzymatic assay for types 1, 2, and 3 17β-HSD and types 1 and 3 3α-HSD". Higher numbers of IC₅₀ are desirable. Blank means that a determination was not done. The % of inhibition at the concentration of 3.10⁻⁶M determined in preliminary screening test is reported in square brackets.

In column 8, the inhibition of human type 3 3α-hydroxysteroid dehydrogenase activity expressed by the concentration which produce 50% of inhibition of enzymatic activity (IC₅₀ in nM) is reported. The manner in which IC₅₀ was determined is described in "Efficacy of Preferred Inhibitors: VI. Enzymatic assay for types 1, 2, and 3 17β-HSD and types 1 and 3 3α-HSD". Higher numbers of IC₅₀ are desirable. Blank means that a determination was not done. The % of inhibition at the concentration of 3.10⁻⁶M determined in preliminary screening test is reported in square brackets.

In column 9, the androgenic activity of preferred type 5 inhibitors expressed as the percentage of stimulation of proliferation of Shionogi cells at concentrations of 10⁻⁷ M (left number) and 10⁻⁶ M (right number) of inhibitor. The manner in which the stimulation is determined is described in "Other Tests; B- Androgenic/Antiandrogenic Activity". Lower numbers are desirable. ND means that a determination was not done.

In column 10, the antiandrogenic activity of preferred type 5 inhibitors expressed by the concentration which produce 50% of inhibition (IC₅₀ in nM) of DHT-induced proliferation of Shionogi cells is reported (bracketed
centered numbers). The percentage of inhibition of DHT-induced proliferation of Shionogi cells at concentrations of $10^{-7}$M (left number) and $10^{-6}$M (right number) of inhibitor is also reported. For example, EM-1403 in Table 4', column 10, the number -54 means that at a concentration of $10^{-6}$M, the stimulation of DHT-induced proliferation of Shionogi cells was 54% reduced. The manner in which the inhibition is determined is described in "Other Tests; B- Androgenic/Antiandrogenic Activity". Lower numbers are desirable. ND means that a determination was not done.

In column 11, the estrogentic activity of preferred type 5 inhibitors expressed as the percentage of stimulation of the proliferation of ZR-75-1 cells at concentrations of $10^{-7}$M (left number) and $10^{-6}$M (right number) of inhibitor. The manner in which the stimulation is determined is described in "Other Tests; C- Estrogenic/Antiestrogenic activity". Lower numbers are desirable. ND means that a determination was not done.

In column 12, the antiestrogenic activity of preferred type 5 inhibitors expressed as percentage of inhibition of E2-induced proliferation of ZR-75-1 cells at a concentrations of $10^{-7}$M (left number) and $10^{-6}$ (right number) of inhibitor is reported. For example, EM-1402-CS in Table 4', column 12, the number -61 means that at a concentration of $10^{-6}$ M, the stimulation of E2-induced proliferation of ZR-75-1 cells was 61% reduced. The manner in which the inhibition is determined is described in "Other Tests; C- Estrogenic/Antiestrogenic Activity". Lower numbers are desirable. ND means that a determination was not done.

In column 13, the binding on androgen receptor expressed as percentage of inhibition of the binding of [3H]R1881 at the concentration of $10^{-8}$M (stared number at $10^{-7}$ M) (left number) and $10^{-4}$M (stared number at $10^{-5}$ M) (right number) of inhibitor is reported. The manner in which the percentage of inhibition is determined is described in "Other Tests; D-Androgen Receptor (AR) Assays". Lower numbers are desirable.

In column 14, the binding on progesterone receptor expressed as percentage of inhibition of the binding of [3H]R5020 at the concentration of $10^{-8}$M (stared number at $10^{-7}$ M) (left number) and $10^{-4}$M (stared number at $10^{-5}$ M) (right number) of inhibitor is reported. The manner in which the percentage of inhibition is determined is described in "Other Tests; E-Progesterone Receptor Assay". Lower numbers are desirable.

In column 15, the binding on glucocorticoid receptor expressed as percentage of inhibition of the binding of [6,7-3H(N)]-dexamethasone at
the concentration of $10^{-4}$M (stared number at $10^{-7}$ M) (left number) and $10^{-6}$M (stared number at $10^{-5}$ M) (right number) of inhibitor is reported. The manner in which the percentage of inhibition is determined is described in "Other Tests; F- Glucocorticoid Receptor Assay".

In column 16, the binding on estrogen receptor expressed as percentage of inhibition of the binding of [H]E2 at the concentration of $10^{-4}$M (stared number $10^{-7}$) (left number) and $10^{-6}$M (stared number $10^{-5}$) (right number) of inhibition is reported. The manner in which the percentage of inhibition is determined is described in "Other Tests; G- Estrogen Receptor (ER) Assay".
EFFICACY OF THE PREFERRED INHIBITORS

A. The preferred inhibitors of the invention are tested for their type 5 17β-HSD inhibitory activity by the following method.

1) Cloning of type 5 17β-HSD cDNA
Using a oligoprimmer pair (5'-GGA-AAT-CGT-GAC-AGG-GAA-TGG-ATT-CCA-AAC-AG-3', 5'-GGA-ATT-CTT-TAT-TGT-ATT-TCT-GGC-CTA-TGG-AGT-GAG-3') derived from human aldoketoreductase (Qin et al., J. Steroid Biochem. Molec. Biol. 46: 673-679, 1993) and polymerase chain reaction (PCR), we have amplified from a human placental λgt11 cDNA library (Clontech Laboratories Inc., Palo Alto, CA) a cDNA fragment that was used as probe to screen again a human placental λgt11 cDNA to get the full length cDNA clone. The amplified cDNA fragment was purified on agarose gel and labeled with [γ-32P]dCTP (Amersham Corp.) using the random primer labeling kit from Pharmacia Inc. PCR amplification reaction medium contained 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 0.1% gelatin, 1 mM MgCl2, 250 μM dNTP, 0.25 μM oligonucleotide primers, and 200 ng of DNA prepared from human placental λgt11 cDNA library. The aqueous phase was overlaid with 75 μL of mineral oil to avoid evaporation. The mixture was heated at 98°C for 5 min, following the temperature reduction to 72°C, 1 unit of Taq DNA polymerase was added. Using a DNA Thermal Cycler (Perkin-Elmer Corp.), 30 cycles of amplification were carried out using a step program (94°C, 1 min; 60°C, 1 min; and 72°C, 1 min)/cycle.

Approximately 5 x 10^5 recombinant phage plaques were screened with 1.5 x 10^6 cpm/ml of the probe. Prehybridization was performed for 4 h at 42°C in 50% formamide, 5X Denhardt's solution (1 X Denhardt's being 0.02% polyvinyl pyrrolidone, 0.02 Ficoll, 0.02 bovine serum albumin), 5 X SSPE (1 X SSPE being 0.18M NaCl, 10 mM NaH2PO4, pH 7.4, 1 mM EDTA), 0.1% SDS, and 100 μg/ml yeast tRNA. The probe was denatured by heating at 100°C for 5 min, before being added to the hybridization solution at 42°C overnight. Filters were then washed twice at room temperature in 2 X SSC (1 X SSC being 0.015 M NaCl, 0.15M sodium citrate, pH 7.0), 0.1% SDS, and twice in 0.1 X SSC, 0.1% SDS at 60°C. Positive recombinant plaques were purified by replating twice and grown in liquid culture. Phage DNA were isolated by centrifugation for 90 min at 105,000 X g, and DNA was then isolated by phenol extraction and precipitation with isopropanol (Karen et al. 1987; In: Current Protocols in Molecular Biology (F.M. Ausubel, R. Brent, E. Kingston, D.D. Moore, T.G.
II) Construction of expression vector, and nucleotide sequence determination

The phage DNA were digested with EcoRI restriction enzymes and the resulting cDNA fragments were inserted into the EcoRI site downstream to the cytomegalovirus (CMV) promoter of the pCMV vector (kindly provided by Dr. Matthew, Cold Spring Harbor, N.Y., USA). Recombinant pCMV plasmids were amplified in Escherichia Coli DH5α competent cells, and were isolated by alkaline lysis procedure (Maniatis et al., In: Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory, 1982). Sequencing of double-stranded plasmid DNA was performed according to the dideoxy chain termination method (Sanger et al., PNAS 74: 5453-5467, 1977) using a T7 DNA polymerase sequencing kit (Pharmacia LKB Biotechnology). To avoid errors, all sequences were determined by sequencing both strands of the DNA. Oligonucleotide primers were synthesized in our laboratory with a 394 DNA/RNA synthesizer (Applied Biosystem).

The pCMV vector contain 576 nucleotides of the pCMV promoter (followed by 432 bp of small t intron (fragment 4713-4570) and polyadenylation signal (2825-2536)of SV40 , followed by 1989 bp of the Fvu II(628) -Aactl (2617) fragment from pUC 19 vector (New England Biolabs) that contains an E.Coli origin of replication and an ampicillln resistance gene for propagation in E.Coli.

III) Cells overexpressing 17β-HSDs in transformed embryonal kidney (293) cells

A. Transient transfection

Transfection was performed by the calcium phosphate procedure (Kingston et al., In: Current Protocols in Molecular Biology (Ausubel et al., eds), pp. 9.1.1-9.1.9, John Wiley & Sons, Inc., New York, 1987) using 1 to 10 µg of recombinant plasmid DNA per 10^6 cells. The total amount of DNA is keep at 10µg of plasmid DNA per 10^6 by completing with pCMV plasmid without insert. The cells were initially plated at 10^4 cells/cm^2 in Falcon culture flasks and grown in Dulbecco’s modified Eagle’s medium containing 10% (vol/vol) fetal bovine serum (Hyclone, Logan, UT) under a humidified atmosphere of air/CO2 (95/5 %) at 37°C supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 100 IU penicillin/ml, and 100 µg streptomycin sulfate/ml.
B. Stable transfection

Cells were cultured in 6-wells falcon flasks to approximately 3 105 cells/well in DMEM. Five μg of pCMV-neo plasmid that expresses type 5 17β-HSD and Neomycin resistant gene, were transfected into 293 cells using lipofectin transfection kit (Life Technologies, Burlington, ON). pCMV-neo vector were constructed by inserting 2685 bp of a BamHI-BamHI DNA fragment that expresses a neomycin resistance gene isolated from the pMAMneo vector (Clontech Laboratories, Palo Alto, CA) at the BamHI site of the pCMV vector. After 6h incubation at 37°C, the transfection medium was removed and 2 ml of DMEM were added. Cells were further cultured for 48h then transferred into a 10 cm petri dishes and cultured in DMEM containing 700μg/ml of G-418 (Life Technologies, Burlington, ON), in order to inhibit the growth of non-transfected cells. Medium containing G-418 was changed every two days until resistant colonies were observed. Resistant clones that incorporated Neomycin resistant gene were tested for 17β-HSD activity. The most actif clone was cultured and keep frozen at -70°C for further inhibition study.

IV) Assay of enzymatic activity


Briefly, 0.1 μM of the indicated 14C-labeled substrate (Dupont Inc. (Canada), namely, DHEA, 4-androsterone-3,17-dione (Δ4), in absence or presence of increasing concentration of preferred inhibitor of the invention, was added to freshly changed culture medium in a 6-well culture plate. After incubation for 1 h, the steroids were extracted twice with 2 ml of ether. The organic phase were pooled and evaporated to dryness. The steroids were solubilized in 50 μl of dichloromethane, applied to Silica gel 60 thin layer chromatography (TLC) plate (Merck, Darmstadt, Germany) then separated by migration in the toluene-acetone (4:1) solvent system. Substrates and metabolites were identified by comparison with reference steroids and revealed by autoradiography and quantitated using the Phosphoimager System (Molecular Dynamics, Sunnyvale, CA). Transfection could be also performed with HeLa, SW-13, 293, COS-1 cells, the preferred cell line is 293 cells.
V. Reversibility of Human Type 5 17β-HSD Inhibitory Activity
The reversibility assay was performed as described above for the standard type 5 17β-HSD enzymatic assay, except that before adding the 14C-labeled substrate, the cells were preincubated with 0.3 μM of the indicated inhibitor for 1 h, (or the indicated time interval), followed by two washes with phosphate saline buffer (PBS).

VI. Enzymatic assay for types 1, 2, and 3 17β-HSD and types 1 and 3 3α-HSD
Enzyme sources. 293 cells transiently transfected with expression vectors encoding types 1, 2 and 3 17β-HSD (Luu-The et al., J. Steroid Biochem. Molec. Biol., 55: 581-587, 1995), types 1 and 2 5α-reductase (Luu-The et al., J. Invest. Dermatol., 102: 221-226, 1994) and types 1 and 3 3α-HSD (Dufort et al. Biochem. Biophys. Res. Commun. 228: 474-479, 1996), using the calcium phosphate procedure (Kingston et al., In: Current Protocols in Molecular Biology. Edited by E.M. Ausbel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, K. Struhl. John Wiley & Sons, New York, pp. 9.1.1-9.1.9, 1991; Luu-The et al., J. Invest. Dermatol., 102: 221-226, 1994). For assays using cell subfractions, cells were sonicated in 50 mM sodium phosphate buffer (pH 7.4), containing 20% glycerol and 1 mM EDTA and centrifuged at 10,000 x g for 30 min before centrifugation for 100,000 x g for 1 h to separate the mitochondrial and microsomal fractions, respectively. The cytosol fractions (100,000 x g supernatant) was used to determine type 1 activity while the microsomal fraction (pellet at 100,000 x g) was used for measurement of types 2 and 3 17β-HSD activities.

Incubation. The enzymatic reaction was carried out at 37°C in 1 ml of 50 mM sodium phosphate buffer, pH 7.4, containing 20% glycerol, 1 mM EDTA, and 2 mM cofactors (NADPH or NAD+) for 1 h in the presence of 0.1 μM 14C-labeled substrate: estrone and 4-dione for types 1 and 2 17β-HSD, respectively, testosterone for type 2 17β-HSD as well as types 1 and 2 5α-reductases, DHT for types 1 and 3 3α-HSD activities, and the indicated concentration of inhibitor.

Extraction of metabolites, migration on TLC and quantification were as described above for the assay of type 5 17β-HSD.
OTHER TESTS

A- In Vivo Assays of Bioavailability of Human Type 5 17β-HSD Inhibitors

1) Principle
The assays of the bioavailability of 17β-HSD Type 5 inhibitors were performed in male Sprague Dawley rats by measuring the plasma concentrations of the compounds after single oral administration of the compounds. The measurements at various time intervals were for values greater than or equal to 1.0 ng/mL and less than or equal to 50 ng/mL.

a) Animals and treatment
Male Sprague-Dawley rats [Crl:CD(SD)Br] weighing 275-350 g were obtained from Charles-River Canada Inc. and housed 2 per cage during the acclimation period and individually during the study period. The animals were maintained under a regimen of 12 hours light: 12 hours dark (lights on at 08:00). Animals received certified Rodent feed (Lab Diet # 5002, pellets) and tap water ad libitum. Rats were fasted (access to water only) starting on the evening prior to dosing.

Each compound to be tested was administered to three animals as a suspension in 0.4% methylcellulose by oral gavage at a dose of 0.5 mg/rat (1.0 ml/rat). Four to eight new compounds were tested each day and one group of animals received megestrol acetate (MGA) under the same conditions on each dosing day as a reference. One blood sample of ~0.7 ml was collected from the jugular vein of rats under Isoflurane-induced anesthesia at 1, 2, 3, 4, and 7 hours post-gavage. Blood samples were immediately transferred into a refrigerated 0.75 ml Microtainer containing EDTA and kept in an ice-water bath until centrifugation at 3000 rpm for 10 minutes. Plasma separation was performed rapidly (less than 50 minutes) after blood collection. One aliquot of 0.25 ml of plasma was then transferred into a borosilicate tube (13 x 100) and was rapidly frozen on dry-ice. Plasma samples were kept at -80°C until measurement of plasma concentration of the inhibitor(s) by LCMS/MS.
2) **LCMS measurements**

*a)* **Apparatus**

1. Vacuum manifold
2. Turbo Vap LV evaporator
3. Mass spectrometer API III or API-300 (PE/Sciex) with associated peripherals
4. Automatic Injector
5. HPLC pump
6. Infusion pump
7. Calibrated pipets

*b)* **Reagents and Solutions**

1. Methanol, HPLC grade
2. Water, Ultrapure (Super Q)
3. Ethanol, reagent grade
4. N-butyl chloride, HPLC grade
5. Acetone, HPLC grade
6. Male rat plasma (EDTA)
7. 17β-HSD type 5 inhibitors in reference standard ethanol solution approximately 100 µg/mL
8. EM 248 Internal Standard reference standard (solution of 50 ng/mL)
9. Mass calibrator solution Polypropylene Glycol (PE/Sciex)

*c)* **Mass Spectrometer Conditions**

Detector: Mass spectrometer API-300 (PE/Sciex)
Interface: Turbo Ion spray inlet (split 1/5)
Auxiliary flow: 4.5L/minute (nitrogen)
Nebulizer Flow: 11
Curtain Gas Flow: 11
Probe Temperature: 460 °C
Pressure: Approximately 3 x 10^{-5} Torr
CAD gas thickness: 3
Count Control: 1
Mobile Phase: Gradient of Methanol with 1 mm Ammonium formate and Water with 1 mm Ammonium formate
Flow Rate: 1 mL/minute
d) **Mass Spectrometer Analysis Parameters for EM-1118**

- Dwell time: 150 msec
- Pause time: 30 msec
- Duration: 4 minute
- MRM mode for EM-1118 analysis: 444.2 and 398.3
- Injection: 10 µL
- Data handling: "API Standard Software" update version.

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e) **Preparation of Standard Solutions**

Stock solutions for each type 5 inhibitor were prepared in methanol and, when not in use, the methanol solutions were stored at -20°C. Calibration curve standard solutions for each compound were prepared in male rat plasma as illustrated in Table 1.

A solution of internal standard in methanol containing EM-248 at 50 ng/mL, was prepared from stock standard solutions of EM-248 stored at -20°C.

<table>
<thead>
<tr>
<th>Concentration of inhibitor 17β-HSD</th>
<th>Volume of solution</th>
<th>Volume of plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std 50 ng/mL</td>
<td>90 µL of 1 µg/mL</td>
<td>1.71 mL</td>
</tr>
<tr>
<td>Std 20 ng/mL</td>
<td>0.8 mL of 50 ng/mL</td>
<td>1.2 mL</td>
</tr>
<tr>
<td>Std 10 ng/mL</td>
<td>0.9 mL of 20 ng/mL</td>
<td>0.9 mL</td>
</tr>
<tr>
<td>Std 5 ng/mL</td>
<td>0.8 mL of 10 ng/mL</td>
<td>0.8 mL</td>
</tr>
<tr>
<td>Std 2 ng/mL</td>
<td>0.6 mL of 5 ng/mL</td>
<td>0.9 mL</td>
</tr>
<tr>
<td>Std 1 ng/mL</td>
<td>0.5 mL of 2 ng/mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Std 0</td>
<td>N/A</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Blank</td>
<td>N/A</td>
<td>0.5 mL</td>
</tr>
</tbody>
</table>
f) **Extraction Procedure for Type 5 Inhibitors From Rat Plasma**

Aliquots of rat plasma (0.250 mL) were transferred to 13 x 100 mm borosilicate tubes. Water (1.0 mL) and internal standard solution (0.1 mL) were added to each sample and vortexed for 2 min. A mixture of N-butyl chloride and acetone (v:v, 7:3) (3 mL) was added to each sample and vortexed for 2 min. This step was repeated and the combined organic phases were evaporated to dryness under nitrogen in a Turbo Vap evaporator at 35° C. The residue was reconstituted with 1 mL of methanol and evaporated in a Turbo Vap evaporator at 35°C. The final extract was reconstituted into 0.1 mL of methanol/water (v:v, 75:25) and then transferred into a conical vial for injection into the mass spectrometer.

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**Assay**

The assay procedure was performed by analyzing, in duplicate, rat plasma samples spiked at six different Type 5 inhibitor concentrations (1, 2, 5, 10, 20 and 50 ng/mL). As example, the results of EM-1118 are presented in Figure 1. The lower limit of quantitation (LOQ) was established at 1.0 ng/mL. Values lower than 1.0 ng/mL were expressed as below limit of quantification (BLQ).

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**Linearity**

The assay procedures for EM-1118 were found to be linear over the 1.0 to 50 ng/mL range. Weighted (1/X) linear regression analysis gave a correlation (r²) of 0.991.

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**Calculation of AUC Values**

For all compounds studied, the area under the plasma concentration versus time curve (AUC) from time 0 to 7 hours post-dosing was determined. AUC₀⁻ий values were calculated by the linear trapezoidal method (model-independent) for each rat and data were expressed as mean AUC₀⁻ий±SEM (n=3).

35

**B- Androgenic/Antiandrogenic Activity**

Androgenic/antiandrogenic activity of some preferred compounds has been measured using the Shionogi mouse mammary carcinoma cells.
Materials
Minimal essential culture medium (MEM), non-essential amino acids, and fetal calf serum were purchased from Flow Laboratories. In order to remove endogenous steroids, serum was incubated overnight at 4 °C with 1% activated charcoal (Norit A, Fisher) and 0.1% Dextran T-70 (Pharmacia). A 2-h supplementary adsorption was performed at 25°C in order to further remove protein-bound steroids. Serum was also inactivated by a 20-min incubation at 56°C.

5α-dihydrotestosterone (DHT) was obtained from Steraloids. The antiandrogen hydroxyflutamide (OH-FLU) was kindly supplied by Drs. T.L. Nagabushan and R. Neri (Schering Corporation, Kenilworth, U.S.A.).

Cell dispersion, culture and cloning
Shionogi male mice bearing androgen-sensitive mammary tumors were obtained from Drs. Keishi Matsumoto, Osaka, Japan, and Yvonne Lefebvre, Ottawa, Canada. For primary culture, tumors were excised and washed in ice-cold sterile 25 mM Hepses buffer (137 mM NaCl; 5 mM KCl; 0.7 mM Na₂HPO₄; 10 mM glucose, pH 7.2). After mincing with scissors, the tumor minces were digested for 2 h at 37°C in Hepses buffer containing 3.8 mg/ml collagenase (Clostridium, Boehringer), 1.5 mg/ml hyaluronidase II (Sigma), and 3% bovine serum albumin fraction V (Schwartz-Mann). Dispersed cells were collected by centrifugation (500 x g for 10 min), washed twice by suspension in minimal essential medium (MEM) containing 5% dextran-coated charcoal-treated fetal calf serum (DCC-FCS), 1% non-essential amino acids, 10 IU/ml penicillin, 50 μg/ml streptomycin, and 100 nM dihydrotestosterone (DHT) (Steraloids).

Cells were plated in the same medium at a density of 75,000 cells/ml in 75 cm² flasks under an atmosphere of 5% carbon dioxide in air at 37°C. The medium was changed weekly. The steroids and antisteroids were dissolved in ethanol and kept in stock solutions chosen to yield final ethanol concentrations less than 0.01% in the culture medium. Such a concentration of ethanol does not affect cell growth.

Cells were subcultured at near-confidence by gentle digestion in a solution of 0.1% pancreatin (Flow Laboratories) in Hepses buffer containing 3 mM ethylenediaminetetraacetic acid (EDTA) (pH 7.2). Cells were pelleted by centrifugation, resuspended in culture medium, counted in a Coulter counter, and replated as described above. Soft agar cloning was performed as described (Stanley et al., Cell 10: 35-44, 1977) in the presence of 100 nM DHT.
Measurement of Cell Growth and Sensitivity to Steroids and Antiestrogens
Cells were plated in 24-well plates at a density of 20,000 cells/well. The indicated increasing concentrations of agents were added to triplicate dishes, and cells were grown for 10-12 days with changes of medium every 3-4 days. Cell number was measured by direct counting in a Coulter counter.

Calculations and Statistical Analysis
ED50 values of action of DHT and glucocorticoids were calculated according to a least-square regression as described (Rodbard, Endocrinology 94: 1427-1431, 1974). Statistical significance was calculated according to a multiple-range test (Kramer, Biometrics 12: 307-310, 1956).

C- Estrogenic/Antiestrogenic Activity
Estrogenic/antiestrogenic activity of some preferred compounds has been measured using the ZR-71-1 human breast cancer cell line as described in more detail below.

Maintenance of Stock Cultures
ZR-71-1 cells (83rd passage) were obtained from the American Type Culture Collection (Rockville, MD) and routinely cultured in phenol red free RPMI 1640 supplemented with 1 nM estradiol (E2), 2 mM L glutamine, 1 mM sodium pyruvate, 15 mM N-2-hydroxyethyl piperazine-N'-2-ethanesulfonic acid, 100 IU penicillin/ml, 100 μg streptomycin/ml, and 10% (v/v) fetal bovine serum (HyClone, Logan, UT) under a humidified atmosphere of 95% air, 5% CO2 at 37 °C. All media and medium supplements were purchased from Sigma. Cells were subcultured weekly by treatment with a pancreatic solution containing EDTA (0.2 g/L). The cell cultures used for the experiments herein described were between passages 89 and 94.

Measurements of Cell Proliferation
Cells in their logarithmic growth phase were harvested, briefly centrifuged, and resuspended in RPMI 1640. Cells were then plated in triplicate in LIMBRO 24-well plastic culture plates (2 cm²/well). Since plating density influences the effect of hormones on ZR-75-1 cell growth, cells were plated at a density of 1 x 10^4 cells/well. After 72 h, medium was replaced with fresh medium containing the inhibitor at the concentration of 3 x 10^-7 and 1 x 10^-6 M in absence or presence of 0.1 M estradiol (E2). Control cultures received the ethanol vehicle only. Cells were then allowed to grow at 37 °C for 10 days with medium changes (of identical
composition) every 2 days. In absence of inhibitors, in 0.1M estradiol (E2)-containing medium, ZR-75-1 cells have doubling time of about 48 h.

After E2 and/or antiestrogen treatment, cells were harvested by addition of 0.5 ml of a pancreatin solution (Sigma) for 5-10 min at 37°C before addition of 0.5 ml of RPMI 1640 containing 5% dextran coated charcoal-free bovine serum in order to block enzymatic action. Cell number (0.10 ml aliquot) was determined by measurement of DNA content as previously described (Simard et al., Endocrinology 126: 3223-3231, 1990).

D- Androgen Receptor (AR) Assays

Tissue Preparation
Male Sprague-Dawley rats (Crl: CD(SD)Br) weighing 200-300g were obtained from Charles-River Inc. (St-Constant, Québec, Canada). The rats were gonadectomized under general anesthesia (Isoflurane) and killed by cervical dislocation 24 hours later. The ventral prostates were rapidly removed, dissected free from adhering tissue and frozen on dry-ice. Prostates were kept at -80°C until assay.

All subsequent steps were performed at 0-4°C. Prostates were homogenized in 5 vol (wt/vol) of buffer A (25 mM Tris-HCl, 1.5 mM EDTA disodium salt, 10 mM α-monomothioglycerol, 10% glycerol, and 10 mM sodium molybdate, pH 7.4), using a Polytron PT-10 homogenizer (Brinkman Instruments, Canada) at a setting of 5 for three periods of 10 sec, with intervals of 10 sec for cooling. The homogenate was then centrifuged at 105,000 x g for 60 min in a Beckman L5-65 ultracentrifuge (Fullerton, CA). The protein concentration of the cytosol fraction was measured according to the method of Bradford (Anal. Biochem. 72: 248-254, 1976), using bovine serum albumin as standard.

Androgen Receptor Assay
Androgen binding was measured using the hydroxylapatite assay (HAP). In brief, the radioactive steroid [3H]R1881 solubilized in ethanol was diluted into buffer A. Aliquots of prostate cytosol preparation (0.1 ml) were then incubated with 8 nM [3H]R1881 (0.1 ml, ~200,000 cpm) in the presence or absence of the indicated concentrations of unlabeled compounds (0.1 ml, prepared in buffer A containing 10% ethanol) for 16-18 h at 0-4°C. Triamcinolone acetonide (150 nM) was added in order to mask progesterone receptors. Unbound steroids were separated by incubation for 40 min at 0-4°C with 0.3 ml HAP prepared in buffer P (50
mM, Tris-HCl, 10 mM KH₂PO₄, pH 7.4) as follows: 10 g HAP were washed with buffer P until the pH of 7.4 and then following centrifugation and decantation of the supernatant, 37.5 ml of buffer P were added. After incubation with HAP and 10 minutes of centrifugation at 1,000 x g, the pellet was washed 3 times with 1 ml buffer P. Thereafter, the radioactivity was extracted from the pellet by incubation at room temperature for 60 minutes with 1 ml EtOH. After centrifugation, the supernatant was decanted into a scintillation vial and the pellet was extracted again with ethanol. Thereafter, 10 ml Formula-989 scintillation liquid was added to pooled supernatant and the radioactivity was measured in a Beckman counter.

Calculations
The results were reported as the percentage of inhibition of the binding of [³H]R1881 at the concentrations of 10⁻⁸ and 10⁻⁶ M of the inhibitor.

E- Progesterone Receptor Assay

Chemicals
[¹⁷α-methyl-³H]-promegestone (R5020) (84 Ci/mmol) and the corresponding unlabeled compound were purchased from New England Nuclear (Lachine, Québec, Canada). All other chemicals were of analytical grade.

Stock solutions of the unlabeled steroids were kept at 4°C in ethanol. The desired steroid solutions were then prepared by appropriate dilution in buffer B (10 mM Tris-HCl, 1.5 mM EDTA, 10 mM α-monoiodoglycerol, pH 7.4) containing 30% ethanol.

Tissue preparation
Female Sprague-Dawley rats weighing 200-300g were obtained from Charles-River Inc. (St-Constant, Québec, Canada). The rats were gonadectomized under general anesthesia (Isoflurane) and killed by cervical dislocation 24 hours later. The uteri are rapidly removed, dissected free from adhering tissue and frozen on dry-ice. Tissues were kept at -80°C until use.

Cytosol preparation
All procedures were performed at 4°C. Tissues were pulverized frozen in dry ice with a Thermovac pulverizer. The samples were then homogenized in 10 vol (w/v) of buffer A (25 mM Tris-HCl, 1.5 mM EDTA, 10 mM α-monoiodoglycerol, 10% glycerol, 10 mM sodium
molybdate, pH 7.4) using a Polytron PT-10 homogenizer (Brinkmann Instruments, Canada) at a setting of 5 for two periods of 10 sec, with intervals of 10 sec for cooling. The homogenate was then centrifuged at 105,000 x g for 90 min. The supernatant was used immediately for assay.

5

**Binding Assays**

Progesterone binding was measured using the dextran-coated charcoal adsorption technique. Incubations were performed at 0-4°C for 16-18 h using 100 µl of cytosol, 100 µl of [3H]-R5020 (5 nM final, which contained 1,000 nM of dexamethasone in order to mask the glucocorticoid receptors) and 100 µl of unlabeled compounds at the indicated concentrations. Each concentration was done in triplicate. Assay was ended with 300 µl of DCC (1% Norit A and 0.1% Dextran T-70 in Buffer B). After 10 min of incubation, tubes were centrifuged at 2,000 x g for 10 min. and decanted in vials with 6 ml of BCS liquid scintillation (New England Nuclear, Dupont). The radioactivity was measured in a Beckman counter at a counting efficiency of 35%.

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**Calculations**

The results were reported as the percentage of the inhibition of the binding of [3H]R5020 at the concentrations of 10^{-8} and 10^{-6}M of the inhibitors.

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**F- Glucocorticoid Receptor Assay**

**Chemicals**

[6,7-3H(N)]-Dexamethasone (39 Ci/mmol) was purchased from New England Nuclear (Lachine, Québec, Canada) while unlabeled dexamethasone was obtained from Steraloids (Wilton, NH). All other chemicals were of analytical grade.

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Stock solutions of the unlabeled steroids were kept at 4°C in ethanol. The desired steroid solutions were then prepared by appropriate dilution in buffer B (10 mM Tris-HCl, 1.5 mM EDTA, 10 mM α-monothioglycerol, pH 7.4) containing 30% ethanol.

25

**Tissue Preparation**

Male Sprague-Dawley rats weighing 200-300g were obtained from Charles-River Inc. (St-Constant, Québec, Canada). The rats were killed by cervical dislocation and the liver were rapidly removed, dissected free from adhering tissue and frozen on dry-ice. Tissues were kept at -80°C until use.
Cytosol preparation
All procedures were performed at 4°C. Tissues were eminced and homogenized in 10 vol (w/v) of buffer A (25 mM Tris-HCl, 1.5 mM EDTA, 10 mM α-monothioglycerol, 10% glycerol, 10 mM sodium molybdate, pH 7.4) using a Polytron PT-10 homogenizer (Brinkmann Instruments, Canada) at a setting of 5 for two periods of 10 sec, with intervals of 10 sec for cooling. The homogenate was then centrifuged at 105,000 x g for 90 min. The supernatant was used immediately for assay.

Binding Assays
Glucocorticoid binding was measured using the dextran-coated charcoal adsorption technique. Incubations were performed at 0-4°C for 16-18 h using 100 μl of cytosol, 100 μl of [3H]-Dexamethasone (5 nM final) and 100 μl of unlabeled compounds at the indicated concentrations. Each concentration was done in triplicate. Assay was ended with 300 μl of DCC (2.5% Norit A and 0.25% Dextran T-70 in Buffer B). After 10 min of incubation, tubes were centrifuged at 2,000 x g for 10 min. and decanted in vials with 6 ml of BCS liquid scintillation (New England Nuclear, Dupont). The radioactivity was measured in a Beckman counter at a counting efficiency of 35%.

Calculations
The results were reported as the percentage of the inhibition of the binding of [3H]-dexamethasone at the concentrations of 10⁻⁸ and 10⁻⁶M of the inhibitor.

G- Estrogen Receptor (ER) Assay

Tissue Preparation
Female Sprague-Dawley rats (Crl: CD(SD)Br) weighing 200-300g were obtained from Charles-River Inc. (St-Constant, Québec, Canada). The rats were gonadectomized under general anesthesia (Isoflurane) and killed by cervical dislocation 24 hours later. The uteri were rapidly removed, dissected free from adhering tissue and frozen on dry-ice. Uteri were kept at -80°C until assay.

All subsequent steps were performed at 0-4°C. Uteri were homogenized in 10 vol (wt/vol) of buffer A (25 mM Tris-HCl, 1.5 mM EDTA disodium salt, 10 mM α-monothioglycerol, 10% glycerol, and 10 mM sodium molybdate, pH 7.4), using a Polytron PT-10 homogenizer (Brinkman Instruments, Canada) at a setting of 5 for three periods of 10 sec, with
intervals of 10 sec for cooling. The homogenate was then centrifuged at 105,000 x g for 60 min in a Beckman L5-65 ultracentrifuge (Fullerton, CA). The protein concentration of the cytosol fraction was measured according to the method of Bradford (Anal. Biochem. 72: 248-254, 1976), using bovine serum albumin as standard.

Estrogen binding was measured using the dextran-coated charcoal adsorption technique as described previously (Asselin et al., Endocrinology, 101: 666-671, 1977; Asselin and Labrie, J. Steroid Biochem., 9: 1079-1082, 1978). Briefly, [3H]E2 solubilized in ethanol were diluted into buffer A. Aliquots of uterine cytosol preparation (0.1 ml) were incubated with 5 nM [3H]E2 (~200,000 cpm, 0.1 ml) in the presence or absence of the indicated concentrations of unlabeled compounds (0.1 ml, prepared in buffer A containing 10% ethanol) for 3 h at room temperature. Unbound steroids were then separated by incubation for 15 min at 0-4°C with 0.3 ml 0.5% Norit-A and 0.05% Dextran T-70 in buffer B (1.5 mM EDTA disodium salt, 10 mM monothioglycerol, and 10 mM Tris-HCl, pH 7.4) and centrifuged at 3,000 x g for 15 min. Aliquots of the supernatant (0.3 ml) were removed for radioactivity measurement. After the addition of 10 ml Formula-989 scintillation liquid (New England Nuclear-DuPont), the radioactivity was measured in a Beckman counter at a counting efficiency of 62%.

Calculations
The results were reported as the percentage of inhibition of the binding of E2 at the concentrations of 10^{-8} and 10^{-6} M of the inhibitor.
Primary criteria in selecting preferred inhibitors include bioavailability, desirable inhibition of type 5 17β-hydroxysteroid dehydrogenase, extent of undesirable inhibition on type 2 17β-hydroxysteroid dehydrogenase and androgenicity. It is believed that the methyl groups in 5' position in EM 1394 and analogous compounds promote selectivity of type 5 inhibition (versus undesirable type 2 inhibition). Of the compounds in the foregoing tables, the most preferred and their molecular structures are set forth below:

EM-1291

EM-1195-CS

CS-243

CS-245
PREFERRED INHIBITORS OF TYPE 3 17β-HYDROXYSTEROID DEHYDROGENASE

Natural androsterone provides inhibition of type 3 17β-hydroxysteroid dehydrogenase with a IC₅₀ = 200 nM. In vitro testing has shown the following compounds to also be capable of inhibiting the type 3-catalyzed conversion of 4-dione to testosterone:

Table 6

<table>
<thead>
<tr>
<th>Name</th>
<th>R</th>
<th>% of Inhibition of the Type 3 17β-HSD Activity *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3.10⁻⁷M</td>
</tr>
<tr>
<td>EM-1071</td>
<td>-O(CH₂)₂OC₂H₅</td>
<td>73</td>
</tr>
<tr>
<td>EM-1065</td>
<td>-S(CH₂)₂SCH₃</td>
<td>63</td>
</tr>
<tr>
<td>EM-1066</td>
<td>-OCH₂SCH₃</td>
<td>63</td>
</tr>
<tr>
<td>EM-1064</td>
<td>-SCH₂OCH₃</td>
<td>62</td>
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<tr>
<td>EM-1074</td>
<td>-O(CH₂)₃CH₃</td>
<td>62</td>
</tr>
<tr>
<td>EM-1073</td>
<td>-O(CH₂)₃OCH₃</td>
<td>51</td>
</tr>
<tr>
<td>EM-1070</td>
<td>-OCH₂OCH₃</td>
<td>57</td>
</tr>
</tbody>
</table>

* The inhibition of human type 3 17β-hydroxysteroid dehydrogenase activity, expressed as the percentage of inhibition of enzymatic activity at 3.10⁻⁷ M (left column) and 3.10⁻⁴ M (right column), is reported. The manner in which the percentage of inhibition was obtained is described in "Efficacy of types 1, 2, and 3 17β-HSD and types 1 and 3 3α-HSD supra". Higher numbers are desirable.
or

Table 7

<table>
<thead>
<tr>
<th>Name</th>
<th>n</th>
<th>Inhibition of the Type 3 17β-HSD Activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IC₅₀ (nM)</td>
</tr>
<tr>
<td>CS-213</td>
<td>1</td>
<td>31 ± 4</td>
</tr>
<tr>
<td>EM-1324-CS</td>
<td>2</td>
<td>31 ± 4</td>
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* The inhibition of human type 3 17β-hydroxysteroid dehydrogenase activity, expressed by the concentration which produce 50% of inhibition of enzymatic activity (IC₅₀ is nM), is reported. The manner in which IC₅₀ was determined is described in "Efficacy of types 1, 2, and 3 17β-HSD and types 1 and 3 3α-HSD". Lower numbers of IC₅₀ are desirable.
EXAMPLES OF SYNTHESIS OF PREFERRED INHIBITORS

The IR spectra herein were taken on a Perkin-Elmer 1600 Series FT-IR spectrophotometer. Proton NMR spectra were recorded on a Brucker AC-F 300 instrument. The following abbreviations have been used: s, singlet; d, doublet; dd, doublet of doublet; t, triplet; q, quadruplet; and m, multiplet. The chemical shifts (δ) were referenced to chloroform (7.26 ppm for 1H and 77.00 ppm for 13C) and were expressed in ppm. Optical rotations were measured at room temperature on a Jasco DIP 360 polarimeter. Mass spectra (MS) were obtained on a V.G. Micromass 16F machine. Thin-layer chromatography (TLC) was performed on 0.25 mm Kieselgel 60F254 plates (E. Merck, Darmstadt, FRG). For flash chromatography, Merck-Kieselgel 60 (230-400 mesh A.S.T.M.) was used. Unless otherwise noted, starting material and reactant were obtained commercially and were used as such or purified by standard means. All solvents and reactants purified and dried were stored under argon. Anhydrous reactions were performed under an inert atmosphere, the set-up assembled and cooled under argon. Organic solutions were dried over magnesium sulfate, evaporated on a rotatory evaporator and under reduced pressure. Starting materials and reagents were available from Aldrich Chemical Company, Inc. (Milwaukee, Wisconsin)

LIST OF ABBREVIATIONS

DHP 3,4-dihydro-2H-pyran
EDTA Ethylenediaminetetraacetic acid
HPLC High pressure liquid chromatography
PTSA p-toluenesulfonic acid
THF Tetrahydrofuran
THP Tetrahydropyranyl
TMS Tetramethylsilyl
Example 1

Synthesis of 6-methyl 4,6-pregnadien/1,4,6-pregnatrien--
17-ol-3,20-dione alkanoates/benzoates

These syntheses are described in Schemes 1 and 2.

Scheme 1
Scheme 2

1) Me₃Al, CuCN
2) 1,3-Dibromo-5,5-dimethyl hydrantoin

Esterification Condition: A or B, C, D, E

7; R' = Me
Example 1A
6-methyl-1,4,6-Pregnatrien-17α-ol-3,20-dione acetate (2; EM-910):
Megestrol acetate (2.5 g; 6.7 mmol) and DDQ (5.07 g; 22 mmol) in
Dioxane (30 mL) were refluxed for 2 h. Solvent was removed and the
mixture in EtOAc was washed with saturated NaHCO₃ solution (3 times).
Solvent was dried (MgSO₄) and evaporated to give the product.
Purification on silica gel column (hexanes/acetone) gave the trienone (2.1
g) in 90% yield; IR (KBr, cm⁻¹) 2953, 2895, 1729, 1709, 1658, 1646, 1610,
1365, 1264, 1247, 887. ¹H NMR (CDCl₃) δ 0.69 (s, 3 H, H-C18), 1.13 (s, 3 H,
H-C19), 1.87 (s, 3H, 6-CH₃), 2.0 (s, 3H), 2.02 (s, 3H), 2.89-2.98 (m,1H), 5.79 (s,
1 H), 6.22 (s, 1H), 6.20-6.24 (m, 1 H, 2-H), 7.04 (d, 1 H, 1-H, J= 10 Hz); ¹³C
NMR (CDCl₃) δ 203.7, 186.4, 170.6, 163.2, 153.4, 134.5, 131.8, 127.7, 121.6,
96.2, 49.1, 47.9, 47.1, 41.1, 37.7, 31.1, 30.3, 26.4, 23.2, 21.5, 20.5 19.2, 14.4.

Example 1B
6-methyl-4,6-Pregnadien-17α-ol-3,20-dione (3): Megestrol acetate (11.2
g) was dissolved in 200 mL of boiling MeOH and to this, was added 2N
NaOH (20 mL). The mixture was refluxed for 5 h. Approx. 100 mL of
MeOH was removed, and water (50 mL) was added and the suspension
was cooled to 0 °C to give a solid, which was filtered and washed with
water (9.6 g, 96%); IR (KBr, cm⁻¹) 3493, 2942, 2767, 1702, 1658, 1646, 1623,
1575. ¹H NMR (CDCl₃) δ 0.75 (s, 3 H, H-C18), 1.06 (s, 3 H, H-C19), 1.81(s,
3H, 6-CH₃), 2.25(s, 3H), 2.96 (s,1H), 5.83 (s, 1 H), 5.95 (s, 1H); ¹³C NMR
(CDCl₃) δ 211.3, 200.0, 164.4, 138.5, 131.1, 121.0, 89.5, 50.3, 48.7, 47.9, 36.9,
36.1, 34.0 33.5, 30.2, 27.7, 23.3, 20.1, 19.8 16.3, 15.2.

Example 1C
6-methyl-4,6-Pregnadien-17α-ol-3,20-dione aklanoates/benzoates (4):
Esterification was conducted by following the method A or B, C, D, E.

Condition A: Using Trifluoromethanesulfonic Anhydride: To a
concentrated solution of the organic acid (1.1 eq.) in CH₂Cl₂ (1 mol/L),
was added trifluoromethanesulfonic anhydride (1.2 eq.) at room
temperature. After 15 min, the mixed anhydride was added to a solution
of megestrol 3 in CH₂Cl₂ (1 mol/L) at 0 °C. The mixture was stirred for 1
h at 0 °C followed by 1 h at room temperature. Saturated solution of
NaHCO₃ was added and the aq. layer was extracted with CH₂Cl₂ (3
times). The combined organic layer was dried and evaporated to give the
dark brown oil, which was purified on a silica gel column using
hexanes/acetone as an eluent to give pure product (yields range from 40
to 80 %)
Condition B; Using Trifluoroacetic Anhydride: Acid (5.61 mmol) was added to trifluoroacetic anhydride (5.33 mmol) in CH₂Cl₂ (5 mL) at room temperature and the solution was stirred for 3 h. The resulting solution was transferred to compound 3 (0.56 mmol) in CH₂Cl₂ (5 mL) at room temperature and the reaction was followed by TLC. The reaction mixture was diluted with CH₂Cl₂ (50 mL) and the organic solution was washed with saturated NaHCO₃ solution and then with saturated NaCl solution, dried (MgSO₄). Evaporation of solvents gave the crude product which was purified by column chromatography using hexanes/acetone as an eluent to give the pure product.

Condition C; Using Acid chloride or Acid anhydride and p-Toluenesulfonic acid: To a solution of megestrol 3 in CH₂Cl₂, was added p-toluenesulfonic acid (1 eq.) followed by acid chloride/acid anhydride (2 to 3 eq.). The reaction mixture was stirred for 8 to 10 h depending upon the alkyl chains. The mixture was poured in ice water and extracted with CH₂Cl₂. The organic layer was washed with saturated NaHCO₃, dried, and solvents removed to give the product which was purified by column chromatography using hexanes/acetone as an eluent.

Condition D; Using Acid chloride or Acid anhydride and phosphoric acid: To a solution of megestrol 3 in CH₂Cl₂, was added 85 % aq phosphoric acid (1 eq.) followed by acid chloride/acid anhydride (2 to 3 eq.). The reaction mixture was stirred for 8 to 10 h depending upon the alkyl chains. The mixture was poured in ice water and extracted with CH₂Cl₂. The organic layer was washed with saturated NaHCO₃, dried, and solvents removed to give the product which was purified by column chromatography using hexanes/acetone as an eluent.

Condition E; Using Acid anhydride and Scandium Trifluoromethanesulfonate: To compound 3 (1.12 mmol), was added propionic acid (47 mmol) and scandium trifluoromethanesulfonate (1 mol %; 5 mg) under argon and left for 3 h while stirring. The mixture was diluted with EtOAc and washed twice with saturated NaHCO₃ solution. Organic solvents were evaporated to give the product which was purified by column chromatography using hexanes/EtOAc as a solvent system to give pure product.

Example 1D
The followings are non-limiting examples of physicochemical characteristics of inhibitors of Example 1.
EM-917 (4; R=n-C3H7): Yield, 74%; IR (NaCl, cm⁻¹) 2950, 2870, 1731, 1661, 1626, 1580, 1458, 1352, 1249, 1159, 1110; ¹H NMR (CDCl₃) δ 0.69 (s, 3H, H-C18), 0.86 (t, 3H, H-C6', J=7 Hz), 1.07 (s, 3H, H-C19), 1.82 (s, 3H, 6-CH3), 2.02 (s, 3H, H-C21), 2.31 (t, 2H, H-C2', J=7 Hz), 2.44-2.56 (m, 2H), 2.92-3.01 (m, 1H), 5.85 (s, 1H), 5.94 (s, 1H); ¹³C NMR (CDCl₃) δ 203.9, 199.8, 173.3, 164.0, 137.9, 131.4, 121.3, 96.2, 50.3, 49.1, 47.6, 37.1, 36.1, 34.4, 34.1, 33.6, 31.3, 31.2, 30.4, 26.4, 24.5, 23.3, 22.3, 20.3, 19.9, 16.4, 14.3, 13.9.

EM-923 (4; R=n-C4H9): Yield, 82%; IR (NaCl, cm⁻¹) 2951, 2872, 1731, 1660, 1626, 1580, 1458, 1387, 1351, 1263, 1160, 1110; ¹H NMR (CDCl₃) δ 0.71 (s, 3H, H-C18), 0.90 (t, 3H, H-C5', J=7 Hz), 1.08 (s, 3H, H-C19), 1.84 (s, 3H, 6-CH3), 2.03 (s, 3H, H-C21), 2.20-2.27 (m, 1H), 2.38 (t, 2H, H-C2', J=7 Hz), 2.40-2.59 (m, 2H), 2.94-3.03 (m, 1H), 5.86 (s, 1H), 5.95 (s, 1H); ¹³C NMR (CDCl₃) δ 203.9, 199.8, 173.3, 164.0, 137.9, 131.4, 121.2, 96.1, 50.3, 49.1, 47.5, 37.1, 36.1, 34.2, 34.1, 33.6, 31.1, 30.4, 26.8, 26.4, 23.3, 22.2, 20.3, 16.4, 14.3, 13.6.

EM-928 (4; R=CH(CH₃)₂): Yield, 58%; IR (NaCl, cm⁻¹) 2947, 2874, 1731, 1660, 1626, 1580, 1469, 1387, 1351, 1272, 1215, 1197, 1154, 1111, 1084, 1060; ¹H NMR (CDCl₃) δ 0.71 (s, 3H, H-C18), 1.08 (s, 3H, H-C19), 1.18 (t, 6H, 2'-CH₃, J=7 Hz), 1.83 (s, 3H, 6-CH₃), 2.01 (s, 3H, H-C21), 2.45-2.60 (m, 3H), 2.93-3.02 (m, 1H), 5.86 (s, 1H), 5.94 (s, 1H); ¹³C NMR (CDCl₃) δ 203.8, 199.8, 176.2, 164.0, 137.9, 131.4, 121.3, 96.0, 50.3, 49.2, 47.7, 37.1, 36.1, 34.2, 34.1, 33.6, 31.2, 30.4, 26.3, 23.3, 20.3, 19.9, 18.8, 18.6, 16.4, 14.4.

EM-948 (4; R=CH₂Cycpenta): Yield, 68%; IR (NaCl, cm⁻¹) 2947, 2867, 1731, 1681, 1626, 1579, 1455, 1352, 1262, 1160; ¹H NMR (CDCl₃) δ 0.66 (s, 3H, H-C18), 1.00 (s, 3H, H-C19), 1.79 (s, 3H, 6-CH₃), 1.98 (s, 3H, H-C21), 2.29 (t, 2H, H-C2', J=8 Hz), 2.38-2.54 (m, 2H), 2.89-2.97 (m, 1H), 2.45-2.60 (m, 3H), 2.93-3.02 (m, 1H), 5.86 (s, 1H), 5.94 (s, 1H); ¹³C NMR (CDCl₃) δ 203.9, 199.8, 173.4, 164.0, 137.9, 131.4, 121.3, 96.1, 50.3, 49.1, 47.5, 39.6, 37.1, 36.1, 34.1, 33.7, 33.6, 32.4, 32.3, 31.1, 30.8, 30.4, 26.4, 25.1, 23.3, 20.3, 19.9, 15.3, 14.3.

EM-949 (4; R=CH₂Ph): Yield, 47%; IR (NaCl, cm⁻¹) 2946, 2870, 1730, 1658, 1625, 1579, 1454, 1351, 1264, 1141; ¹H NMR (CDCl₃) δ 0.67 (s, 3H, H-C18), 1.07 (s, 3H, H-C19), 1.86 (s, 3H, 6-CH₃), 1.90 (s, 3H, H-C21), 2.00-2.04 (m, 1H), 2.17-2.19 (m, 1H), 2.43-2.64 (m, 2H), 2.90-2.98 (m, 1H), 3.65 (s, 2H, H-C2'), 5.90 (s, 2H), 7.22-7.33 (m, 5H, Ar); ¹³C NMR (CDCl₃) δ 203.6, 199.9, 170.8, 164.0, 137.9, 133.4, 131.4, 129.3, 128.7, 127.3, 121.3, 96.8, 50.4, 48.9, 47.7, 41.9, 37.1, 36.1, 34.2, 33.5, 31.0, 30.2, 26.2, 23.2, 20.3, 19.9, 16.4, 14.3.
EM-950 (4; R=Cyhex); Yield, 49 %; IR (NaCl, cm⁻¹) 2948, 2869, 1726, 1660, 1625, 1578, 1475, 1351, 1271, 1193, 1155, 1110; ¹H NMR (CDCl₃) δ 0.72 (s, 3H, H-C₁₈), 1.09 (s, 3H, H-C₁₉), 1.65 (s, 3H, 6-CH₃), 2.03 (s, 3H, H-C₂¹), 2.21-2.24 (m, 1H), 2.41-2.63 (m, 1H), 2.72-2.79 (m, 1H, H-C₂'), 2.94-3.03 (m, 1H), 5.88 (s, 1H), 5.95 (s, 1H); ¹³C NMR (CDCl₃) δ 203.9, 199.8, 175.9, 164.0, 138.0, 131.4, 121.3, 96.0, 50.3, 49.2, 47.6, 44.0, 37.1, 36.1, 34.1, 33.6, 31.2, 30.5, 29.7, 29.6, 26.3, 25.7, 25.6, 23.4, 20.3, 19.9, 16.4, 14.3.

EM-978 (4; R=Cyhex); Yield, 48 %; IR (NaCl, cm⁻¹) 2935, 2857, 1726, 1660, 1625, 1579, 1449, 1351, 1316, 1247, 1159; ¹H NMR (CDCl₃) δ 0.71 (s, 3H, H-C₁₈), 1.09 (s, 3H, H-C₁₉), 1.65 (s, 3H, 6-CH₃), 2.03 (s, 3H, H-C₂¹), 2.24-2.37 (m, 1H), 2.42-2.64 (m, 1H), 2.94-3.03 (m, 1H), 5.88 (s, 1H), 5.95 (s, 1H); ¹³C NMR (CDCl₃) δ 203.8, 199.8, 175.3, 164.0, 138.0, 131.4, 121.3, 95.9, 50.3, 49.2, 47.6, 43.2, 37.1, 36.1, 34.0, 33.6, 31.3, 30.5, 28.8, 28.7, 26.3, 25.6, 25.3, 23.4, 20.3, 19.9, 16.4, 14.4.

EM-979 (4; R=t-Bu); Yield, 82 %; IR (NaCl, cm⁻¹) 2949, 2872, 1724, 1660, 1626, 1580, 1152; ¹H NMR (CDCl₃) δ 0.71 (s, 3H, H-C₁₈), 1.09 (s, 3H, H-C₁₉), 1.22 (s, 9H, Z′-CH₃), 1.84 (s, 3H, 6-CH₃), 2.01 (s, 3H, H-C₂¹), 2.21-2.25 (m, 1H), 2.42-2.62 (m, 2H), 2.95-3.04 (m, 1H), 5.87 (s, 1H), 5.94 (s, 1H); ¹³C NMR (CDCl₃) δ 203.7, 199.8, 177.5, 164.0, 137.9, 131.4, 121.3, 95.9, 50.4, 49.4, 47.8, 39.0, 37.1, 36.1, 34.0, 33.6, 31.4, 30.4, 26.9, 26.2, 23.3, 20.3, 19.9, 16.4, 14.3.

EM-996 (4; R=(CH₂)sBr); Yield, 43 %; IR (NaCl, cm⁻¹) 2946, 2868, 1731, 1660, 1626, 1579, 1457, 1387, 1352, 1260, 1194, 1123, 1110; ¹H NMR (CDCl₃) δ 0.70 (s, 3H, H-C₁₈), 1.07 (s, 3H, H-C₁₉), 1.83 (s, 3H, 6-CH₃), 2.03 (s, 3H, H-C₂¹), 2.35 (t, 2H, H-C₂', J=7 Hz), 2.93-3.02 (m, 1H), 3.37 (t, 2H, H-C₆', J=7 Hz), 5.85 (s, 1H), 5.94 (s, 1H); ¹³C NMR (CDCl₃) δ 203.8, 199.8, 172.9, 164.0, 137.8, 131.4, 121.2, 96.2, 50.2, 49.0, 47.5, 37.0, 36.0, 34.1, 34.0, 33.5, 33.3, 32.2, 31.1, 30.4, 27.6, 26.5, 23.9, 23.2, 20.2, 19.8, 16.3, 14.3.

EM-1003 (4; R=(CH₂)sBr); Yield, 13 %; IR (NaCl, cm⁻¹) 2947, 1731, 1659, 1625, 1579, 1444, 1124; ¹H NMR (CDCl₃) δ 0.74 (s, 3H, H-C₁₈), 1.01 (s, 3H, H-C₁₉), 1.85 (s, 3H, 6-CH₃), 2.07 (s, 3H, H-C₂¹), 2.55 (t, 2H, H-C₂', J=5 Hz), 2.96-3.05 (m, 1H), 3.47 (t, 2H, H-C₆', J=7 Hz), 5.89 (s, 1H), 5.96 (s, 1H); ¹³C NMR (CDCl₃) δ 203.6, 199.7, 172.0, 163.9, 137.7, 131.3, 121.2, 96.4, 50.1, 48.9, 47.5, 37.0, 36.0, 33.9, 33.5, 32.5, 32.4, 31.0, 30.4, 27.2, 26.5, 23.2, 20.2, 19.8, 16.3, 14.2.

EM-1029 (4; R=(CH₂)sCl); Yield, 30 %; IR (NaCl, cm⁻¹) 2947, 2871, 1730, 1659, 1625, 1579, 1458, 1445, 1353, 1271, 1059; ¹H NMR (CDCl₃) δ 0.67 (s,
3H, H-C18), 1.04 (s, 3H, H-C19), 1.79 (s, 3H, 6-CH₃), 2.00 (s, 3H, H-C21), 2.16-2.23 (m, 1H), 2.34 (t, 2H, H-C'2', J=7 Hz), 2.89-2.98 (m, 1H), 3.49 (t, 2H, H-C'5', J=7 Hz), 5.81 (s, 1H), 5.91 (s, 1H); ¹³C NMR (CDCl₃) δ 203.6, 199.6, 172.5, 163.9, 137.8, 131.2, 121.0, 96.2, 50.1, 48.9, 47.4, 44.2, 36.9, 35.9, 33.9, 33.4, 31.6, 30.9, 30.3, 26.4, 23.1, 21.9, 20.1, 19.7, 16.2, 14.1.

EM-1044 (4; R=CH₃PhCl(p)); Yield, 38 %; IR (KBr, cm⁻¹) 2948, 1734, 1656, 1625, 1578, 1492, 1459, 1352, 1262, 1154, 1089, 1017; ¹H NMR (CDCl₃) δ 0.67 (s, 3H, H-C18), 1.07 (s, 3H, H-C19), 1.86 (s, 3H, 6-CH₃), 1.92 (s, 3H, H-C21), 2.15-2.22 (m, 1H), 2.41-2.67 (m, 2H), 2.88-2.96 (m, 1H), 3.61 (s, 2H, H-C'2'), 5.89 (s, 2H), 7.18 (d, 2H, J=8.3 Hz), 7.27 (d, 2H, J=8.3 Hz); ¹³C NMR (CDCl₃) δ 203.6, 199.9, 170.3, 164.0, 137.7, 133.4, 131.8, 131.5, 130.7, 128.8, 121.3, 96.9, 50.4, 48.9, 47.8, 41.1, 37.1, 36.1, 34.1, 33.6, 31.0, 30.3, 26.4, 23.1, 20.3, 19.9, 16.4, 14.3.

EM-1049 (4; R=CH₂PhOMe(p)); Yield, 40 %; IR (KBr, cm⁻¹) 2947, 2871, 1729, 1657, 1625, 1578, 1513, 1459, 1352, 1302, 1249, 1151, 1035; ¹H NMR (CDCl₃) δ 0.64 (s, 3H, H-C18), 1.05 (s, 3H, H-C19), 1.83 (s, 3H, 6-CH₃), 1.87 (s, 3H, H-C21), 1.96-2.08 (m, 1H), 2.13-2.20 (m, 1H), 2.39-2.62 (m, 2H), 2.85-2.93 (m, 1H), 3.56 (s, 2H, H-C'2'), 3.72 (s, 3H, p-OCH₃), 5.86 (s, 1H), 5.89 (s, 1H), 6.83 (d, 2H, J=8.5 Hz), 7.18 (d, 2H, J=8.5 Hz); ¹³C NMR (CDCl₃) δ 203.8, 199.9, 171.1, 164.1, 158.9, 137.9, 131.4, 130.3, 125.4, 121.3, 114.0, 96.7, 55.3, 50.3, 48.9, 47.7, 40.9, 39.1, 37.1, 36.1, 34.1, 33.6, 31.0, 30.2, 26.2, 23.2, 20.3, 19.9, 16.4, 14.3.

EM-1107 (4; R=CH₃PhOCO-t-Bu(p)); Yield, 38 %; IR (NaCl, cm⁻¹) 2972, 2872, 1731, 1659, 1625, 1579, 1508, 1263, 1202, 1118; ¹H NMR (CDCl₃) δ 0.67 (s, 3H, H-C18), 1.07 (s, 3H, H-C19), 1.33 (s, 9H, tert-Butyl), 1.85 (s, 3H, 6-CH₃), 1.91 (s, 3H, H-C21), 3.64 (s, 2H, H-C'2'), 5.88 (s, 1H), 5.93 (s, 1H), 6.97-7.0 (m, 2H), 7.25-7.28 (m, 2H); ¹³C NMR (CDCl₃) δ 203.7, 200.0, 177.0, 170.6, 164.1, 150.4, 138.0, 131.4, 130.6, 130.2, 121.8, 121.3, 96.9, 50.3, 49.0, 47.7, 41.2, 39.1, 37.1, 36.1, 34.0, 33.6, 30.9, 30.2, 27.1, 26.3, 23.2, 20.2, 19.9, 16.4, 14.3.

CS-251 (4; R=(CH₃O)COCH₃); Yield, 26 %; IR (NaCl, cm⁻¹) 2949, 2873, 1732, 1660, 1626, 1580, 1463, 1356, 1258, 1060; ¹H NMR (CDCl₃) δ 0.63 (s, 3H, H-C18), 1.00 (s, 3H, H-C19), 1.77 (s, 3H, 6-CH₃), 1.96 (s, 3H, H-C'5'), 2.05 (s, 3H, H-C21), 2.29 (t, 2H, J=7 Hz), 2.43 (t, 2H, J=7 Hz), 5.77 (s, 1H), 5.87 (s, 1H); ¹³C NMR (CDCl₃) δ 207.5, 203.6, 199.6, 172.5, 163.8, 137.7, 131.1, 120.9, 96.0, 50.0, 48.8, 47.4, 42.0, 36.8, 35.8, 33.8, 33.4, 33.1, 30.8, 30.2, 29.7, 26.3, 23.1, 20.0, 19.6, 18.4, 16.1, 14.1.
CS-256 (4; R=CH₂PhF(p)); Yield, 32 %; IR (KBr, cm⁻¹) 2948, 2872, 1734, 1657, 1625, 1578, 1510, 1458, 1352, 1264, 1224, 1152; ¹H NMR (CDCl₃) δ 0.65 (s, 3H, H-C18), 1.05 (s, 3H, H-C19), 1.84 (s, 3H, 6-CH₃), 1.88 (s, 3H, H-C21), 1.96-2.02 (m, 1 H), 2.13-2.21(m, 1H), 2.38-2.49 (m, 2H), 2.87-2.95 (m, 1H), 3.59 (s, 2H, H-C2'), 5.86 (s, 1H), 5.89 (s, 1 H), 6.93-6.99 (m, 2H), 7.18-7.82 (m, 2H); ¹³C NMR (CDCl₃) δ 203.6, 199.9, 170.6, 164.0, 163.7, 160.5, 137.8, 131.4, 130.9, 130.8, 129.12, 129.07, 121.3, 115.7, 115.4, 96.9, 50.4, 48.9, 47.7, 40.9, 37.0, 36.1, 34.1, 33.6, 31.0, 30.2, 26.3, 23.1, 20.3, 19.9, 16.4, 14.3.
Example 2

Synthesis of 1α-alkyl-6-methyl-4,6-pregnadien-17α-ol-3,20-dione alkanoates/benzoates

These syntheses are described in Scheme 2

Example 2A
1α-alkyl-6-methyl-4,6-Pregnadien-17α-ol-3,20-dione acetate (5): The following example is a representative: To trienone 2 (500 mg; 1.33 mmol) in THF (30 mL), were added CuBr (12 mg; 0.08 mmol), trimethylaluminum (2.6 mL; 5.2 mmol) and chlorotrimethylsilane (0.659 mL; 5.2 mmol) under argon. The reaction mixture was stirred for 1 h. Excess of reagents was destroyed by the slow addition of MeOH at 0 °C. The mixture was extracted with CH₂Cl₂ dried and solvents removed. Purification on silica gel column using hexanes/EtOAc as an eluent gave the 1α-methyl compound, EM-952 (466 mg) in 90 % yield; IR (KBr, cm⁻¹) 2972, 2892, 1734, 1715, 1661, 1625, 1368, 1259; ¹H NMR (CDCl₃) δ 0.66 (s, 3 H, 18-CH₃), 0.93 (d, 3H, 1-CH₃, j=7 Hz), 1.12 (s, 3 H, 19-CH₃), 1.88(s, 3H, 6-CH₃), 1.99(s, 3H), 2.05(s, 3H), 2.78-2.97 (m,2H), 5.81 (s, 1 H), 5.89 (s, 1H); ¹³C NMR (CDCl₃) δ 203.6, 199.2, 170.5, 160.3, 137.8, 131.8, 120.5, 96.3, 49.0, 47.3, 44.6, 41.1, 39.2, 36.8, 35.6, 30.99, 30.2, 26.2, 23.2, 21.5, 19.7, 19.6, 18.9, 14.8, 14.2.

Example 2B
1α-alkyl-6-methyl-4,6-Pregnadien-17α-ol-3,20-dione (6): The following example is a representative: To 1α-Methylidienone 5 (517 mg; 1.3 mmol) in MeOH (30 mL), was added aq NaOH (52 mg in 1 mL of water and the mixture was refluxed for 1 h. The mixture was diluted with brine and extracted with CH₂Cl₂ (3x50 mL), dried, and solvents were removed under reduced pressure to the 17α-hydroxy compound 6 (430 mg) in 93 % yield; IR (KBr, cm⁻¹) 3445, 2972, 2892, 1703, 1647, 1622, 1576, 1259; ¹H NMR (CDCl₃) δ 0.75 (s, 3 H, H-C18), 0.91 (d, 3H, 1-CH₃, j=7 Hz), 1.14 (s, 3 H, H-C19), 1.81(s, 3H, 6-CH₃), 2.25(s, 3H COCH₃), 2.69-2.88 (m,2H), 3.0 (s, 1H), 5.83 (s, 1 H), 5.93 (s, 1H); ¹³C NMR (CDCl₃) δ 211.3, 199.6, 160.9, 138.5, 131.8, 120.5, 89.6, 48.9, 48.2, 44.8, 42.1, 39.2, 36.8 35.7, 33.5, 30.2, 27.7, 23.3, 19.8, 19.6, 18.9, 15.3, 14.8.
Example 2C
1α-alkyl-6-methyl-4,6-Pregnadien-17α-ol-3,20-dione aklanoates/benzoates (7):
Esterification was conducted by following the method A or B, C, D, E of Example 1.

Example 2D
The following is a non-limiting example of physicochemical characteristics of inhibitors of Example 2.

EM-1022 (7; R=Cypent); Yield 54%; IR (CDCl₃) 1733, 1717, 1652, 1623, 1576 cm⁻¹; ¹H NMR (CDCl₃) δ 0.69 (s, 3 H), 0.95 (d, 3 H, J=7 Hz), 1.15 (s, 3 H), 1.82 (s, 3 H), 2.00 (s, 3 H), 2.71-3.00 (m, 3 H), 5.84 (s, 1 H), 5.91 (s, 1 H); ²(3, 3 H), ¹³C NMR (CDCl₃) δ 203.8, 199.3, 175.9, 160.4, 137.9, 131.9, 120.6, 95.9, 49.3, 47.6, 44.7, 43.7, 42, 39.2, 36.9, 35.7, 31.2, 30.4, 29.7, 29.5, 26.2, 25.7, 25.6, 23.3, 19.9, 19.7, 19, 14.8, 14.3.

EM-1059 (7; R=CH₂Ph); Yield 74%; IR (CDCl₃) 1731, 1651, 1576 cm⁻¹; ¹H NMR (CDCl₃) δ 0.59 (s, 3 H), 0.92 (d, 3 H, J=7 Hz), 1.08 (s, 3 H), 1.79 (s, 3 H), 1.84 (s, 3 H), 2.85 (m, 2 H), 3.59 (s, 2 H), 5.81 (s, 2 H) 7.19 (m, 5 H); ¹³C NMR (CDCl₃) δ 203.6, 199.3, 170.7, 160.5, 137.9, 133.4, 131.9, 129.3, 128.6, 127.2, 120.6, 96.7, 49.1, 47.8, 44.7, 42.1, 41.8, 39.2, 39.9, 35.9, 35.8, 30.8, 30.1, 26.1, 23.1, 19.9, 19.7, 19, 14.9, 14.2.

CS-209 (7; R=CH(CHOH)₂); Yield 33%; IR (CDCl₃) 1731, 1659, 1624, 1577 cm⁻¹; ¹H NMR (CDCl₃) δ 0.72 (s, 3 H), 0.98 (d, 3 H, J=7 Hz), 1.18 (s, 3 H), 1.18 (d, 3 H, J=7 Hz), 1.20 (d, 3 H, J=7 Hz), 1.85 (s, 3 H), 2.03 (s, 3 H), 2.56-2.66 (m, 1 H), 2.85-3.05 (m, 2 H), 5.88 (s, 1 H), 5.94 (s, 1 H); ¹³C NMR (CDCl₃) δ 203.7, 199.4, 176.3, 160.4, 137.9, 132, 120.6, 95.9, 49.3, 47.7, 44.8, 42, 39.2, 36.7, 35.7, 34, 31.2, 30.4, 26.2, 23.3, 19.9, 19.7, 19, 18.7, 18.6, 14.9, 14.3.

CS-243 (7; R=CH₂CH₂); Yield 67%; IR (CDCl₃) 1732, 1716, 1659, 1625, 1577 cm⁻¹; ¹H NMR (CDCl₃) δ 0.68 (s, 3 H), 0.94 (d, 3 H, J=7 Hz), 1.12 (t, 3 H, J=5.8 Hz), 1.14 (s, 3 H), 1.81 (s, 3 H), 2 (s, 3 H), 2.80-2.90 (m, 2 H), 5.83 (s, 1 H), 5.91 (s, 1 H); ¹³C NMR (CDCl₃) δ 203.8, 199.4, 173.9, 160.5, 137.9, 132, 120.7, 96.49.1, 47.5, 44.6, 41.9, 39.1, 36.9, 35.6, 31, 30.2, 27.7, 26.2, 23.2, 19.8, 19.6, 18.9, 14.8, 14.2, 8.9.

CS-245 (7; R=(CH₂)₃C₄H₈); Yield 70%; IR (CDCl₃) 1731, 1715, 1660, 1625, 1577 cm⁻¹; ¹H NMR (CDCl₃) δ 0.67 (s, 3 H), 0.91 (t, 3 H, J=7 Hz), 0.94 (d, 3 H, J=7 Hz), 1.14 (s, 3 H), 1.81 (s, 3 H), 1.99 (s, 3 H), 2.29 (t, 2 H, J=7 Hz),
2.80-2.90 (m, 2 H), 5.82 (s, 1 H), 5.9 (s, 1 H); $^1$H NMR (CDCl$_3$) $\delta$ 203.7, 199.3, 173.1, 160.4, 137.9, 131.9, 120.6, 96, 49.1, 47.5, 44.6, 42, 39.2, 36.9, 36.2, 35.7, 31, 30.3, 26.2, 23.2, 19.8, 19.6, 18.9, 18.2, 14.8, 14.2, 13.6.
Example 3

6-methyl-4,6-Pregnadien-17α-ol-3,20-dione 4′-carboxethoxybutyrate (EM-1007): To a solution of megestrol 3 (400 mg; 1.17 mmol) in CH₂Cl₂, was added p-toluenesulfonic acid (90 mg; 0.47 mmol.) followed by glutaryl chloride (2.1 g; 12.5 mmol) at 20 °C. After addition, the reaction mixture was stirred for 7.5 h. The mixture was cooled to 0 °C and quenched with excess of MeOH. After 10 min, the mixture was poured in brine and extracted with CH₂Cl₂. The organic layer was washed with saturated NaHCO₃, dried, and solvents removed to give the product which was purified by column chromatography using hexanes/acetone: 80/20 as an eluent to give EM-1007 (161 mg) in 30% yield; IR (NaCl, cm⁻¹) 2949, 2873, 1732, 1860, 1626, 1580, 1443, 1363, 1260, 1198, 1144; ¹H NMR (CDCl₃) δ 0.72 (s, 3H, H-C18), 1.09 (s, 3H, H-C19), 1.85 (s, 3H, 6-CH₃), 1.96 (t, 2H, H-C4', J=7 Hz), 2.06 (s, 3H, H-C21), 2.21-2.27 (m, 1H), 2.39 (t, 2H, H-C2', J=7 Hz), 2.95-3.04(m, 1H), 3.67 (s, 3H, 5'-OCH₃), 5.88 (s, 1H), 5.95 (s, 1H); ¹³C NMR (CDCl₃) δ 203.6, 199.6, 173.0, 172.3, 163.9, 137.8, 131.2, 121.0, 96.2, 51.4, 50.0, 48.8, 47.4, 36.9, 35.9, 33.8, 33.4, 33.2, 32.7, 30.9, 30.2, 26.3, 23.1, 20.1, 19.7, 16.2, 14.1.
Example 4

6-methyl-17α-pentylxyloxy-4,6-Pregnadien-3,20-dione (EM-1127): Powered potassium hydroxide (263 mg; 4.7 mmol) was added to DMSO (4 mL) under argon. After 5 min, megestrol (3) (400 mg; 1.17 mmol) was added followed by 1-iodopentane (305 µl; 2.3 mmol). The reaction mixture was stirred for 1 h and water (20 mL) was added, and extracted with CH₂Cl₂ (3x20 mL). The organic layer was washed with brine (20 mL), dried, and solvents removed to give the crude product which was purified on silica gel column using hexanes/EtOAc as eluent to give pure product (33 mg) in 7% yield: IR (NaCl, cm⁻¹) 2932, 2868, 1706, 1662, 1625, 1579; ¹H NMR (CDCl₃) δ 0.63 (s, 3 H, H-C18), 0.88 (t, 3 H, J=6.9 Hz), 1.06 (s, 3 H, H-C19), 1.82 (s, 3H, 6-CH₃), 2.11 (s, 3H, COCH₃), 2.42-2.56 (m, 3H), 2.91 (app. q, 1H), 3.34 (app. q, 1H), 5.85 (s, 1 H), 5.95 (s, 1H); ¹³C NMR (CDCl₃) δ 21.2, 200.1, 164.5, 138.7, 131.0, 121.0, 95.9, 64.4, 50.1, 48.5, 48.1, 37.2, 36.1, 33.9, 33.6, 30.7, 29.9, 28.3, 26.5, 23.5, 23.1, 22.5, 20.4, 19.8, 16.3, 14.7, 14.0.
Example 5

16α-substituted 4,6-androstadien-3,17-dione derivatives

These syntheses are described in Scheme 3.

Scheme 3
Example 5A

General method for 16α-substituted 4,6-androstadien-3,17-dione (13)

16α-substituted 5-androsten-3,17-dione 3-ethyleneketal (11) Under argon atmosphere, a solution of 5-androsten-3,17-dione 3-ethyleneketal 10 in anhydrous THF (3.3% W/V) was cooled at 0 °C and treated with a 1.0 M solution of lithium bis(trimethylsilyl)amid in THF (1.0 equiv) and HMPA (2.0 equiv). The solution was stirred 20 min at room temperature, cooled at -78 °C, and treated with 1.2 equiv of alkyl iodide. The reaction mixture was allowed to reach room temperature and stirred overnight (for R = isobutyl). For smaller R group (e.g.: R=Pr), the reaction was allowed to reach -40 °C and stirred 3 h. The reaction was quenched with saturated ammonium chloride and diluted with ethyl acetate. The organic phase was washed with brine, dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by flash chromatography (hexanes to hexanes-acetone 19:1) to provide compound 11 (e.g., R=iso-butyl, 8%): 1H NMR (300 MHz, CDCl3) δ 0.88 (d, J=6.3 Hz, 3H), 0.92 (s, 3H), 0.92 (d, J=6.1 Hz, 3H), 1.05 (s, 3H), 1.08-1.84 (m, 17H), 2.11 (m, 2H), 2.46 (m, 1H), 2.58 (m, 1H), 3.95 (m, 4H), 5.37 (m, 1H).

16α-substituted 4-androsten-3,17-dione (12). Under argon atmosphere, a solution of compound 11 and p-toluenesulfonic acid (0.11 equiv) in acetone (1.0% W/V) was refluxed for 2.5 h. The crude mixture was warmed to room temperature, diluted with distilled water, and evaporated (acetone). The aqueous phase was extracted with dichloromethane. The organic phase was washed two times with saturated sodium bicarbonate and brine, dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by flash chromatography (hexanes-acetone 49:1 to hexanes-acetone 19:1) to provide compound 12 (e.g., R=iso-butyl, 81%): 1H NMR (300 MHz, CDCl3) δ 0.88 (d, J=6.5 Hz, 3H), 0.92 (d, J=6.5 Hz, 3H), 0.96 (s, 3H), 1.21 (s, 3H), 0.98-2.02 (m, 16H), 2.38 (m, 5H), 5.75 (s, 1H).

16α-substituted 4,6-androstadien-3,17-dione (13). Under argon atmosphere, a solution of compound 12 and chloranil (4.8 equiv) in anhydrous tert-butanol (2.6% W/V) was refluxed for 3 h. The reaction mixture was cooled at room temperature and the suspension was filtered to remove excess of chloranil and then evaporated. The crude mixture was diluted with dichloromethane and the obtained solution was washed 3 times with distilled water, 4 times with 5% sodium hydroxide, and 4 times with distilled water. The organic phase was dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by 2 flash chromatographies (hexanes to hexanes-acetone 9:1; and hexanes-ethyl acetate 97:3 to hexanes-ethyl acetate 17:5) to provide compound 13.
(e.g., EM-1273-CS, R=isobutyl, 29%): IR (CHCl₃) 3010, 2957, 2871, 1733, 1656, 1619, 1467 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 0.89 (d, j=6.5 Hz, 3H), 0.93 (d, j=6.4 Hz, 3H), 1.01 (s, 3H), 1.14 (s, 3H), 1.05-2.05 (m, 13H), 2.34-2.58 (m, 4H), 5.70 (s, 1H), 6.18 (m, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 14.45, 16.32, 19.92, 21.35, 23.44, 26.57, 27.45, 31.52, 33.87, 36.13, 36.97, 40.02, 43.06, 46.56, 48.88, 50.86, 124.16, 128.76, 138.37, 162.91, 199.30, 221.43.
Example 6

16-Symmetrical-disubstituted 4,6-androstadien-3,17-dione and 4,6-estradien-3,17-dione derivatives

Scheme 4
Example 6A
General method for 16-symmetrical-disubstituted 4,6-androstadien-3,17-dione and 4,6-estradien-3,17-dione (17)

16-symmetrical-disubstituted 5-androsten-3,17-dione 3-ethyleneketal (15). Under argon atmosphere, a solution of 5-androsten-3,17-dione 3-ethyleneketal (14) in anhydrous THF (2.4% W/V) was treated with sodium hydride in 60% dispersion in mineral oil (10 equiv) and alkyl dihalide (1.5 equiv) or alkyl halide (10 equiv) and refluxed for 20 h. The reaction mixture was cooled at room temperature and quenched with ethanol, and then the solvents were evaporated. The crude mixture was diluted with dichloromethane and washed with saturated ammonium chloride and brine. The organic phase was dried over magnesium sulfate, filtered, and evaporated. The crude compound 16 (e.g. R1=CH3, R2=-(CH2)n-) was directly used for the next step: 1H NMR (300 MHz, CDCl3) δ 0.88 (s, 3H), 1.05 (s, 3H), 1.0-2.2 (m, 24H), 2.58 (m, 1H), 3.95 (m, 4H), 5.37 (m, 1H). When alkyl halide was used, compounds 15 were purified by flash chromatography.

16-symmetrical-disubstituted 4-androsten-3,17-dione (16). The same procedure for compound 12 was used, starting from compound 15. The crude mixture was purified by flash chromatography (hexanes-acetone 19:1 to hexanes-acetone 4:1) to provide compound 16 (e.g. EM-844, R1=CH3, R2=-(CH2)n-, 74%). A sample was recrystallized from hexanes-acetone 4:1: IR (KBr) 2950, 2925, 2858, 1730, 1674, 1617, 1448 cm⁻¹; 1H NMR (300 MHz, CDCl3) δ 0.89 (s, 3H), 1.18 (s, 3H), 0.93-2.04 (m, 19H), 2.26-2.44 (m, 4H), 5.70 (s, 1H); 13C NMR (75 MHz, CDCl3) δ 14.03, 17.36, 20.31, 25.37, 25.93, 30.93, 31.89, 32.58, 33.89, 34.71, 35.65, 38.18, 38.66, 39.21, 39.93, 48.00, 48.54, 53.94, 56.15, 124.06, 170.39, 199.22, 225.41.

16-symmetrical-disubstituted 4,6-androstadien-3,17-dione (17). A solution of compound 16 in acetic anhydride (12.4 equiv) and pyridine (1.1 equiv) was treated with acetyl chloride (13.0 equiv), refluxed for 3 h, cooled at room temperature, and stirred overnight. The reaction mixture was evaporated and coevaporated 2 times with ethanol and 1 time with toluene. The crude dienol acetate was dissolved in wet DMF (97%). The solution was treated with N-bromosuccinimide (1.03 equiv), stirred for 45 min, treated with lithium carbonate (2.4 equiv) and lithium bromide (1.02 equiv), and heated at 100 °C for 3 h. The reaction mixture was cooled at room temperature, treated with saturated ammonium chloride, and extracted 3 times with ethyl acetate. The combined organic phase was dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by flash chromatography (hexanes to hexanes-
acetone 19-1) and recrystallization (hexanes-acetone 49-1) to provide compound 17 (e.g. CS-195, R¹=CH₃, R²=-(CH₂)₄-, 61%): IR (CHCl₃) 2947, 2872, 1731, 1657, 1619 cm⁻¹; ¹H RMN (300 MHz, CDCl₃) δ 0.98 (s, 3H), 1.14 (s, 3H), 1.2-2.05 (m, 18H), 2.35-2.65 (m, 3H), 5.70 (s, 1H), 6.17 (m, 2H); ¹³C RMN (75 MHz, CDCl₃) δ 14.04, 16.33, 20.00, 25.38, 25.93, 31.86, 33.87, 36.17, 36.65, 37.73, 39.20, 39.98, 46.57, 48.84, 50.91, 56.24, 124.12, 128.70, 138.68, 163.00, 199.32, 224.69.

Example 6B
16-symmetrical-disubstituted 1,4,6-androstatrien-3,17-dione (18). Under argon atmosphere, a solution of compound 17 and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (1.7 equiv) in toluene (4.3% W/V) was refluxed for 5 h. The reaction mixture was cooled at room temperature, diluted with ethyl acetate, and washed with saturated sodium carbonate and brine. The organic phase was dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by flash chromatography (hexanes to hexanes-acetone 19-1) and triturated in hexanes to provide compound 18 (e.g. EM-1039, R¹=CH₃, R²=-(CH₂)₄-, 52%): IR (CHCl₃) 2946, 1732, 1652, 1603 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 1.01 (s, 3H), 1.23 (s, 3H), 1.29-2.04 (m, 16H), 2.45 (m, 1H), 6.03 (m, 2H), 6.29 (m, 2H), 7.06 (d, J=10.1 Hz, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 14.14, 20.76, 21.22, 25.37, 25.93, 31.84, 37.20, 37.70, 39.18, 39.99, 41.18, 46.75, 48.40, 48.58, 56.22, 124.28, 128.34, 128.49, 135.99, 152.41, 161.78, 186.13, 224.32.

Example 6C
1α-methyl-16-symmetrical-disubstituted 4,6-androsten-3,17-dione (19). Under argon atmosphere, a solution of compound 18 and cuprous bromide (0.01 equiv) in anhydrous THF (15% W/V) was treated with a 2.0 M solution of trimethylaluminum in toluene (1.1 equiv) and stirred 1 h at room temperature. The reaction mixture was hydrolyzed with water and the solid filtered off and washed. The crude mixture was purified by flash chromatography (hexanes-acetone 19-1) to provide compound 19 (e.g. EM-1077, R¹=CH₃, R²=-(CH₂)₄-, 55%): IR (CHCl₃) 3014, 2948, 2873, 1731, 1651, 1618, 1253 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 0.95 (d, J=6.8 Hz, 3H), 0.99 (s, 3H), 1.23 (s, 3H), 1.25-2.05 (m, 16H), 2.19-2.38 (m, 3H), 2.85 and 2.91 (dd, J=5.4 and 17.8 Hz, 1H), 5.71 (s, 1H), 6.17 (m, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 14.10, 14.85, 19.02, 19.47, 25.40, 25.96, 31.84, 35.82, 36.63, 37.77, 39.23, 39.32, 40.02, 42.38, 45.47, 46.79, 49.00, 56.22, 123.70, 129.54, 138.41, 159.77, 198.95, 224.71.
Example 6D
16-symmetrical-disubstituted 4-androsten-3,17-dione-6-methylene (22)
A solution of 16-symmetrical-disubstituted 4-androsten-3,17-dione (16),
sodium acetate (4.4 equiv), diethoxymethane (89 equiv), and phosphorus
oxychloride (15 equiv) in chloroform (3.3% W/V) was refluxed for 1 h.
The reaction mixture was cooled at rt, quenched slowly with saturated
sodium carbonate, and diluted in chloroform. The organic phase was
washed with distilled water and brine. The crude mixture was purified
by flash chromatography (hexanes-ethyl acetate 9-1 to hexanes-ethyl
acetate 4-1) and recrystallization (acetone-hexanes 1-9) to provide
compound XI (e.g. EM-921, R¹=CH₃, R²=-(CH₂)₅, 59%): IR (KBr) 2931,
2857, 1726, 1673, 1596, 1444, 1267, 1227 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ
0.91 (s, 3H), 1.11 (s, 3H), 1.14-2.08 (m, 22H), 2.35-2.50 (m, 2H), 2.58 and
2.26 (dd, J=3.3 and 12.9 Hz, 1H), 4.98 (d, J=1.9 Hz, 1H), 5.09 (d, J=2.0 Hz,
1H), 5.92 (s, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 14.29, 17.10, 20.24, 22.40,
22.55, 25.42, 31.87, 31.92, 33.00, 33.79, 34.77, 35.10, 36.65, 39.02, 39.18, 48.25,
48.56, 50.47, 52.80, 114.44, 121.91, 145.54, 168.43, 199.61, 223.84.

Example 6E
16-symmetrical-disubstituted 4,6-androsten-3,17-dione-6-methyl(23)
A solution of 16-symmetrical-disubstituted 4-androsten-3,17-dione-6-
methylene (22) in benzene (0.67% W/V) was treated with acetic
anhydride (15 equiv) and a solution of p-toluenesulfonic acid (5 equiv) in
benzene (8.4% W/V), and refluxed for 3.5 h. The reaction mixture was
cooled at rt, quenched with distilled water, and diluted with benzene.
The organic phase was washed 1 time with distilled water, 2 times with
5% sodium bicarbonate, and 4 times with brine. The organic phase was
dried over magnesium sulfate, filtered, and evaporated. The crude
mixture was purified by flash chromatography (hexanes-ethyl acetate 9-1
to hexanes-ethyl acetate 4-1) and recrystallization (acetone-hexanes 1-9)
to provide compound 23 (e.g. EM-925, R¹=CH₃, R²=-(CH₂)₅, 60%): IR
(KBr) 2926, 2858, 1726, 1662, 1629, 1582, 1444, 1268 cm⁻¹; ¹H NMR (300
MHz, CDCl₃) δ 0.97 (s, 3H), 1.11 (s, 3H), 1.20-2.62 (m, 26H), 5.89 (s, 1H),
6.06 (s, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 14.31, 16.39, 19.89, 19.98, 22.46,
22.58, 25.40, 31.81, 32.01, 32.53, 33.61, 34.02, 36.22, 36.63, 46.13, 49.27, 50.59,
51.13, 121.45, 131.85, 136.56, 163.88, 199.82, 223.45.
Example 7

16-symmetrical-disubstituted 1,4-androistadien-3,17-dione (21). The same procedure for compound 18 was used, starting from compound 16. The reflux period was 20 h instead of 5 h. The crude mixture was purified by 2 flash chromatographies (hexanes to hexanes-acetone 9:1) to provide compound 21 (e.g. EM 1123, R1=CH3, R2=-(CH2)4-, 74%). A sample was recrystallized in acetone-hexanes (1:49): IR (CHCl3) 3014, 2946, 2871, 1730, 1661, 1622, 1603 cm⁻¹; ¹H NMR (300 MHz, CDCl3) δ 0.96 (s, 3H), 1.26 (s, 3H), 1.14-2.05 (m, 19H), 2.35-2.60 (m, 2H), 6.09 (s, 1H), 6.23 and 6.26 (dd, j=2.0 and 10.2 Hz), 7.05 (d, j=10.2 Hz, 1H); ¹³C NMR (75 MHz, CDCl3) δ 14.16, 18.72, 22.14, 25.38, 25.94, 31.81, 32.50, 32.59, 34.71, 38.37, 39.23, 39.98, 43.49, 48.16, 52.45, 56.13, 124.13, 127.72, 155.34, 168.37, 186.22, 225.19.
Example 8

16-asymmetrical-disubstituted 6-methyl-4,6-androstadien-3,17-dione (27)

These syntheses are described in Scheme 5.
Example 8A
General method for 16-asymmetrical-disubstituted 4,6-androstadien-3,17-dione (27)

16-monosubstituted 5-androsten-3,17-dione 3-ethyleneketal (24). Under argon atmosphere, a solution of 5-androsten-3,17-dione 3-ethyleneketal (10) in anhydrous THF (4.6% W/V) was cooled at -20 °C, treated with a 1.0 M solution of lithium bis(trimethylsilyl)amide in THF (1.0 equiv), warmed to room temperature for 15 min, cooled at -78 °C, and treated with alkyl iodide (1.05 equiv). The reaction mixture was warmed to room temperature, stirred for 2 h, quenched with saturated ammonium chloride, and evaporated (THF). The aqueous phase was extracted with dichloromethane. The combined organic phase was washed with brine, dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by flash chromatography (hexanes-acetone 99:1 to hexanes-acetone 19:1) to provide compound 24 (e.g. R^1=CH_3, 7:3 16α/β ratio, 69%): 1H NMR (300 MHz, CDCl_3) δ 0.84 (s, 0.9H), 0.91 (s, 2.1H), 1.04 (s, 3H), 1.09 (d, J=6.5 Hz, 2.1H), 1.20 (d, J=7.0 Hz, 0.9 H), 1.25-1.86 (m, 13H), 2.05-2.16 (m, 3H), 2.56 (m, 2H), 3.95 (m, 4H), 5.36 (m, 1H).

16-asymmetrical-disubstituted 5-androsten-3,17-dione 3-ethyleneketal (25). Under argon atmosphere, a solution of compound 24 in anhydrous THF (4.0% W/V) was cooled at -40 °C, treated with 0.5 M solution of potassium bis(trimethylsilyl)amide in toluene (1.2 equiv), warmed to room temperature for 15 min, cooled at -78 °C, and treated with 1.5 equiv of alkyl iodide. The reaction mixture was allowed to reach room temperature, refluxed for 2 h, and cooled at room temperature. The reaction mixture was quenched with saturated ammonium chloride, evaporated (THF), and extracted with dichloromethane. The combined organic phase was washed with brine, dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by flash chromatography (hexanes-acetone 99:1 to hexanes-acetone 19:1) to provide compound 25 (e.g. R^1=CH_3, R^2=isobutyl, 56%): 1H NMR (300 MHz, CDCl_3) δ 0.85 (d, J=6.6 Hz, 3H), 0.90 (d, J=6.6 Hz, 3H), 0.93 (s, 3H), 1.05 (s, 3H), 1.15 (s, 3H), 1.27-2.16 (m, 19H), 2.56 (m, 1H), 3.95 (m, 4H), 5.37 (m, 1H).

16-asymmetrical-disubstituted 4-androsten-3,17-dione (26). The same procedure for compound 12 was used, starting from compound 25. The crude mixture was purified by flash chromatography (hexanes-acetone 49:1 to hexanes-acetone 19:1) to provide compound 26 (e.g. EM-1133, R^1=CH_3, R^2=isobutyl, 96%): IR (KBr) 2951, 2869, 1734, 1676, 1616, 1454 cm⁻¹; 1H NMR (300 MHz, CDCl_3) δ 0.86 (d, J=6.6 Hz, 3H), 0.90 (d, J=6.6 Hz,
16-asymmetrical-disubstituted 4,6-androstan-3,17-dione (27). The same procedure for compound 13 was used, starting from compound 26. The crude mixture was purified by flash chromatography (hexanes-acetone 49:1 to hexanes-acetone 19:1) to provide compound 27 (e.g. EM-1134, R₁=CH₃, R₂=iso-butyl, 57%): IR (KBr) 2954, 2870, 1735, 1665, 1618, 1584, 1466 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 0.88 (d, J=6.7 Hz, 3H), 0.91 (d, J=6.6 Hz, 3H), 1.01 (s, 3H), 1.14 (s, 3H), 1.20 (s, 3H), 1.22-2.65 (m, 16H), 5.71 (s, 1H), 6.18 (m, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 15.10, 16.29, 19.82, 24.56, 24.89, 25.15, 25.97, 31.99, 33.78, 33.87, 34.80, 36.15, 36.59, 45.77, 46.27, 48.98, 50.92, 124.07, 128.77, 138.70, 163.05, 199.34, 224.32.

Example 8B

16α-alkylhalide-16β-alkyl 4,6-androstan-3,17-dione (27b). Under argon atmosphere, a solution of 16α-hydroxyalkyl-16β-alkyl 4,6-androstan-3,17-dione (27a) and triphenylphosphine (1.2 equiv) in dichloromethane (10% W/V) was cooled at 0 °C and treated with N-chlorosuccinimide (or N-bromosuccinimide) (1.2 equiv). The reaction mixture was warmed to room temperature for 30 min and diluted with dichloromethane. The organic phase was washed with brine, dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by 2 flash chromatographies (hexanes-ethyl acetate 9:1 to hexanes-ethyl acetate 7:3; and dichloromethane to dichloromethane-ethyl acetate 9:1) to provide compound 27b (e.g. EM-1135, R₁=CH₃, R₂=(CH₂)₃Cl, 51%): IR (CHCl₃) 3010, 2946, 2873, 1733, 1657, 1619 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 1.02 (s, 3H), 1.14 (s, 3H), 1.21 (s, 3H), 1.26-2.11 (m, 14H), 2.35-2.65 (m, 3H), 3.49 (m, 2H), 5.71 (s, 1H), 6.17 (m, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 14.91, 16.32, 19.80, 25.16, 27.97, 31.78, 33.80, 33.89, 34.83, 35.25, 36.16, 36.60, 45.19, 46.01, 48.10, 49.36, 50.89, 124.21, 128.89, 138.43, 162.92, 199.40, 223.36.
Example 9

17α-acyloxy-6-methyl-4,6-androstadien-3-one-17β-carboxamide (34)

These syntheses are described in Scheme 6.

Scheme 6

1. H$_2$O$_2$
2. H$_2$SO$_4$
3. PTSA, (CH$_3$CO)$_2$O
4. TMS urea, CH$_3$Cl, pyridine
5. K$_3$CO$_3$
6. 10% HCl, acetone, CH$_3$OH

R$^1$, R$^2$, R$^3$
Example 9A
General method for 17α-acyloxy-6-methyl-4,6-androstan-3-one-17β-carboxamide (34)

4-androsten-17α-ol-3-one-17β-carboxylic acid methyl ester (29). A solution of 4-pregnen-17,21-diol-3,20-dione (28) (6.93 g, 0.0200 mol) in methanol (500 mL) was treated with a 0.075 M periodic acid (300 mL, 0.0225 mol) and 2.5 M sulfuric acid (70 mL, 0.175 mol) and stirred 3 h. The reaction mixture was evaporated (methanol) and filtered. The crude mixture was dried to afford 4-androsten-17α-ol-3-one-17β-carboxylic acid (6.50 g). A solution of the carboxylic acid (4.56 g, 0.0137 mol) and 6 drops of concentrated sulfuric acid in methanol (250 mL) was refluxed for 30 h. The reaction mixture was cooled to room temperature, evaporated, diluted with ethyl acetate, dichloromethane, and water, and treated with sodium bicarbonate. The mixture was extracted 3 times with ethyl acetate. The combined organic phase was washed with brine, dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by flash chromatography (hexanes to hexanes-ethyl acetate 3:2) to provide the methyl ester 29 (4.12 g, 87%): 1H NMR (300 MHz, CDCl3) δ 0.72 (s, 3H), 1.18 (s, 3H), 0.94-1.89 (m, 13H), 2.01 (m, 1H), 2.36 (m, 4H), 2.65 (m, 1H), 2.85 (s, 1H), 3.75 (s, 3H), 5.72 (s, 1H).

Example 9B
6-methylene-4-androsten-17α-ol-3-one-17β-carboxylic acid methyl ester (30). A suspension of sodium acetate (6.65 g, 0.081 mol), diethoxymethane (201 mL, 1.62 mol) and phosphorus oxychloride (25 mL, 0.27 mol) in chloroform (250 mL) was refluxed for 1 h, treated with a solution of compound 29 (6.02 g, 0.018 mol) in chloroform (50 mL), and refluxed for 2 h. The reaction mixture was cooled to room temperature, diluted with water, and extracted with dichloromethane. The combined organic phase was stirred in the presence of sodium carbonate for 1 h, decanted, washed with water and brine, dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by flash chromatography (hexanes to hexanes-ethyl acetate 1:1) to provide the methyl ester 30 (3.60 g, 56%): 1H NMR (300 MHz, CDCl3) δ 0.71 (s, 3H), 1.08 (s, 3H), 1.13-2.08 (m, 13H), 2.45 (m, 3H), 2.67 (m, 1H), 2.85 (m, 1H), 3.76 and 3.77 (2s, 2.2 and 0.8H), 4.93 (d, J=1.9 Hz, 1H), 5.05 (d, J=1.6 Hz, 1H), 5.90 (s, 1H).
Example 9C
17α-acetoxy-6-methyl-4,6-androstadien-3-one-17β-carboxylic acid methyl ester (31). The same procedure for compound 23 was used, starting from compound 30. The crude mixture was purified by flash chromatography (hexanes to hexanes-ethyl acetate 7-3) to provide compound 31 (EM-1010, 75%): IR (KBr) 2967, 2871, 1742, 1668, 1628, 1584, 1458, 1366, 1260, 1245 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 0.77 (s, 3H), 1.09 (s, 3H), 1.23-2.02 (m, 11H), 1.84 (broad s, 3H), 2.04 (s, 3H), 2.24 (m, 1H), 2.42-2.65 (m, 2H), 2.98 (m, 1H), 3.71 (s, 3H), 5.87 (s, 1H), 5.96 (s, 1H); ¹³C NMR (75 MHz, CDCl₃) 14.43, 16.38, 19.86, 20.15, 21.05, 23.36, 30.35, 31.90, 33.61, 34.08, 36.12, 37.23, 48.01, 48.40, 50.36, 52.08, 90.58, 121.22, 131.37, 138.02, 164.12, 170.25, 170.41, 199.88.

Example 9D
6-methyl-4,6-androstadien-17α-ol-3-one-17β-carboxylic acid (32). A solution of compound 31 (3.00 g, 7.50 mmol) in methanol (225 mL) and 10% potassium carbonate (62 mL, 0.045 mol) was refluxed for 21 h. The crude mixture was cooled at room temperature, evaporated (methanol), acidified with 10% hydrochloric acid, and diluted with water. The precipitate was filtered, washed with water, and dried. The filtrate was extracted with dichloromethane. The organic phase was washed 3 times with brine, dried over magnesium sulfate, filtered, and evaporated. A solution of the combined solids in methanol-dichloromethane 1:1 (300 mL) was treated with 10% hydrochloric acid (60 mL), stirred for 3 h, and evaporated (methanol and dichloromethane). The aqueous phase was extracted 3 times with dichloromethane. The combined organic phase was washed 4 times with brine, dried over magnesium sulfate, filtered, and evaporated to provide carboxylic acid 32 (2.05 g, 80%); ¹H NMR (CDCl₃, CD₂OD) δ 0.68 (s, 3H), 0.95 (s, 3H), 1.05-1.88 (m, 13H), 1.68 (s, 3H), 2.09 (m, 1H), 2.55 (m, 1H), 5.69 (s, 1H), 5.89 (s, 1H).

Example 9E
6-methyl-4,6-androstadien-17α-ol-3-one-17β-carboxamide (33). A solution of compound 32 and 1.3-bis(trimethylsilyl)urea (1.5 equiv) in dichloromethane-pyridine 8:1 (5.7% W/V) was refluxed for 21 h. The reaction mixture was cooled at room temperature, filtered, evaporated (dichloromethane), dissolved in dichloromethane, filtered, and evaporated. A solution of the silylated α-hydroxycarboxylic acid in chloroform-dimethylformamide 99:1 (5.1% W/V) was cooled at 0 °C, treated with oxalyl chloride (1.2 equiv), stirred for 1 h, and warmed to room temperature for 30 min. The reaction mixture was cooled at 0 °C, treated with pyridine (4 equiv), and amine (3 equiv), stirred for 1 h, and warmed to room temperature for 3 h. The reaction mixture was diluted
with water and extracted with ethyl acetate. The combined organic phase was washed with 10% hydrochloric acid and brine, dried over magnesium sulfate, filtered, and evaporated. A solution of the crude mixture in methanol-acetone 1-1 (10 mL) was treated with 10% hydrochloric acid (2 mL) and stirred for 3 h. The reaction mixture was evaporated and extracted 3 times with ethyl acetate. The combined organic phase was washed 2 times with brine, dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by flash chromatography (hexanes to hexanes-ethyl acetate 2:1) to provide compound 33 (e.g. R1=Me, R2=Et, 41%). 1H NMR (300 MHz, CDCl3) δ 0.83 (broad s, 3H), 1.09 (s, 3H), 1.13 (t, J=7.0 Hz, 3H), 1.22-2.23 (m, 12H), 1.84 (s, 3H), 2.45-2.63 (m, 3H), 3.08 (m, 5H), 3.80 (m, 1H), 5.86 (s, 1H), 5.98 (s, 1H).

Example 9F
17α-acyloxy-6-methyl-4,6-androstadien-3-one-17β-carboxamide (34). A solution of compound 33 and p-toluenesulfonic acid (0.14 equiv) in acid chloride (4.7% W/V) was stirred 10 h at room temperature. The reaction mixture was quenched with methanol and water, stirred for 30 min, and extracted with ethyl acetate. The combined organic phase was washed with saturated sodium bicarbonate and brine, dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by 2 flash chromatographies (hexanes to hexanes-ethyl acetate 3:2 and dichloromethane to dichloromethane-acetone 49:1) to provide compound 34 (e.g. EM-1181-CS, R1=Me, R2=Et, R3=(CH2)3Cl, 50%); IR (KBr) 2944, 2871, 1738, 1659, 1640, 1631, 1580 cm⁻¹; 1H NMR (300 MHz, CDCl3) δ 0.84 (s, 3H), 1.10 (m, 6H), 1.25-2.25 (m, 14H), 1.85 (s, 3H), 2.40-2.63 (m, 4H), 2.89 and 2.99 (2s, 0.7 and 2.3H), 3.43 (m, 3H), 3.62 (t, J=6.1 Hz, 2H), 5.88 (s, 1H), 5.98 (s, 1H); 13C NMR (75 MHz, CDCl3) δ 11.49, 14.70, 16.32, 19.85, 20.54, 22.91, 27.12, 30.99, 32.93, 33.57, 33.99, 34.10, 35.44, 36.02, 37.28, 43.87, 45.18, 48.51, 48.91, 50.04, 94.76, 121.15, 131.28, 138.10, 164.07, 167.31, 170.78, 199.87.
Example 10

17β-acyl-17α-acyloxy-6-methyl-4,6-androstadien-3-one

These syntheses are described in Scheme 7.

Scheme 7
General method for 17β-acyl-17α-acyloxy-6-methyl 4,6-androstadien-3-one (40)

Example 10A

5

5-androsten-17α-ol-3-one-17β-carboxylic acid 3-ethyleneketal methyl ester (35). A solution of 4-androsten-17α-ol-3-one-17β-carboxylic acid methyl ester (29) (5.16 g, 0.0154 mol) and ethylene glycol (2.87 g, 0.0463 mol) in benzene (500 mL) was refluxed with a Dean-Stark apparatus for 1 h, treated with p-toluene sulfonic acid (100 mg, 0.53 mmol), and refluxed for 6.5 h. The reaction mixture was cooled to room temperature, diluted with ethyl acetate, washed with saturated sodium bicarbonate, and 2 times with water, dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by flash chromatography (hexanes to hexanes-ethyl acetate 7:3) to provide the ketal 35 (5.12 g, 85%): 1H NMR (300 MHz, CDCl3) δ 0.70 (s, 3H), 1.03 (s, 3H), 1.08-1.82 (m, 15H), 2.01 (m, 1H), 2.10 and 2.15 (dd, J=2.6 and 14.2 Hz, 1H), 2.61 (m, 2H), 2.78 (s, 1H), 3.75 (s, 3H), 3.94 (m, 4H), 5.35 (m, 1H).

Example 10B

17α-(2'-tetrahydro-2'H-pyran-3'-yl)-5-androsten-3-one-17β-carboxylic acid 3-ethyleneketal methyl ester (36). A solution of compound 35 (4.94 g, 0.0126 mol) in 3,4-dihydro-2H-pyran (100 mL) was cooled at 0°C, treated with p-toluene sulfonic acid (300 mg, 1.6 mmol), and stirred for 5 h. The reaction mixture was diluted with ethyl acetate, washed with saturated sodium bicarbonate and 2 times with water, dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by flash chromatography (hexanes to hexanes-ethyl acetate 21:4) to quantitatively provide the diketal 36 (6.10 g): 1H NMR (300 MHz, CDCl3) δ 0.66 (s, 1.1H), 0.67 (s, 1.9 H), 1.01 (s 3H), 1.09-2.15 (m, 22H), 2.47 (m, 3H), 3.37 (m, 0.7H), 3.51 (m, 1.3H), 3.65 (s, 1.7H), 3.69 (s, 1.3H), 3.93 (m, 4H), 4.56 (m, 0.6H), 4.77 (m, 0.4H), 5.34 (m, 1H).

Example 10C

17α-(2'-tetrahydro-2'H-pyran-3'-yl)-5-androsten-3-one-17β-carboxylic acid 3-ethyleneketal (37). Compound 36 (3.73 g, 0.00786 mol) was treated with 1 N potassium tert-butoxide in dimethyl sulfoxide (118 mL). The reaction mixture was stirred 4 h at room temperature, diluted with water, acidified to pH 2, and extracted 3 times with ethyl acetate. The combined organic phase was washed with brine and water, dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by flash chromatography (hexanes to ethyl acetate) to provide the carboxylic acid
37 (2.50 g, 69%): ¹H NMR (300 MHz, CDCl₃) δ 0.76 (s, 2.0H), 0.80 (s, 1.0H), 0.82-2.15 (m, 22H), 1.03 (s, 3H), 2.38-2.58 (m, 3H), 3.4-3.6 (2m, 2H), 3.95 (m, 4H), 4.68 (m, 1H), 5.35 (m, 1H).

**Example 10D**

17β-acyl-4-androsten-17α-ol-3-one (38). Under argon atmosphere, a solution of compound 37 in dry benzene (3.0% W/V) was cooled at 0 °C, treated with an organolithium solution (7.0 equiv), and stirred 20 h at room temperature. The reaction mixture was cooled to 0 °C, quenched with saturated ammonium chloride, diluted with water, and extracted 3 times with ethyl acetate. The combined organic phase was washed with brine, dried over magnesium sulfate, filtered, and evaporated. The crude mixture was dissolved in methanol-acetone 1:1 (2% W/V), treated with 2.5 M hydrochloric acid, and refluxed for 1 h. The reaction mixture was cooled at room temperature, evaporated (solvents), diluted in water, and extracted 3 times with ethyl acetate. The combined organic phase was washed with brine and water, dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by flash chromatography (hexanes to hexanes-ethyl acetate 3:1) to provide compound 38 (e.g. R¹=Bn, 42%): ¹H NMR (300 MHz, CDCl₃) δ 0.74 (s, 3H), 0.96-1.88 (m, 13H), 1.17 (s, 3H), 2.02 (m, 1H), 2.35 (m, 4H), 2.76 (m, 2H), 3.77 (d, J=16.5 Hz, 1H), 4.07 (d, J=16.4 Hz, 1H), 5.72 (s, 1H), 7.16-7.33 (m, 5H).

**Example 10E**

17β-acyl-6-methylene-4-androsten-17α-ol-3-one (39). A solution of compound 38, paraformaldehyde (5.4 equiv), and N-methylanilinium trifluoroacetate (4.8 equiv) in THF (2.9% W/V) was refluxed for 2.5 h. The reaction mixture was cooled at room temperature, treated with 2.5 M hydrochloric acid, stirred for 15 min, and extracted 3 times with ethyl acetate. The combined organic phase was washed with water, saturated sodium bicarbonate, and brine, dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by flash chromatography (hexanes to hexanes-ethyl acetate 4:1) to provide compound 39 (e.g. R¹=Bn, 33%): ¹H NMR (300 MHz, CDCl₃) δ 0.75 (s, 3H), 1.09 (s, 3H), 1.15-2.08 (m, 13H), 2.45 (m, 3H), 2.78 (m, 2H), 3.78 (d, J=16.3 Hz, 1H), 4.07 (d, J=16.3 Hz, 1H), 4.94 (s, 1H), 5.06 (s, 1H), 5.91 (s, 1H), 7.17-7.34 (m, 5H).
Example 10F
17β-acyl-17α-acyloxy-6-methyl-4,6-androstan-3-one (40). The same procedure for compound 23 was used, starting from compound 39. The crude mixture was purified by 2 flash chromatographies (dichloromethane to dichloromethane-acetone 99:1 and hexanes to hexanes-ethyl acetate 17:3) to provide compound 40 (e.g. EM-1106, R1=Hex, R2=CH3, 45%). IR (KBr) 2943, 2857, 1736, 1718, 1656, 1628, 1579, 1266, 1236; 1H NMR (300 MHz, CDCl3)  δ 0.72 (s, 3H), 1.09 (s, 3H), 1.26-2.25 (m, 12H), 1.86 (s, 3H), 2.14 (s, 3H), 2.43-2.64 (m, 2H), 3.06 (m, 1H), 3.68 (m, 2H), 5.89 (s, 1H), 5.97 (s, 1H), 7.16-7.33 (m, 5H); 13C NMR (75 MHz, CDCl3) δ 14.52, 16.39, 19.94, 20.35, 21.35, 23.30, 30.76, 31.21, 33.64, 34.07, 36.12, 37.11, 45.02, 47.91, 49.06, 50.23, 96.42, 121.29, 126.85, 128.32, 129.94, 131.49, 133.88, 137.93, 164.08, 170.76, 199.97, 203.41.
Example 11

17α-acyloxy-6-methyl-4,6-androsten-3-one-17β-carboxyester

These syntheses are described in Scheme 8.

Scheme 8
Example 11A
4-androsten-17α-ol-3-one-17β-carboxyester (41). The same procedure for
compound 29 was used and provide compound 41 (e.g., compound 41,
R1=CH3).

Example 11B
6-methylene-4-androsten-17α-ol-3-one-17β-carboxester (42). The same
procedure for compound 30 was used and provide compound 42 (e.g.
compound 42, R1=CH3).

Example 11C
17α-acyl-6-methyl-4,6-androstadien-17α-ol-3-one-17β-carboxester (43).
The same procedure for compound 31 was used and provide compound
43 (e.g. compound 43, R1,R2=CH3).
Example 12

15α-substituted 4,6-androstadien-3,17-dione derivatives

These synthesis are described in Scheme 9.

Scheme 9

1. LHMDS, TMSCl
2. Pb(OAc)$_2$

44

CR$^1$R$^2$CR$^3$CR$^4$R$^5$Mg Br

KJL, 18C6
THF

45

46

TsOH
Acetone

Chloranil
t-BuOH

47

48
Example 12A
5,15-androstadien-3,17-dione 3-ethyleneketal (44). Under argon atmosphere, a solution of 5-androsten-3,17-dione 3-ethyleneketal 10 (4.95 g, 0.0150 mol) in anhydrous THF (150 mL) was cooled at 0 °C and treated with 1.0 M solution of lithium bis(trimethylsilyl)amide in THF (18 mL, 0.018 mol). The solution was stirred 20 min at room temperature, cooled at -78 °C, and treated with chlorotrimethylsilane (2.24 mL, 0.0176 mol). The reaction was allowed to warm to room temperature, then evaporated. The residue was diluted with dichloromethane, washed two times with water and brine, dried over magnesium sulfate, filtered, and evaporated. A solution of the crude silylenol ether in a dichloromethane-acetonitrile 2:5 (140 mL) was treated with palladium acetate (4.04 g, 0.0180 mol) and refluxed for 30 min. The reaction mixture was cooled to room temperature and evaporated. The black residue was purified by flash chromatography (hexanes-ethyl acetate 4:1) to provide the enone 44 (3.49 g, 71%): 1H NMR (300 MHz, CDCl3) δ 1.085 (s, 3H), 1.09 (s, 3H), 1.21-1.93 (m, 11H), 2.15 (dd, J=2.7 and J=14.1 Hz, 1H), 2.26-2.34 (m, 2H), 2.58 (d, J=14.1 Hz, 1H), 3.95 (m, 4H), 5.40 (d, J=5.1 Hz, 1H), 6.04 (dd, J=3.2 and J=6 Hz, 1H), 7.50 (d, J=5.8 Hz, 1H).

Example 12B
17α-substituted allyl 5,15-androstadien-17β-ol-3-one 3-ethyleneketal (45). Under argon atmosphere, a solution of compound 44 in anhydrous THF (10% W/V) was cooled at 0 °C, treated with a solution of substituted allylmagnesium bromide or chloride in THF or ether (3 equiv) and stirred 3 h. The reaction mixture was quenched with saturated ammonium chloride and diluted with ethyl acetate. The organic phase was washed with brine, dried over magnesium sulfate, filtered, and evaporated. The crude residue was purified by flash chromatography (hexanes-ethyl acetate 9-1 to hexanes-ethyl acetate 4:1) to provide compound 45 (e.g., R1, R2, R3, R4, R5=H, 70%): 1H NMR (300 MHz, CDCl3) δ 0.92 (s, 3H), 1.06 (s, 3H), 1.11-1.81 (m, 11H), 1.95 (d, J=9.3 Hz, 1H), 2.10-2.21 (m, 3H), 2.30-2.36 (m, 1H), 2.57 (d, J=5 Hz, 1H), 3.95 (m, 4H), 5.10-5.16 (m, 2H), 5.36 (broad d, J=5 Hz, 1H), 5.61 (dd, J=3.1 and 5.7 Hz, 1H), 5.83 (d, J=5.8 Hz, 1H), 5.84-5.94 (m, 1H).
Example 12C
15α-substituted allyl-5-androsten-3,17-dione 3-ethyleneketal (46).
Under argon atmosphere, a solution of compound 45 in dry THF (10% W/V) was cooled at 0 °C, treated with potassium hydride in 35%
dispersion in mineral oil (2 equiv), and stirred 30 min. Then, the resulting
suspension was treated with 18-crown-6 ether (1.5 equiv), allowed to
reach room temperature, and stirred 2 h. The black mixture was
quenched with saturated ammonium chloride then diluted with ethyl
acetate. The organic phase was washed with brine, dried over
magnesium sulfate, filtered, and concentrated. The oily residue was
purified by flash chromatography (hexanes-ethyl acetate 4:1) to provide
compound 46 (e.g., R¹, R², R³, R⁴, R⁵=H, 71%): "H NMR (300 MHz, CDCl₃)
δ 0.94 (s, 3H), 1.06 (s, 3H), 1.1-1.5 (m, 5H), 1.5-2.0 (m, 11H), 2.14 (d, J=14.1
Hz, 1H), 2.19-2.29 (m, 2H), 2.62 (dd, J=8.3 and 19.3 Hz, 2H), 3.95 (m, 4H),
4.66 (dd, J=16 Hz, 2H), 5.35 (s, 2H).

Example 12D
15α-substituted allyl 4-androsten-3,17-dione (47). The same procedure
for compound 12 was used, starting from compound 46. The crude
mixture was purified by flash chromatography (hexanes-ethyl acetate 9:1
to hexanes-ethyl acetate 4:1) to provide compound 47 (e.g., EM-1284, R¹,
R², R³, R⁴, R⁵=H, 85%): IR (CHCl₃) 3020, 2954, 1733, 1664, 1617, 1275, 1230
cm⁻¹; "H NMR (300 MHz, CDCl₃) δ 0.97 (s, 3H), 1.21 (s, 3H), 0.99-1.60 (m,
4H), 1.60-2.05 (m, 10H), 2.05-2.50 (m, 4H), 2.65 (dd, J=8.7 and 16.2 Hz, 2H),
5.03 (m, 2H), 5.67-5.73 (m, 2H); "C NMR (75 MHz, CDCl₃) δ 15.30, 17.62,
20.26, 31.09, 32.40, 32.57, 33.91, 35.79, 35.88, 36.59, 38.73, 40.17, 42.42, 49.94,
53.91, 54.36, 116.78, 123.72, 135.93, 169.93, 199.15, 219.08.

Example 13E
15α-substituted allyl 4,6-androstadien-3,17-dione (48). The same
procedure for compound 13 was used, starting from compound 47. The
crude residue was purified by 2 flash chromatographies (hexanes-acetone
9:1 and hexanes-ethyl acetate 17:3) to provide compound 48 (e.g., EM-
1261, R¹, R², R³, R⁴, R⁵=H, 61%): IR (CHCl₃) 3013, 2943, 2865, 1734, 1655,
1619, 1229 cm⁻¹; "H NMR (CDCl₃, 300 MHz) δ 1.02 (s, 3H), 1.12 (s, 3H),
1.30-1.55 (m, 4H), 1.64-2.20 (m, 6H), 2.30-2.80 (m, 6H), 5.03-5.08 (m, 2H),
5.65-5.85 (m, 2H), 6.17 (dd, J=2.7 and 10.0 Hz, 1H), 6.42 (dd, J=1.8 and 10.0
Hz, 1H); "C NMR (75 MHz, CDCl₃) δ 15.41, 16.31, 19.79, 31.12, 33.85,
33.95, 35.29, 36.03, 38.40, 39.96, 42.25, 50.57, 52.61, 117.31, 123.97, 128.29,
135.19, 139.01, 162.35, 199.12, 218.20.
Example 13

(E)-16-monosubstituted methylene 4,6-androsten-3,17-dione

These syntheses are described in Scheme 10.

Scheme 10
Example 13A
16-mono-substituted hydroxymethyl 5-androsten-3,17-dione 3-ethyleneketal (49). Under argon atmosphere, a solution of 5-androsten-3,17-dione 3-ethyleneketal (10) in anhydrous THF (3.3% W/V) was cooled at 0 °C and treated with a 1.0 M solution of lithium bis(trimethylsilyl)amide in THF (1.0 equiv) and HMPA (2.0 equiv). The solution was stirred 20 min at room temperature, cooled at -78 °C, and treated with 1.2 equiv of aldehyde. The reaction mixture was allowed to reach room temperature and stirred over 3 h. The reaction was quenched with saturated ammonium chloride and diluted with ethyl acetate. The organic phase was washed with brine, dried over magnesium sulfate, filtered, and evaporated. The crude mixture was used to carry out the next step without further purification (e.g., R²=isopropyl, quantitative yield): 1H NMR (300 MHz, CDCl₃) δ 0.89-0.99 (m, 9H), 1.01-2.00 (m, 14H), 2.2-2.6 (m, 6H), 3.5-4.0 (m, 5H), 5.72 (m, 2H).

Example 13B
(E)-16-mono-substituted methylene 4-androsten-3,17-dione (50). The same procedure for compound 12 was used, starting from compound 49. The crude mixture was purified by flash chromatography (hexanes-ethyl acetate 97:3 to hexanes-ethyl acetate 17:3) to provide compound 50 (e.g., R²=isopropyl, 19%): 1H NMR (300 MHz, CDCl₃) δ 0.88 (s, 3H), 0.99 (d, J=6.6 Hz, 3H), 1.00 (d, J=6.6 Hz, 3H), 1.18 (s, 3H), 1.1-1.5 (m, 5H), 1.6-1.8 (m, 3H), 1.8-2.1 (m, 4H), 2.2-2.6 (m, 6H), 5.70 (s, 1H), 6.38 (m, 1H).

Example 13C
(E)-16-mono-substituted methylene 4,6-androstadien-3,17-dione (51). The same procedure for compound 13 was used, starting from compound 50. The crude mixture was purified by 2 flash chromatographies (hexanes-ethyl acetate 19:1 to hexanes-ethyl acetate 7:3) to provide compound 51 (e.g., EM-1353, R²=isopropyl, 60%): IR (CHCl₃) 2961, 2868, 1722, 1656, 1619, 1465, 1374, 1269, 1209, 914, 733 cm⁻¹; 1H NMR (300 MHz, CDCl₃) δ 0.89 (s, 3H), 1.05 (d, J=6.4 Hz, 3H), 1.07 (d, J=5.9 Hz, 3H), 1.16 (s, 3H), 1.25-1.55 (m, 4H), 1.71-1.80 (m, 2H), 1.94-2.05 (m, 2H), 2.18-2.28 (m, 1H), 2.40-2.65 (m, 4H), 2.74 and 2.79 (dd, J=6.5 and 15.2 Hz, 1H), 5.71 (s, 1H), 6.19 (s, 2H), 6.49 (m, 2H); 13C NMR (75 MHz, CDCl₃) δ 14.26, 16.29, 19.97, 21.79, 25.74, 29.19, 31.21, 33.73, 33.83, 36.13, 36.56, 46.39, 48.50, 50.74, 124.13, 128.76, 133.68, 138.44, 144.27, 162.88, 199.25, 208.08.
Example 14

4,6-androstadien-3,17-dione-16-spirobicyclo [3'.1'.0'] hexane

These syntheses are described in Scheme 11.
Example 14A
5-androsten-3,17-dione-16-spirobicyclo[3\'.1\'.0\']-hexane 3-ethyleneketal (52). Under argon atmosphere, a solution of 5-androsten-3,17-dione 3-ethyleneketal (10) (3.96 g, 0.0120 mol), potassium tertbutoxide (0.70 g, 0.059 mol) and 4-pentynyltriphenylphosphonium iodide (2.90 g, 0.0635 mol) in dry toluene (3% W/V) was heated at 100 °C for 1 h. The reaction mixture was cooled at room temperature, poured into ice-water and extracted several times with ethyl acetate. The combined organic phase was washed with water and brine, dried over magnesium sulfate, filtered, and evaporated. The obtained solid was purified by flash chromatography (hexanes-ethyl acetate 9-1 to hexanes-ethyl acetate 4-1) to provide spirobicykletal 52 (0.95 g, 20%): 1H NMR (300 MHz, CDCl3) δ 0.94 (s, 3H), 1.06 (s, 3H), 1.15-2.04 (m, 22H), 2.16 (dd, J=2.7 and J=14.1 Hz, 1H), 2.57 (m, 2H), 3.96 (m, 4H), 5.38 (s, 1H).

Example 14B
4-androsten-3,17-dione-16-spirobicyclo[3\'.1\'.0\']-hexane (53). The same procedure for compound 12 was used, starting from compound 52. The crude mixture was purified by flash chromatography (hexanes-ethyl acetate 9-1) to provide spirobicycloenone 53 (50%): 1H NMR (300 MHz, CDCl3) δ 0.95 (s, 3H), 1.18 (s, 3H), 1.02-2.10 (m, 22H), 2.25-2.45 (m, 3H), 5.8 (s, 1H).

Example 14C
4,6-androstadien-3,17-dione-16-spirobicyclo[3\'.1\'.0\']-hexane (EM-1299). The same procedure for compound 13 was used, starting from compound 53. The crude mixture was purified by flash chromatography (hexanes-ethyl acetate 9-1 to hexanes-ethyl acetate 4-1) to provide spirobicyclodienone EM-1299 (51%): IR (CHCl3) 3008, 2943, 2878, 1730, 1643, 1611 cm⁻¹; 1H NMR (300 MHz, CDCl3) δ 1.01 (s, 3H), 1.14 (s, 3H), 1.15-1.80 (m, 13H), 1.84-2.15 (m, 5H), 2.38-2.54 (m, 3H), 5.70 (s, 1H), 6.14-6.24 (m, 2H); 13C NMR (75 MHz, CDCl3) δ 14.51, 16.32, 19.92, 23.12, 23.24, 25.77, 31.21, 33.83, 35.35, 36.16, 36.28, 47.54, 48.58, 50.87, 124.12, 128.70, 138.65, 163.01, 199.31, 218.66.
Example 15

15α, 16α, (E-ring)-4,6-androstadien-3,7-dione derivatives

These syntheses are described in Scheme 12.

Scheme 12
Example 15A
15α-[1-(3-hydroxypropyl)]-5-androsten-17β-ol-3-one 3-ethyleneketal (54). Under argon atmosphere, a solution of compound 46 (1.20 g, 3.24 mmol) in dry THF (6 mL) was cooled at 0 °C and treated with a 0.5 M solution of 9-BBN in THF (20 mL, 10 mmol). The reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was cooled at 0 °C and treated with few drops of methanol, 3 N sodium hydroxide (5 mL) and 30% hydrogen peroxide (5 mL). The reaction mixture was vigorously stirred 2 h and neutralized with 10% hydrochloric acid. After evaporating of the solvent, the aqueous phase was extracted with dichloromethane. The organic phase was washed with brine, dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by flash chromatography (dichloromethane-ethyl acetate 9:1 to dichloromethane-ethyl acetate 4:1) to provide the diol 54 (0.97 g, 77%): 1H NMR (300 MHz, CDCl3) δ 0.78 (s, 3H), 1.04 (s, 3H), 1.07-1.89 (m, 19H), 2.11 (dd, J=2.6 and 14 Hz, 2H), 2.55 (d, J=14.1 Hz, 1H), 3.60 (m, 3H), 3.92 (m, 4H), 5.32 (s, 1H).

Example 15B
15α-[1-(3-tosyloxypropyl)]-5-androsten-17β-ol-3-one 3-ethyleneketal (55). Under argon atmosphere, a solution of compound 54 (558 mg, 1.43 mmol) in dry pyridine (5 mL) was cooled at 0 °C, treated with p-toluenesulfonyl chloride (328 mg, 1.72 mmol), and stirred 5 h. The reaction mixture was diluted with ethyl acetate and washed with 10% hydrochloric acid and brine. The organic phase was dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by flash chromatography (hexanes-ethyl acetate 9:1) to give the hydroxytosylate 55 (389 mg, 50%): 1H NMR (300 MHz, CDCl3) 0.74 (s, 3H), 1.04 (s, 3H), 1.06-1.76 (m, 19H), 2.12 (dd, J=2 and 15 Hz, 2H), 2.45 (s, 3H), 2.55 (d, J=15 Hz, 1H), 3.58 (t, J=8 Hz, 1H), 3.92-4.12 (m, 4H), 5.29 (s, 1H), 7.34 (d, J=8.4 Hz, 2H), 7.75 (d, J=8.2 Hz, 2H).

Example 15C
15α-[1-(3-tosyloxypropyl)]-5-androsten-3,17-dione 3-ethyleneketal (56). Under argon atmosphere, a solution of compound 55 (258 mg, 0.474 mmol) in dry dichloromethane (10 mL) was treated with 4-methylmorpholine N-oxide (82 mg, 0.70 mmol) and molecular sieves 4Å, stirred 30 min, treated with a catalytic amount of tetrapropylammonium perruthenate (5 mg) and stirred 2 h. Filtrating and evaporating of the reaction mixture afforded a black residue which was purified by flash chromatography (hexanes-ethyl acetate 7:3) to provide the ketotosylate 56 (129 mg, 50%): 1H NMR (300 MHz, CDCl3) δ 0.74 (s, 3H), 1.05 (s 3H), 1.08-
2.16 (m, 20H), 2.45 (s, 3H), 2.54-2.66 (m, 2H), 3.90-4.00 (m, 4H), 4.03 (t, J=6.4 Hz, 2H), 5.30 (s, 1H), 7.35 (d, J=8.4 Hz, 2H), 7.79 (d, J=8.2 Hz, 2H).

Example 15D
15α,16α-(1,3-propylene)-5-androsten-3,17-dione 3-ethyleneketal (57).
Under argon atmosphere, a solution of compound 56 in dry THF (10 mL) was cooled at 0 °C and treated with 1 M solution of lithium bis(trimethylsilyl)amide in THF (0.50 mL, 0.50 mmol) and HMPA (57.5 μL, 0.330 mmol). The reaction mixture was stirred 10 min, quenched with saturated ammonium chloride, evaporated (THF) and extracted with dichloromethane. The organic phase was washed with brine, dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by flash chromatography (hexanes-ethyl acetate 4:1 to hexanes-ethyl acetate 7:3) to provide the pentacyclic ketone 57 (96 mg, 78%): 1H NMR (300 MHz, CDCl3) δ 0.95 (t, J=10.4 Hz, 1H), 1.02 (s, 3H), 1.07 (s, 3H), 1.13-1.88 (m, 17H), 2.12 (dd, J=2.6 and 14.2 Hz, 2H), 2.55 (m, 2H), 2.99 (m, 1H), 3.95 (m, 4H), 5.3 (s, 1H).

Example 15E
15α,16α-(1,3-propylene)-4-androsten-3,17-dione (58). The same procedure for compound 12 was used, starting from compound 57. The crude mixture was purified by flash chromatography (hexanes-ethyl acetate 4:1) to provide pentacyclic enone 58 (~100%): 1H NMR (300 MHz, CDCl3) δ 0.85 (t, J=10.6 Hz, 1H), 0.94-1.02 (m, 1H), 1.05 (s, 3H), 1.22 (s, 3H), 1.24-1.87 (m, 14H), 2.06 (m, 2H), 2.31-2.48 (m, 3H), 2.58 (m, 1H), 3.01 (m, 1H), 5.30 (s, 1H).

Example 15F
15α,16α-(1,3-propylene)-4,6-androstadien-3,17-dione (59). The same procedure for compound 13 was used, starting from compound 58. The crude mixture was purified by 2 flash chromatographies (hexanes-acetone 9:1 and hexanes-ethyl acetate 97:3 to hexanes-ethyl acetate 17:3) to provide the pentacyclic dienone 59 (60%). The 1H NMR spectrum shows the presence of pentacyclic enone 58 in 10%: 1H NMR (300 MHz, CDCl3) δ 1.04 (t, J=10 Hz, 1H), 1.09 (s, 3H), 1.13 (s, 3H), 1.22-1.86 (m, 12H), 1.99 (m, 1H), 2.16-2.60 (m, 3H), 2.72 (q, J=8.2 Hz, 1H), 3.05 (m, 1H), 5.70 (s, 1H), 6.18 (dd, J=9.8 and 2.5 Hz, 1H), 6.32 (d, J=10 Hz, 1H).
Example 16

6,17α,β-dimethyl/1α,6,17αβ-trimethyl-D-Homo-4,6-androstadien/1,4,6-androstatrien-17α-ol-3, 17-dione alkanoate

These syntheses are described in Scheme 13.

Scheme 13
Example 16A
6,17αβ-dimethyl-D-Homo-4,6-androstan-17αα-ol-3,17-dione (60): The following example is a representative: Megestrol (44.1 g, 129 mmol) was dissolved in THF (550 mL). Sodium hydride (10.2 g; 3.2 eq.) was added portion-wise at 0 °C. After addition, the mixture was stirred for 9 h at room temperature and cooled to 0 °C, and water (300 mL) was added slowly. Approximately THF (300 mL) was evaporated under vacuum and another 300 mL of water was added. The suspension was cooled to 0 °C. Solid was filtered and washed with water. Solid, thus obtained was triturated with boiling MeOH (150 mL) and cooled to room temperature. This gave pure product which was filtered and washed with MeOH, and dried (36.4 g; 82 % yield).

Example 16B
6,17αβ-dimethyl-D-Homo-1,4,6-androstatrien-17αα-ol-3,17-dione (61): Compound 60 (5 g; 14.7 mmol) and DDQ (10 g; 3 eq) in dioxane (60 mL) were refluxed for 3 h. Solvent was removed and the mixture in EtOAc was washed with saturated NaHCO₃ solution (3 times). Solvent was dried (MgSO₄) and evaporated to give the product. Purification on silica gel column (hexanes/acetone) gave the trienone 61 (3.7 g) in 76% yield.

Example 16C
1α,6,17αβ-trimethyl-D-Homo-4,6-androstan-17αα-ol-3,17-dione (63): This compound was prepared by following the methods, described above.

Example 16D
6,17αβ-dimethyl/1α,6,17αβ-trimethyl-D-Homo-4,6-androstadien-17αα-ol-3,17-dione alkanoate (64): The alcohols were esterified by using the conditions A, B, C, D or E.

Example 16E
The followings are non-limiting examples of physicochemical characteristics of inhibitors of Example 16.

EM-1078 (64, R=H, R’=(CH₂)₄CH₃); Yield, 66 %; IR (KBr, cm⁻¹) 2948, 2871, 1735, 1660, 1624, 1577, 1458, 1375, 1317, 1269, 1188, 1099; ¹H NMR (CDCl₃) δ 0.80 (s, 3H, H-C18), 0.96 (t, 3H, H-C4, J=7 Hz), 1.06 (s, 3H, H-C19), 1.34 (s, 3H, 17α-CH₃), 1.87 (s, 3H, 6-CH₃), 2.01-2.06 (m, 2H), 2.32 (t, 2H, H-C2', J=7 Hz), 2.69 (s, 1H), 6.13 (s, 1H); ¹³C NMR (CDCl₃) δ 208.0, 200.0, 172.5, 163.7, 136.5, 131.8, 121.0, 87.6, 49.5, 45.4, 40.4, 37.3, 36.3, 36.1, 33.7, 33.4, 31.8, 26.1, 20.2, 19.8, 18.3, 16.2, 13.6, 13.0.
EM-1091 (64, R'=H, R=CH₂CH₃): Yield, 53%; IR (KBr, cm⁻¹) 2944, 1734, 1664, 1625, 1581, 1444, 1352, 1273, 1194, 1098; ¹H NMR (CDCl₃) δ 0.77 (s, 3H, H-CH₃), 1.01 (t, 3H, H-C₃, J=7 Hz), 1.30 (s, 3H, 17a-CH₃), 1.83 (s, 3H, 6-CH₃), 2.34 (q, 2H, H-C₂', J=7 Hz), 5.84 (s, 1H), 6.10 (s, 1H); ¹³C NMR (CDCl₃) δ 207.8, 199.8, 173.2, 163.5, 136.4, 131.9, 121.4, 121.2, 49.7, 45.5, 40.6, 37.4, 36.2, 33.9, 33.6, 31.9, 27.9, 26.1, 20.2, 19.9, 16.3, 13.7, 13.1, 9.0.

EM-1098 (64, R'=H, R=CH₂Ph): Yield, 37%; IR (KBr, cm⁻¹) 2946, 2869, 1735, 1657, 1624, 1578, 1456, 1268, 1127, 1098; ¹H NMR (CDCl₃) δ 0.72 (s, 3H, H-CH₃), 1.01 (s, 3H, H-C₁₈), 1.30 (s, 3H, H-C₁₈), 1.90 (s, 3H, 6-CH₃), 3.65 (s, 2H, H-C₂'), 5.93 (s, 1H), 7.26 (s, 5H, Aromatics); ¹³C NMR (CDCl₃) δ 207.5, 199.9, 169.7, 165.6, 136.5, 131.6, 129.3, 128.6, 127.2, 121.0, 88.2, 49.6, 45.5, 42.0, 39.9, 37.2, 37.1, 36.1, 33.9, 33.5, 31.8, 25.8, 20.1, 20.2, 19.9, 19.2, 13.6, 14.0.

EM-1146 (64, R'=H, R=CH₂PhOCO-t-Bu(p)): Yield, 14%; IR (KBr, cm⁻¹) 2955, 2871, 1736, 1660, 1624, 1582, 1508, 1458, 1393, 1270, 1202, 1166, 1117; ¹H NMR (CDCl₃) δ 0.69 (s, 3H, H-C₁₈), 1.05 (s, 3H, H-C₁₈), 1.31 (s, 3H, 17a-CH₃), 1.34 (s, 9H, t-Butyl), 1.88 (s, 3H, 6-CH₃), 3.64 (s, 2H, H-C₂'), 5.90 (s, 1H), 6.10 (s, 1H), 6.97 (d, 2H, J=8.3 Hz), 7.28 (d, 2H, J=8.3 Hz); ¹³C NMR (CDCl₃) δ 207.7, 199.9, 177.0, 169.7, 163.6, 150.5, 136.7, 131.7, 130.5, 130.4, 121.9, 121.2, 88.4, 49.8, 45.6, 41.4, 40.2, 39.1, 37.4, 37.2, 36.2, 33.8, 33.6, 31.8, 27.1, 25.9, 20.2, 19.9, 16.3, 13.7, 13.1.

CS-259 (64, R'=H, R=(CH₂)₂Br): Yield, 32%; IR (KBr, cm⁻¹) 2943, 2869, 1734, 1660, 1624, 1579, 1458, 1375, 1270, 1194, 1099; ¹H NMR (CDCl₃) δ 0.81 (s, 3H, H-C₁₈), 1.06 (s, 3H, H-C₁₈), 1.34 (s, 3H, 17a-CH₃), 1.87 (s, 3H, 6-CH₃), 1.89-2.07 (m, 1H), 2.38 (t, 2H, H-C₂', J=7 Hz), 3.40 (t, 2H, H-C₆', J=7 Hz), 5.89 (s, 1H), 6.13 (s, 1H); ¹³C NMR (CDCl₃) δ 207.8, 199.8, 172.2, 163.5, 136.4, 131.8, 121.0, 87.8, 49.5, 45.3, 40.4, 37.3, 36.1, 34.1, 33.7, 33.4, 32.1, 31.8, 27.5, 26.0, 23.8, 20.2, 19.8, 16.1, 13.6, 13.0.

CS-260 (64, R'=H, R=(CH₂)₄CH₃): Yield, 41%; IR (KBr, cm⁻¹) 2953, 2870, 1735, 1660, 1624, 1579, 1458, 1375, 1317, 1270, 1098; ¹H NMR (CDCl₃) δ 0.79 (s, 3H, H-C₁₈), 0.87 (s, 3H, H-C₆'), 1.32 (s, 3H, 17a-CH₃), 1.86 (s, 3H, 6-CH₃), 2.00-2.06 (m, 2H), 2.33 (t, 2H, H-C₂', J=7 Hz), 5.88 (s, 1H), 6.12 (s, 1H); ¹³C NMR (CDCl₃) δ 208.0, 199.9, 172.7, 163.6, 136.5, 131.9, 121.2, 87.7, 49.7, 45.5, 40.5, 37.4, 36.2, 34.4, 33.8, 33.5, 31.9, 31.3, 26.1, 24.5, 22.3, 20.2, 19.9, 16.2, 13.9, 13.7, 13.1.
CS-237 (64, R'=H, R=CH₃): Yield 67%; IR (CDCl₃) 1735, 1652, 1609, 1582 cm⁻¹; ¹H NMR (CDCl₃) δ 0.79 (s, 3 H), 1.1 (s, 3 H), 1.29 (s, 3 H), 1.89 (s, 3 H), 2.02 (s, 3 H), 2.41-2.50 (m, 1 H), 5.96 (s, 1 H) 6.17 (s, 1 H), 6.24 (d, 1 H, J=10 Hz), 7.06 (d, 1 H, J=10 Hz); ¹³C NMR (CDCl₃) δ 207.6, 186.3, 169.9, 162.7, 153.1, 132.7, 132.3, 127.8, 121.5, 87.7, 77.4, 77, 76.6, 47.2, 45, 41.1, 40.7, 38, 37.2, 31.7, 26.1, 21.3, 21.1, 20.2, 19.5, 13.6, 13.

CS-240 (64, R=CH₃): Yield 70%; IR (CDCl₃) 1737, 1651, 1622, 1578 cm⁻¹; ¹H NMR (CDCl₃) δ 0.78 (s, 3 H), 0.96 (d, 3 H, J=7 Hz), 1.12 (s, 3 H), 1.31 (s, 3 H), 1.84 (s, 3 H), 2.01 (s, 3 H), 2.80-2.90 (dd, 1 H, J=5.1, 17.5 Hz), 5.85 (s, 1 H) 6.1 (s, 1 H); ¹³C NMR (CDCl₃) δ 207.8, 199.4, 169.4, 159.9, 136.5, 132.5, 120.6, 87.9, 45.5, 44, 42, 40.6, 39.3, 37.4, 37.3, 35.5, 31.8, 26, 21.2, 20.2, 19.3, 18.8, 14.8, 13.7, 13.

EM-1117 (64, R=Cyhex): Yield 38%; IR (CDCl₃) 1731, 1703, 1658, 1619, 1578 cm⁻¹; ¹H NMR (CDCl₃) δ 0.80 (s, 3 H), 0.99 (d, 3 H, J=7 Hz), 1.14 (s, 3 H), 1.31 (s, 3 H), 1.87 (s, 3 H), 5.89 (s, 1 H) 6.1 (s, 1 H); ¹³C NMR (CDCl₃) δ 208.1, 199.7, 174.7, 160.2, 136.7, 132.5, 120.6, 87.4, 45.7, 44.2, 43, 42.8, 42.1, 40.9, 39.4, 37.4, 35.6, 32, 29.7, 28.9, 28.8, 28.7, 26.2, 25.6, 25.3, 25.2, 20.3, 19.4, 18.9, 14.9, 13.8, 13.2.

EM-1121 (64, R=(CH₂)₃CH₃): Yield 70%; IR (CDCl₃) 1735, 1657, 1622, 1578 cm⁻¹; ¹H NMR (CDCl₃) δ 0.77 (s, 3 H), 0.85 (t, 3 H, J=7 Hz), 0.94 (d, 3 H, J=7 Hz), 0.87 (s, 3 H), 1.11 (s, 3 H), 1.83 (s, 3 H), 2.36 (t, 2 H, J=4 Hz), 2.47 (m, 1 H), 2.80-2.88 (dd, 1 H, J=5.3, 17.8 Hz), 5.84 (s, 1 H) 6.09 (s, 1 H); ¹³C NMR (CDCl₃) δ 207.9, 199.4, 172.7, 160, 136.5, 132.4, 120.5, 87.6, 45.5, 44, 42, 40.7, 39.2, 37.4, 37.3, 35.5, 34, 31.9, 26.7, 26, 22.1, 20.2, 19.3, 18.8, 14.8, 13.7, 13.6, 13.

EM-1142 (64, R=(CH₂)₄CH₃): Yield 39%; IR (CDCl₃) 1735, 1657, 1621, 1578 cm⁻¹; ¹H NMR (CDCl₃) δ 0.80 (s, 3 H), 0.87 (t, 3 H, J=7 Hz), 0.98 (d, 3 H, J=7 Hz), 1.15 (s, 3 H), 1.33 (s, 3 H), 1.87 (s, 3 H), 2.84-2.91 (dd, 1 H, J=5.1, 17.6 Hz), 5.89 (s, 1 H) 6.1 (s, 1 H); ¹³C NMR (CDCl₃) δ 208.10, 199.6, 172.8, 160.1, 136.6, 132.5, 120.6, 87.7, 45.6, 44.2, 42.1, 40.8, 39.4, 37.5, 37.6, 34.4, 33.8, 32, 31.2, 26.1, 24.4, 22.3, 20.3, 19.4, 18.9, 14.9, 13.9, 13.2.

EM-1143 (64, R=CH(CH₃)₂): Yield 34%; IR (CDCl₃) 1733, 1657, 1622, 1578 cm⁻¹; ¹H NMR (CDCl₃) δ 0.80 (s, 3 H), 0.96 (d, 3 H, J=7 Hz), 1.14 (s, 3 H), 1.17 (d, 3 H, J=7 Hz), 1.18 (d, 3 H, J=7 Hz), 1.31 (s, 3 H), 1.86 (s, 3 H), 2.84-2.92 (dd, 1 H, J=5.1, 17.6 Hz), 5.87 (s, 1 H) 6.1 (s, 1 H); ¹³C NMR (CDCl₃) δ 207.8, 199.5, 175.8, 160, 136.5, 132.6, 120.7, 87.5, 61.9, 45.8, 44.2, 42.1, 40.9, 39.4, 37.4, 35.6, 34.1, 32, 26.2, 20.3, 19.4, 18.9, 18.7, 14.9, 14.1, 13.8, 13.1.
EM-1144 (64, R=CH₂Ph); Yield 34%; IR (CDCl₃) 1734, 1654, 1623, 1578 cm⁻¹; ¹H NMR (CDCl₃) δ 0.72 (s, 3 H), 0.99 (d, 3 H, J=7 Hz), 1.06 (s, 3 H), 1.29 (s, 3 H), 1.89 (s, 3 H), 2.83-2.90 (dd, 1 H, J=5, 17.4 Hz), 3.66 (s, 2 H), 5.91 (s, 1 H), 5.96 (s, 1 H); ¹³C NMR (CDCl₃) δ 207.6, 199.5, 169.7, 160, 136.6, 133.6, 132.2, 129.4, 128.7, 127.2, 120.5, 88.1, 45.7, 44, 42, 40.1, 39.2, 37.2, 37.1, 35.6, 31.8, 25.8, 20.2, 19.3, 18.8, 14.9, 13.7, 13.

EM - 1154 (64, R=Cypent); Yield 30%; IR (CDCl₃) 1731, 1657, 1622, 1578 cm⁻¹; ¹H NMR (CDCl₃) δ 0.79 (s, 3 H), 0.97 (d, 3 H, J=7 Hz), 1.14 (s, 3 H), 1.31 (s, 3 H), 1.86 (s, 3 H), 2.79-2.84 (m, 2 H), 5.87 (s, 1 H), 6.1 (s, 1 H); ¹³C NMR (CDCl₃) δ 208.1, 199.5, 175.5, 160, 136.6, 132.6, 120.7, 87.5, 45.7, 44.2, 43.8, 42.1, 40.9, 39.4, 37.4, 37.3, 35.6, 32, 29.9, 29.6, 26.2, 25.8, 25.7, 20.3, 19.4, 18.9, 14.9, 13.8, 13.1.
Example 17

Synthesis of 4-aza-androstan-3-one-spiro γ/δ-lactone derivatives

These syntheses are described in Scheme 14.

Scheme 14

[Diagram of chemical reactions and structures]
Example 17A

General Procedure for 66a and 66c:
A flask was charged with HC=C(CH_2)_nOTHP (n=2 or 3) (41.5 mmol) and dry THF (300 mL) under argon, and cooled to -50 °C in a cold bath. To this solution, was added n-BuLi (1.6 M, 41.4 mmol) via a syringe slowly, and the solution was allowed to warm up to 0 °C over a course of 2 h. Then, the solution was cooled to -78 °C, and the ketone 65a (10.4 mmol) was added as a solid. The reaction mixture was stirred and allowed to warm up to room temperature over a course of 3 h, and stirred for additional 2 h at room temperature. Saturated aqueous NH_4Cl solution (50 mL) was added. The resulting two phase was separated, and water phase was extracted with CH_2Cl_2 (2 x 100 mL). The combined solvents were removed and the residue was extracted with CH_2Cl_2 (2 x 150 mL). Solution was washed with brine and dried over MgSO_4. Solvent was removed on a rotary evaporator. The purification of the residue by flash column gave the product as a white solid.

17β-Hydroxy-17α-[4'-{2''-tetrahydro-2''H-pyranlyloxy]butyn-1'-yl]-4-aza-5α-androstan-3-one (66a): Yield, 73%; IR (KBr, cm⁻¹) 3520-3210 (br), 2906, 2838, 1642; ¹H NMR (CDCl₃) δ 0.81 (s, 3H, 18-CH₃), 0.88 (s, 3H, 19-CH₃), 2.52 (dd, 2H, J=4.8, 11.2 Hz), 2.61 (t, 2H, J=7.7 Hz), 3.01 (dd, 1H, J=3.2,12.2 Hz), 3.48-3.56 (m, 2H), 3.78-3.86 (m, 2H), 4.64 (t, 1H, J=3.1 Hz), 6.14 (br, s, 1H); ¹³C NMR (CDCl₃) 172.4, 98.6, 84.6, 83.1, 79.6, 65.8, 62.0, 60.7, 50.9, 49.9, 35.7, 35.6, 33.3, 32.6, 30.5, 29.6, 29.2, 29.0, 28.5, 27.2, 25.4, 22.9, 20.8, 20.3, 19.3, 12.8, 11.3.

17β-Hydroxy-17α-[5'-{2''-tetrahydro-2''H-pyranlyloxy}pentyn-1'-yl]-4-aza-5α-androstan-3-one (66c): Yield, 70%; IR (KBr, cm⁻¹) 3242, 3136, 2906, 2842, 1642; ¹H NMR (CDCl₃) δ 0.78 (s, 3H, 18-CH₃), 0.86 (s, 3H, 19-CH₃), 2.28-2.58 (m, 3H), 3.0 (dd, 1H, J=3.4, 12.3 Hz), 3.39-3.47 (m, 2H), 3.74-3.84 (m, 2H), 4.55 (br s, 1H), 6.44 (s, 1H); ¹³C NMR (CDCl₃, ppm) δ 172.3, 98.7, 85.3, 84.0, 79.5, 65.8, 62.1, 60.7, 50.9, 49.8, 46.9, 38.9, 35.6, 33.3, 32.5, 30.5, 29.3, 29.0, 28.9, 28.5, 27.1, 25.3, 22.9, 20.8, 19.4, 15.6, 12.8, 11.3.

Example 17B

17β-Hydroxy-17α-[4'-{2''-tetrahydro-2''H-pyranlyloxy]butyn-1'-yl]-4-methyl-4-aza-5α-androstan-3-one (66b): A flask was charged with HC=C(CH_2)_nOTHP (3.05g, 19.8 mmol) and anhydrous THF (200 mL) under argon, and cooled to -50 °C in a cold bath. To this solution was added n-BuLi (1.6 M, 19.8 mmol) and the solution was allowed to warm up to 0 °C over a course of 2 h. Then, the solution was cooled to -78 °C, and ketone 65b in dry THF (100 mL) was added via a cannula. The...
reaction mixture was stirred under argon and allowed to warm up to room temperature over a course of 3 h, and stirred for additional 2 h at room temperature. Saturated aqueous NH₄Cl solution (50 mL) was added. The resulting two phase was separated, and water phase was extracted with CH₂Cl₂ (2 x 100 mL). The combined solvents were removed and the residue was extracted with CH₂Cl₂ (2 x 150 mL). Solution was washed with brine and dried over MgSO₄. Solvent was removed on a rotary evaporator. The purification of the residue by a flash column gave the product as a white solid (3.18 g, 7.12 mmol, 72%); IR (KBr, cm⁻¹) 3530-3130 (br), 2920, 2850, 1620; ¹H NMR (CDCl₃ δ 0.84 (s, 3H, 18-CH₃), 0.90 (s, 19-CH₃), 2.54 (t, 2H, J=7.1 Hz), 2.93 (s, 3H), 3.54 (dd, 1H, J=3.3, 12.9 Hz), 3.51-3.59 (m, 2H), 3.78-3.89 (m, 2H), 4.11-4.64 (m, 1H); ¹³C NMR (CDCl₃, ppm) δ 172.1, 98.2, 84.1, 83.1, 79.4, 65.9, 65.2, 62.1, 60.9, 60.7, 53.8, 50.7, 49.8, 46.5, 38.3, 35.6, 35.4, 33.1, 31.3, 29.4, 28.3, 27.5, 26.8, 25.7, 21.4, 20.9, 20.6, 12.7, 11.4.

Example 17C
General procedure for 67a - 67c: Compound 66 (5 mmol) was dissolved in methanol (100 mL) and amberlyst-15 (0.4 g) was added. The reaction mixture was stirred for 2 to 3 h (monitored by TLC) at room temperature and filtered. Solvent was removed on a rotary evaporator; and purification of the residue by flash column gave product as a white solid.

17β-Hydroxy-17α-[4'-hydroxybutyn-1'-yl]-4-aza-5α-androstan-3-one (67a): Yield, 87%; IR (KBr, cm⁻¹) 3490-3108 (br), 2910, 2832, 1636; ¹H NMR (CDCl₃ δ 0.80 (s, 3H, 18-CH₃), 0.86 (s, 3H, 19-CH₃), 2.45 (t, 2H, J=6.9 Hz), 3.02 (dd, 1H, J=3.4, 12.1 Hz), 3.67 (t, 2H, J=6.8 Hz), 6.23 (br, s, 1H); ¹³C NMR (CDCl₃, ppm) δ 172.6, 85.6, 82.7, 79.3, 60.8, 60.6, 50.8, 49.7, 49.2, 46.8, 38.8, 35.6, 33.1, 32.6, 29.0, 28.4, 27.0, 23.1, 22.9, 20.7, 12.8, 11.2.

17β-Hydroxy-17α-[4'-hydroxybutyn-1'-yl]-4-methyl-4-aza-5α-androstan-3-one (67b): Yield, 91%; IR (KBr, cm⁻¹) 3540-3120, 2904, 2836, 1624; ¹H NMR (CDCl₃ δ 0.83 (s, 3H, 18-CH₃), 0.89 (s, 3H, 19-CH₃), 2.43-2.51 (m, 4H), 2.92 (s, 3H), 3.35 (dd, 1H, J=3.4, 12.5 Hz), 3.69 (t, 2H, J=6.2 Hz); ¹³C NMR (CDCl₃, ppm) δ 171.1, 85.6, 82.8, 79.4, 65.7, 60.9, 60.7, 51.6, 49.9, 46.9, 38.9, 36.4, 34.9, 32.8, 29.7, 29.6, 29.2, 29.0, 25.2, 23.2, 22.9, 20.8, 12.9, 12.4.
17β-Hydroxy-17α-[5'-hydroxypentyn-1'-yl]-4-aza-5α-androstan-3-one (67a): Yield, 84%; IR (KBr, cm⁻¹) 3530-3082, 2902, 2842, 1618; ¹H NMR (CDCl₃) δ 0.83 (s, 3H, 18-CH₃), 0.89 (s, 3H, 19-CH₃), 2.24 (t, 2H, J=6.9 Hz), 2.29 (dd, 2H, J= 4.7, 5.5 Hz), 2.95 (dd, 1H, J=3.8, 12.6 Hz), 3.59 (t, 2H, J=6.3 Hz), 6.26 (br, s 1H); ¹³C NMR (CDCl₃, ppm) δ 173.0, 84.9, 83.9, 79.2, 60.7, 60.4, 50.7, 49.7, 46.7, 38.7, 35.4, 32.9, 31.1, 28.9, 28.8, 28.1, 26.7, 22.8, 20.6, 15.1, 12.7, 11.1.

Example 17D

General procedure for 68a - 68c: A flask was charged with compound 67 (5 mmol), 10 mmol % of Pd/C, CH₃OH (30 mL), ethyl acetate (120 mL) and stir bar, and then the compound was hydrogenated using a balloon. The reaction mixture was stirred at room temperature for 3 h and filtered. Solvent was removed on a rotary evaporator, and purification of the residue by flash column gave product as a white solid.

17β-Hydroxy-17α-[4'-hydroxybutan-1'-yl]-4-aza-5α-androstan-3-one (68a): Yield, 65%; ¹H NMR (CDCl₃) δ 0.87 (s, 3H, 18-CH₃), 0.91 (s, 3H, 19-CH₃), 2.39-2.44 (m, 2H), 3.04 (dd, 1H, J=4.2, 11.9 Hz), 3.67 (t, 2H, J=5.8 Hz), 5.93 (br, s 1H).

17β-Hydroxy-17α-[4'-hydroxybutan-1'-yl]-4-methyl-4-aza-5α-androstan-3-one (68b): Yield, 81%; ¹H NMR (CDCl₃) δ 0.87 (s, 3H, 18-CH₃), 0.89 (s, 3H, 19-CH₃), 2.41-2.46 (m, 2H), 2.92 (s, 3H), 3.02 (dd, 1H, J=3.5, 12.5 Hz), 3.68-7.26 (br, s, 2H).

17β-Hydroxy-17α-[5'-hydroxypentan-1'-yl]-4-aza-5α-androstan-3-one (68c): Yield, 76%; IR (thin film, cm⁻¹) 1663 (s); ¹H NMR (CDCl₃) δ 0.87 (s, 3H, 18-CH₃), 0.92 (s, 3H, 19-CH₃), 2.41-2.46 (m, 2H), 3.08 (dd, 1H, J=4.8, 11.5 Hz), 3.62 (br, s, 2H), 5.48 (br, s, 1H); ¹³C NMR (CDCl₃) δ 172.3, 83.2, 62.8, 60.8, 51.2, 50.1, 46.6, 36.6, 35.9, 35.8, 34.3, 33.4, 32.7, 31.4, 29.4, 28.6, 27.3, 26.6, 23.6, 23.3, 20.9, 14.5, 11.4.

Example 17E

General procedure for 69a and 69b: Alcohol 68 (5 mmol) was dissolved in 150 mL of acetone and the solution was cooled to 0 °C in an ice bath. Jones' reagent (0.5 M, 12.5 mmol) was added dropwise. After addition, the mixture was stirred for 30 min at 0 °C. Then 100 mL of water was added and product was extracted with CH₂Cl₂ (3 x 150 mL). The combined organic layer was washed with saturated aqueous NaHCO₃ solution, water and brine, and then dried over MgSO₄. Solvents were
removed on a rotary evaporator and the purification of the residue by flash column gave product as a white solid.

4-aza-5α-androstan-3-one-17(R)-spiro-2’-(6’-oxo)tetrahydropyran (69a):

Yield, 58%; IR (thin film, cm⁻¹) 1725 (s), 1663 (s). ¹H NMR (CDCl₃) δ 0.92 (s, 3H, 18-CH₃), 0.98 (s, 3H, 19-CH₃), 5.53 (s, 1H, br, NH); ¹³C NMR (CDCl₃) δ 172.2, 171.9, 93.0, 59.5, 51.0, 49.3, 47.1, 35.7, 35.4, 33.8, 33.4, 31.7, 29.3, 29.2, 28.5, 27.8, 27.1, 23.6, 20.6, 15.8, 14.4, 11.3. Anal. Calcd for C₂₂H₃₅NO₃: C 73.50; H 9.25, N 3.90. Found: C 73.23; H 9.29, N 3.78.

Example 17F

General procedure for 71a and 71b: A flask was charged with 69 (1.11 mmol), DDQ (1.11 mmol) and anhydrous dioxane (8 mL).

Bis(trimethylsilyl) trifluoroacetamide (BSTFA) (4.56 mmol) was added via a syringe. The reaction mixture was stirred at room temperature under argon for 18 h, and then refluxed for 8 h. The mixture was poured into CH₂Cl₂ (80 mL), and the solution was washed with saturated NaHCO₃ (2 x 50 mL) and Brine. The organic layer was dried over MgSO₄. Solvents were removed on a rotary evaporator and the residue purified by a flash column to give product as a white solid.

4-aza-5α-androst-1-en-3-one-17(R)-spiro-2’-(6’-oxo)tetrahydropyran (71a): Yield, 52%; IR (thin film, cm⁻¹) 1715 (s), 1674 (s), 1599 (m). ¹H NMR (CDCl₃) δ 0.95 (s, 3H, 18-CH₃), 0.96 (s, 3H, 19-CH₃), 5.76 (d, 1H, J=10.0 Hz, CH=CH), 6.67 (br, s, 1H, NH), 6.76 (d, 1H, J=10.0 Hz, CH=CH); ¹³C NMR (CDCl₃) δ 172.0, 166.9, 151.0, 123.0, 92.9, 59.6, 49.3, 47.4, 47.2, 39.3, 35.6, 33.8, 31.7, 29.4, 29.1, 27.8, 25.6, 23.7, 20.7, 15.7, 14.5, 11.9.

4-methyl-4-aza-5α-androst-1-en-3-one-17(R)-spiro-2’-(6’-oxo)tetrahydropyran (71b): Yield, 25%; IR (thin film, cm⁻¹) 1725 (s), 1661 (s), 1604 (m); ¹H NMR (CDCl₃) δ 0.92 (s, 3H, 18-CH₃), 0.98 (s, 3H, 19-CH₃), 2.93 (s, 3H, NMe), 5.83 (d, 1H, J=9.9 Hz, CH=CH), 6.67 (d, 1H, J=9.9 Hz, CH=CH); ¹³C NMR (CDCl₃) δ 171.9, 165.5, 148.5, 123.2, 92.9, 63.7, 49.2,
Example 17G

17β-Hydroxy-17α-[4′-carboxybutan-1′-yl]-4-methyl-4-aza-5α-androstan-3-one (70): Alcohol 68c (1.82 g, 5.00 mmol) was dissolved in 100 mL of acetone and the solution was cooled to 0 °C in an ice bath. Jones' reagent (0.5 M, 25.0 mL, 12.5 mmol) was added dropwise. Then saturated aqueous NaHCO₃ (150 mL) and ethyl acetate (100 mL) were added and the mixture was stirred vigorously for overnight. Two phases were separated and the water phase was acidified using 1 M HCl. The acidic solution was extracted with CH₂Cl₂ (3 x 120 mL). The combined organic layer were washed with water and brine, and then dried over MgSO₄. Removal of solvents gave the product as a white solid (1.25 g, 3.20 mmol, 64%); IR (thin film, cm⁻¹) 1704 (s), 1632 (s); ¹H NMR (pyridine-ds) δ 1.22 (s, 3H, 18-CH₃), 1.45 (s, 3H, 19-CH₃), 8.41 (s, 1H, NH); ¹³C NMR (pyridine-ds) δ 176.1, 171.4, 82.6, 60.9, 51.5, 50.5, 47.2, 37.5, 36.2, 35.9, 35.2, 34.5, 34.0, 32.1, 29.9, 29.4, 27.5, 26.8, 24.2, 24.1, 21.3, 15.4, 11.4.

Example 17H

Preparation of the lactone 72: To acid 70 (100 mg, 0.258 mol) in 20 mL of CH₂Cl₂ was added oxalyl chloride (1.2 eq, 42 mL) at 0 °C under argon, and the reaction mixture was stirred for 1 h. After 1h, no more acid was detected on the TLC. Then, pyridine (4 eq, 50 mL) was added and the mixture was stirred for 48 h at room temperature. The product was extracted with ethyl acetate, dried over MgSO₄ and purified by a flash column using CH₂Cl₂/MeOH (gradient, 2 to 8 %) as an eluent to give the lactone 72 as a white solid (43 mg, 45%); IR (thin film, cm⁻¹) 1714 (s), 1660 (s); ¹H NMR (CDCl₃, ppm) δ 0.88 (s, 3H, 18-CH₃), 0.96 (s, 3H, 19-CH₃), 6.57 (s, 1H, NH); ¹³C NMR (CDCl₃, ppm) δ 175.6, 172.8, 60.7, 51.2, 48.8, 36.1, 35.6, 33.6, 33.3, 30.9, 30.6, 29.7, 28.5, 27.1, 24.2, 24.1, 23.1, 22.6, 20.6, 15.3, 14.1, 11.4.
Example 18

Syntheses of unsubstituted-A-ring-1,3,5(10)-estratrien-17-spiro-5-lactone derivatives

These syntheses are described in Scheme 15.

Scheme 15

a: Tf2O pyridine  
b: HCOOH, PPh3, Pd(OAc)2, Et3N  
c: tetrahydro-2-(butynyloxy)-2-H-pyran, THF, n-BuLi, 0 °C  
d: H2, 10% Pd on charcoal  
e: p-TSA, methanol, room temperature  
f: 1) Jones' reagent, acetone; 2) isopropyl alcohol  
g: n-BuLi, diisopropylamine, THF, CH3I, room temperature
Example 18A
Synthesis of 3-deoxyoxygenated-estrone (74). At 0 °C, 1.0 g (3.7 mmol) of estrone (73) was dissolved in 50 mL of dry pyridine and 1.24 mL (7.4 mmol) of trifluoroacetic anhydride (TFA) was added slowly to the solution. After 1 h, the crude solution was poured into a cold aqueous solution of CuSO₄ (1M) and the organic phase washed with the same solution until the blue color disappear. Next, the solution was extracted with EtOAc and the organic phase washed with water, and dried over MgSO₄. After evaporation of the solvent, the crude estrone-triflate was dissolved in 50 mL of dry DMF. To the resulting mixture, we added 1.54 mL (11 mmol) of Et₃N, 0.41 mL (11 mmol) of HCOOH, 0.143 mg (0.55 mmol) of PPh₃, and 30.8 mg (0.14 mmol) of Pd(OAc)₂. After 3 h at room temperature, the solution was quenched with aqueous HCl (5%) and extracted with CH₂Cl₂. The organic phase was washed with water, dried over MgSO₄, and solvents evaporated to dryness. The crude compound was purified by flash chromatography with hexanes/EtOAc (9:1) as eluent to give 615 mg (65% two steps) of 13,5(10)-estratrien-17-one (74). White solid; IR ν (film): 1738 (C=O); ¹H NMR (CDCl₃) δ 0.94 (s, 3H, 18-CH₃), 2.94 (m, 2H, 6-CH₂), 7.16 (m, 3H, 1-CH, 2-CH, 3-CH), 7.32 (d, J=5.9 Hz, 1H, 4-CH); ¹³C NMR (CDCl₃) δ 13.76 (C-18), 21.51 (C-15), 25.61 (C-11), 26.42 (C-7), 29.29 (C-6), 31.56 (C-12), 35.77 (C-16), 38.04 (C-8), 44.40 (C-9), 47.88 (C-13), 50.49 (C-14), 125.22 (C-3), 125.73 (C-2 and C-1), 128.98 (C-4), 136.37 (C-5), 139.62 (C-10), 220.63 (C-17).

Example 18B
Synthesis of alcohol 75. To a solution of tetrahydro-2-(butyloxy)-2-H-pyran (0.94 mL, 5.9 mmol) in 30 mL of dry THF was added at 0 °C, 3.53 mL of n-BuLi 1.6 M (5.7 mmol), and the mixture was stirred for 40 min. A solution of 3-deoxyoxygenated-estrone (74) (500 mg, 1.96 mmol) in 10 mL of THF was then added dropwise at -78 °C and the mixture stirred for 11 h. After this time, a solution of aqueous NaHCO₃ (5%) was added and aqueous phase was extracted with EtOAc. Organic layer was washed with brine, and dried over MgSO₄. After evaporation of solvent, the crude compound was purified by flash chromatography with hexane/EtOAc (9:1) as eluent to give 566 mg (68%) of 17β-hydroxy-17α-[4'-[(tetrahydro-2'H-pyran-3-yl)oxy] butenyl]-13,5(10)-estratrien (75). Colorless oil; IR ν (film): 3438 (OH, alcohol), 2233 very weak (C=O); ¹H NMR (CDCl₃) δ 0.88 (s, 3H, 18-CH₃), 2.57 (t, J=7.0 Hz, 2H, C=CCH₂), 2.87 (m, 2H, 6-CH₂), 3.55 and 3.86 (2m, 4H, CH₂ of side chain and CH₂O of THP), 4.68 (sep, 1H, CH of THP), 7.13 (m, 3H, 1-CH, 2-CH, 3-CH), 7.31 (d, J=6.7 Hz, 1H, 4-CH); ¹³C NMR (CDCl₃) δ 12.72 (C-18), 19.21 (C-4" of THP), 20.30 (C-3'), 22.76 (C-15), 25.37 (C-5" of THP), 26.18 (C-11), 27.13 (C-7), 29.49 (C-6), 30.48 (C-3" of THP), 32.87 (C-12), 38.98 (C-16), 39.14 (C-8), 43.07 (C-9), 47.07 (C-13), 49.53 (C-14),...
61.95 (C-2'' of THP), 65.78 (C-4'), 79.81 (C-17), 83.07 (C-2), 84.68 (C-1'), 98.58 (C-1'' of THP), 125.25 (C-3), 125.47 (C-2), 125.51 (C-1), 128.91 (C-4), 136.62 (C-5), 140.22 (C-10).

Example 18C

Synthesis of lactone 77

a) Reduction of triple bond (75 -> 78a). To a solution of compound 75 (650 mg, 1.6 mmol) in乙酸乙酯 was added 40 mg of palladium on activated charcoal (10%), and the mixture was stirred at room temperature overnight under an hydrogen atmosphere. After this time, it was filtered through celite, washed with乙酸乙酯, and evaporated to dryness to give the compound 78a as a white foam.

b) Hydrolysis of THP group (78a -> 78b). The crude alcohol 78a was dissolved in 60 mL of methanol and 20 mg of p-TSA was added. After 2 h at room temperature, water was added to the mixture, the methanol was evaporated, and the resulting mixture extracted with乙酸乙酯. The organic phase was washed with water and dried over MgSO₄. Then the solvent was evaporated to dryness to give 455 mg of crude diol 78b.

c) Jones' oxidation with lactonization (78b -> 77) The crude diol 78b (450 mg) was dissolved in 30 mL of acetone and 0.9 mL of Jones' reagent (2.7M) was added dropwise at 0 °C. After the addition was completed, the mixture was stirred at room temperature for 2 h. Then 2 mL of isopropyl alcohol was added and the resulting green solution was evaporated to dryness. The solid was dissolved in water and乙酸乙酯, and the mixture extracted with乙酸乙酯. Organic layer was washed with brine and dried over MgSO₄. After evaporation of solvent, the crude compound was purified by flash chromatography with hexanes/乙酸乙酯 (8:2) as eluent to give 392 mg (65%, three steps) of 1,3,5(10)-estratrien-17(R)-spiro-2''-(6''-oxo)tetrahydropyran (77). White solid; IR ν (KBr): 1732 (C=O, lactone); 1H NMR (CDCl₃) δ 1.03 (s, 3H, 18-CH₃), 2.88 (m, 2H, 6-CH₂), 7.12 (m, 3H, 1-CH, 2-CH, 3-CH), 7.29 (d, J=5.8 Hz, 1H, 4-CH); 13C NMR (CDCl₃) δ 14.03 (C-18), 15.59 (C-2'), 23.22 (C-15), 25.54 (C-11), 27.13 (C-1), 27.63 (C-7), 29.17 (C-6 and C-3'), 31.69 (C-12), 33.67 (C-16), 38.56 (C-8), 43.83 (C-9), 46.92 (C-13), 48.64 (C-14), 92.93 (C-17), 124.92 (C-3), 125.39 (C-1 and C-2), 128.70 (C-1), 136.18 (C-10), 139.56 (C-5), 171.70 (C-4'); El-HRMS: calcd for C₂₂H₃₇O₅ 324.20892, found 324.20702.
Example 18D
Synthesis of lactones 76a and 76b. A mixture of 127 μL (1.0 mmol) of disopropylamine, 0.5 mL (0.80 mmol) of n-BuLi (1.6M) and 5 mL of dry THF was stirred at 0 °C for 30 min. The solution was cooled at -78 °C, and 75 mg (0.233 mmol) of lactone 77 in 10 mL of dry THF was added dropwise. After 1 h, 90 μL of CH3I was added and the mixture stirred overnight and let warm to room temperature. Then, the solution was quenched with water and extracted with EtOAc. The organic phase was washed with water and dried over MgSO4. After evaporation of the solvent, the crude compound was purified by flash chromatography with hexanes/EtOAc (95:5) as eluent to give 28 mg (36%, two steps) of monomethylated lactone 76a and 33 mg (40%, two steps) of dimethylated lactone 76b.

1,3,5(10)-estratrien-17(R)-spiro-2'(5'-methyl-6'-oxo)tetrahydropyran (76a). [major isomer only]. White solid; IR v (film): 1734 (C=O, lactone); 1H NMR (CDCl3) δ 1.02 (s, 3H, 18-CH3), 1.25 (d, J=6.8 Hz, 3H, CHCH3), 2.56 (m, 1H, CHCH3), 2.87 (m, 2H, 6-CH2), 7.12 (m, 3H, 1-CH, 2-CH, 3-CH), 7.29 (d, J=6.1 Hz, 1H, 4-CH); 13C NMR (CDCl3) δ 14.34 (C-18), 17.26 (CHCH3), 23.52 (C-15), 24.44 (C-2'), 26.78 (C-11), 27.28 (C-1'), 27.40 (C-7), 29.41 (C-6), 32.01 (C-12), 33.51 (C-3'), 34.00 (C-16), 38.84 (C-8), 44.14 (C-9), 47.21 (C-13), 48.76 (C-14), 92.75 (C-17), 125.22 (C-3), 125.56 (C-1 and C-2), 128.97 (C-4), 135.51 (C-10), 139.87 (C-5), 175.84 (C-4').

1,3,5(10)-estratrien-17(R)-spiro-2'(5',5'-dimethyl-6'-oxo)tetrahydropyran (76b). White solid; IR v (film): 1724 (C=O, lactone); 1H NMR (CDCl3) δ 1.02 (s, 3H, 18-CH3), 1.28 (s, 6H, 2x CH3), 2.88 (m, 2H, 6-CH2), 7.13 (m, 3H, 1-CH, 2-CH, 3-CH), 7.30 (d, J=6.2 Hz, 1H, 4-CH); 13C NMR (CDCl3) δ 14.41 (C-18), 23.29 (C-15), 25.59 (C-1'), 25.84 (C-11), 27.39 (C-7), 27.54 and 27.76 (2x CH3), 29.42 (C-6), 31.51 (C-12), 32.02 (C-16), 34.82 (C-2'), 37.79 (C-3'), 38.87 (C-8), 44.15 (C-9), 47.25 (C-13), 48.84 (C-14), 93.55 (C-17), 125.21 (C-3), 125.52 (C-2), 125.56 (C-1), 128.99 (C-4), 135.52 (C-10), 139.87 (C-5), 177.82 (C-4').
Example 19

Synthesis of 2-nitro-1,3,5(10)-estratrien-17-spiro-5-lactone derivatives

These syntheses are described in Scheme 16.

Scheme 16

- Reaction conditions:
  - a. NaN₃, HNO₃, AcOH
  - b. TBDMSCl, imidazole
  - c. HCl/CH₂Cl₂/THF, Me₂I
  - d. H₂, Pd/C, CO₂, MeOH
  - e. AcOH, CO₂, CH₂Cl₂/CH₂Cl₂, CH₂OH, H₂O
  - f. Jones reagent
  - g. LDA, DMF
  - h. R₂CO₃, CH₂Cl₂/CH₂Cl₂, CH₂OH, Δ

- Compounds and intermediates:
  - EM-1118
  - EM-1125
  - EM-1124
Example 19A
3-hydroxy-2-nitro-1,3,5(10)-estratrien-17-one (79a). The titled compound was prepared as described by Stabenracht and Knuppen[2]. The procedure is described below.

Estrone (37, 18.004 g, 66.6 mmol) was dissolved in boiling acetic acid (540 mL) and allowed to cool down to 50°C. The nitration mixture was prepared from 70% nitric acid (4.5 mL, 70 mmol), water (10 mL) and a few crystal of sodium nitrite, warmed up to 50 °C and added dropwise to the solution of estrone with stirring. After stirring overnight at room temperature, the yellow precipitate was filtered by suction and recrystallized from 92% aqueous acetic acid. 4-nitro derivative (6.800 g, 32%) was thus obtained as a pale yellow solid. IR (v) 3227 (OH), 2931, 2864, 1723 (C=O), 1626, 1584, 1523, 1458, 1404, 1374, 1295, 1264, 1245, 1211, 1169, 1085, 1062, 1027, 954, 930, 908, 881, 823, 796, 719, 654, 588, 556, 530, 494 cm⁻¹; ¹H NMR (Pyridine-ds) δ 0.85 (3H, s, 18'-CH₃), 2.85 (2H, d, 6'-CH₂), 5.00 (1H, s, OH), 7.11 (1H, d, J=8.7 Hz, 2'-CH), 7.26 (1H, d, J=8.7 Hz, 1'-CH); ¹³C NMR (Pyridine-ds) δ 13.8 (C-18), 21.6 (C-15), 24.4 (C-11), 25.7 (C-7), 26.2 (C-12), 32.0 (C-6), 35.9 (C-16), 37.7 (C-8), 44.0 (C-14), 47.9 (C-13), 50.1 (C-9), 115.4 (C-2), 128.4 (C-1), 129.0 (C-10), 131.8 (C-5), 148.4 (C-3), 219.2 (C-17).

The reaction filtrate from above was evaporated under reduced pressure and the residue was recrystallized from EtOH/H₂O 8.5:1.5. A brown solid (7.854 g) was obtained which was further purified by flash chromatography on SiO₂ column (EtOAc/hexanes, gradient 8-20%) to give pure compound 79a (6.284 g, 30%) as a yellow solid. IR (ν): 3300 (OH), 2933, 2864, 1737 (C=O), 1630, 1562, 1522, 1480, 1431, 1372, 1311, 1252, 1216, 1146, 1084, 1054, 1035, 1008, 905, 832, 762, 722, 662, 600, 520 cm⁻¹. ¹H NMR (Pyridine-ds) δ 0.85 (3H, s, 18'-CH₃), 2.76 (2H, d, 6'-CH₂), 4.99 (1H, s, OH), 6.98 (1H, s, 4'-CH), 7.96 (1H, s, 1'-CH); ¹³C NMR (Pyridine-ds) δ 13.8 (C-18), 21.7 (C-15), 25.8 (C-11), 26.1 (C-7), 29.6 (C-12), 31.9 (C-6), 35.9 (C-16), 37.8 (C-8), 43.5 (C-14), 47.9 (C-13), 50.3 (C-9), 119.8 (C-4), 122.2 (C-1), 132.8 (C-10), 147.8 (C-2), 152.6 (C-3), 219.1 (C-17).

Example 19B
3-(3-tert-butyldimethylsilyloxy)-2-nitro-1,3,5(10)-estratrien-17-one (79b). A solution of 2-nitro-estrone (79a, 1.118g, 3.55 mmole), imidazole (0.670g, 9.84 mmole) and TBDMSCl (0.781g, 5.18 mmole) in dry DMF (50 mL) was stirred under Ar(g) overnight. The mixture was then poured onto ice/water (80 mL). The white precipitate was filtered, washed with water and then dried in vacuo to give (79b) as a yellowish powder (1.447 g, 95%). [α]D +123.9° (c 1.03, CHCl₃); IR (NaCl) 2933, 2860, 1736 (s,C=O),
1617, 1561, 1518, 1492, 1408, 1351, 1291, 1256, 1054, 909, 832, 790, 697 cm⁻¹;

1H NMR δ 0.24 (6H, s, Si(CH₃)₂), 0.92 (3H, s, 18-CH₃), 1.01 (9H, s, SiC(CH₃)₃), 1.40-1.78 (6H, m), 1.90-2.35 (5H, m), 2.37-2.60 (2H, m), 2.90 (2H, m, 6-CH₂), 6.67 (1H, s, 4-CH), 7.76 (1H, s, 1-CH); ¹³C NMR δ 220.2, 147.2, 143.8, 139.6, 133.3, 122.6, 122.1, 50.3, 47.9, 43.6, 37.8, 35.8, 31.3, 29.4, 26.1, 25.7, 25.6, 21.5, 18.2, 13.8, -4.4.

**Example 19C**
3-(tert-butyl(dimethyl)silyloxy)-17β-hydroxy-2-nitro-17α-(4′-(2′-
tetrahydro-2′H-pyranloyloxy)-butynyl)-1,3,5(10)-estratriene (80). To a stirred solution of tetrahydro-2′-(butynloxy)-2′H-pyran (1.71 mL, 10.91 mmole) in dry THF (75 mL) under Ar (g) at -35 °C was dropwise added (MeLi 1.4M in ether 7.80 mL, 10.92 mmole). The solution was stirred for 45 min. after which was added at -35 °C a solution of ketone 79b (1.294 g, 3.01 mmole) in dry THF (20 mL). After 75 min. ice (20 g) and saturated aqueous NaHCO₃ (70 mL) were added to the reaction mixture and the aqueous phase was extracted with EtOAc. The combined organic layers were washed with brine, dried with magnesium sulfate, filtered and then concentrated in vacuo. The crude yellow oil was purified on SiO₂ (40 g, 2.8 EtOAc/hexanes) to give compound 80 as a yellow foam (1.617 g, 92%). [α]D²⁵ 57.5° (c 0.72, CHCl₃); IR (NaCl) 3423 (broad, OH), 2936, 2870, 2366, 1654, 1630, 1578, 1560, 1527, 1481, 1458, 1438, 1313, 1268, 1121, 1080, 1032, 899, 869, 761, 669 cm⁻¹; ¹H NMR δ 0.23 (6H, s, Si(CH₃)₂), 0.87 (3H, s, 18-CH₃), 1.00 (9H, s, SiC(CH₃)₃), 1.20-2.35 (20H, m), 2.55 (2H, t, J=6.9Hz, CCCH₂), 2.84 (2H, m, 6-CH₂), 3.55 (2H, m, CH₂O of chain), 3.85 (2H, m, CH₂O of THF), 4.65 (1H, m, CH of THF), 6.65 (1H, s, 4-CH), 7.76 (1H, s, 1-CH); ¹³C NMR δ 147.0, 144.2, 139.5, 133.9, 122.6, 122.0, 98.8, 84.5, 83.4, 79.8, 65.8, 62.2, 49.4, 47.1, 43.2, 38.9, 32.6, 30.6, 29.6, 26.7, 26.2, 25.6, 25.4, 22.8, 20.4, 19.4, 18.2, 12.7, -4.4.

**Example 19D**
3-(tert-butyl(dimethyl)silyloxy)-17β-hydroxy-2-nitro-17α-(4′-(2′-
tetrahydro-2′H-pyranloyloxy)-butyl)-1,3,5(10)-estratriene (81). A solution of compound 80 (2.00g, 3.42 mmol) and 5% Pd/CaCO₃ (400 mg) in dry MeOH (400 mL) was stirred under H₂(g) atmosphere (balloon) for 1 h. The mixture was then filtered through celite and the filtrate rotary evaporated. The residue was purified on silica gel (2.8 EtOAc/hexanes) to give compound 81 as a white foamy solid (1.483 g, 74%). [α]D²⁵ +31.3° (c 0.90, CHCl₃); IR (NaCl) 3458 (broad, OH), 2935, 2860, 1616, 1563, 1518 (NO₂), 1491, 1408, 1348 (NO₂), 1291, 1256, 1119, 1070, 1023, 925, 893, 832, 784, 672 cm⁻¹; ¹H NMR δ 0.23 (6H, s, Si(CH₃)₂), 0.91 (3H, s, 18-CH₃), 1.00 (9H, s, SiC(CH₃)₃), 1.20-2.38 (26H, m), 2.85 (2H, m, 6-CH₂), 3.48 (2H, m,
CH$_2$O of chain), 3.83 (2H, m, CH$_2$O of THP), 4.59 (1H, m, CH of THP), 6.65 (1H, s, 4-CH), 7.75 (1H, s, 1-CH): $^{13}$C NMR δ 146.9, 144.1, 139.4, 134.0, 122.4, 121.9, 98.9, 83.2, 67.6, 67.6, 62.4, 62.4, 49.3, 46.6, 36.3, 34.1, 31.3, 30.7, 30.3, 29.5, 26.9, 26.0, 25.5, 25.4, 23.3, 20.4, 19.6, 18.1, 14.3, -4.4.

**Example 19E**

17α-(4'-hydroxybutyl)-3,17β-dihydroxy-2-nitro-1,3,5(10)-estratriene (82). A solution of compound 81 (300 mg, 0.510 mmol) in 5% HCl in MeOH (10 mL) was stirred at room temperature and under argon atmosphere for 12 h. The reaction mixture was then poured into NaHCO$_3$/ice and MeOH was evaporated under reduced pressure. The aqueous phase was extracted with EtOAc and the combined organic layers were washed with brine, dried over MgSO$_4$ and then evaporated to dryness. This gave a crude yellow foam (198 mg, 100%). Purification by flash chromatography (column loaded with CH$_2$Cl$_2$ and then eluted with EtOAc/CH$_2$Cl$_2$ 2:8, 4:6, 1:1, 6:4, 9:1) gave compound 82 as a yellow solid (127.0 mg, 64%). Rf 0.21 (8:2 EtOAc/Hexanes); M.p. 184-6 °C; [α]$^2$ +58.8° (c. 1.00, CHCl$_3$); IR (v) 3335, 2934, 2865, 1735, 1719, 1654, 1630, 1576, 1522, 1479, 1434, 1373, 1305, 1266, 1169, 1112, 1067, 1033, 1000, 896, 874, 762, 659 cm$^{-1}$; $^{1}$H NMR δ 0.90 (3H, s), 3.69 (2H, d, $J$=5.7 Hz), 6.84 (1H, s), 7.98 (1H, s), 10.42 (1H, s); $^{13}$C NMR δ 14.3, 19.8, 23.3, 26.1, 26.8, 29.8, 31.2, 33.3, 34.3, 36.1, 39.0, 43.2, 46.5, 49.4, 61.7, 62.8, 83.4, 118.8, 121.4, 131.6, 133.7, 149.2, 152.8.
Example 19F
2-Nitro-1,3,5(10)-estraatriene-3-ol-17(R)-spiro-2'-6'-oxotetrahydropyran (EM-1124). To a stirred solution of compound 82 (128 mg, 0.33mmole) in dry acetone (25 mL) at 0 °C was slowly added a first 1.1 equivalent of Jones' reagent (1.25 M, 0.29 mL, 0.80 mmol). The orange solution was then stirred for 0.5 h then a second equivalent was added. The dark solution was stirred for a further 0.5 h then quenched with isopropanol (green precipitate formed). The mixture was stirred for 10 min. then filtered through celite and the filtrate rotary evaporated. The residue was taken in EtOAc then washed with aq. sat. NaHCO₃, H₂O, brine, dried (MgSO₄), filtered, rotovapd. The crude solid was purified by flash chromatography on SiO₂ (3:7 EtOAc/Hexanes) to give EM-1124 (108 mg, 85%) as a yellow solid. M.p. 213 °C; [α]D +90.0° (c 0.70, CHCl₃); IR (NaCl) 3198, 2934, 2876, 2245, 1720 (s, C=O, lactone), 1630, 1577, 1522, 1480, 1434, 1378, 1314, 1267, 1234, 1199, 1169, 1151, 1120, 1070, 1036, 1024, 992, 914, 851, 759, 732, 662, 585 cm⁻¹; 1H NMR δ 1.02 (3H, s, 18-CH₃), 1.20-2.23 (16H, m), 2.25-2.65 (3H, m), 2.90 (2H, m, 6-CH₂), 6.85 (1H, s, 4-CH), 7.97 (1H, s, 1-CH), 10.41 (1H, s, OH phenol); 13C NMR δ 171.9, 152.8, 148.9, 133.3, 131.7, 121.5, 118.9, 93.0, 48.8, 47.1, 43.1, 38.4, 33.9, 31.6, 29.7, 29.4, 27.9, 26.8, 25.8, 23.4, 15.8, 14.2.

Example 19G
2-Nitro-1,3,5(10)-estraatrien-3-ol-17(R)-spiro-2'(5'-methyl-6'-oxotetrahydropyran (EM-1126, EM-1131). LDA was prepared as follows: To a stirred solution of diisopropylamine (92 µL, 71 mg, 0.70 mmol) in dry THF (5 mL) at -78 °C under Ar(g) was added n-BuLi (1.2 M/Hexane, 580 µL, 0.68 mmol) and the solution was then stirred at 0 °C for 25 min. then cooled down to -78 °C. A solution of EM-1124 (66 mg, 0.17 mmol) in dry THF (5 mL) was added and the resulting dark orange solution was then stirred for 30 min. Dry HMPA (2 mL) was added and after 15 min., MeI (107 µL, 243 mg, 1.71 mmol). The solution was then stirred for a further 4 h. The reaction was quenched with aqueous saturated NH₄Cl and extracted with EtOAc. The organic phase was washed with 1 M aqueous CuSO₄ (4X), H₂O, aqueous 1M Na₂SO₃, brine, dried (MgSO₄), filtered then rotary evaporated to give a crude solid (103 mg). Purification by flash chromatography on SiO₂ (1:9 → 2:8 EtOAc/Hexanes) provided first EM-1126 (11 mg, 16%) closely followed by EM-1131 (34 mg, 34%) both as yellow solids. EM-1126: M.p. 204-6 °C; [α]D +73.4° (c 1.67, CDCl₃); IR ν 3422 (br, OH), 2937, 2874, 1725 (vs, CO), 1630, 1577, 1525, 1479, 1458, 1432, 1378, 1311, 1269, 1249, 1205, 1188, 1150, 1118, 1088, 1071, 1007, 990, 934, 896, 760, 731, 668, 585, 495 cm⁻¹; 1H NMR δ 1.03 (3H, s), 1.30 (3H, d, J=7.1 Hz), 1.31-1.77 (10H, m), 1.89-2.03 (5H, m), 2.15 (1H, td, J=7.1 Hz, J=5.0 Hz), 2.30-2.50 (2H, m), 2.90 (2H, dd, J=8.3 Hz,
EM-1131 (5'-epimer of EM-1126, real configuration not determined): M.p. 206-8 °C; [α]25D +62.6 ° (c 0.68, CDCl3); IR ν 3422 (br, OH), 3192, 2934, 2876, 2858, 2824, 1721 (vs, CO), 1631, 1578, 1522, 1482, 1458, 1346, 1377, 1314, 1271, 1237, 1204, 1173, 1120, 1103, 1082, 1051, 1019, 1002, 933, 901, 877, 860, 759, 663, 638, 600, 495 cm⁻¹; ¹H NMR δ 7.01 (3H, s), 1.24 (3H, d, J = 7.0 Hz), 1.31-1.80 (10H, m), 1.89-2.20 (6H, m), 2.35 (1H, br s), 2.55 (1H, sextuplet, J = 7.5 Hz), 2.89 (2H, t, J = 5.2 Hz), 6.84 (1H, s), 7.96 (1H, s), 10.41 (H, s, OH); ¹³C NMR δ 175.8, 152.6, 148.9, 133.3, 131.6, 121.4, 118.8, 92.5, 77.4, 77.0, 76.6, 48.5, 47.0, 43.0, 38.4, 33.8, 33.4, 31.6, 29.7, 27.16, 26.7, 25.8, 24.3, 23.6, 17.2, 14.3.

Example 19H
2-Nitro-1,3,5(10)-estratrien-3-ol-17(R)-spiro-2′-(5′,5′-dimethyl-6′-oxo)tetrahydropryan (EM-1125). LDA was prepared as follows: To a stirred solution of diisopropylamine (206 µL, 159 mg, 1.57 mmol) in dry THF (12 mL) at -78 °C under Ar(g) was added n-BuLi (1.2 M/Hexane, 1.28 mL, 1.53 mmol) and the solution was then stirred at 0 °C for 20 min. then cooled down to -78 °C. A solution of a mixture of EM-1126 and EM-1131 (153 mg, 0.38 mmol) in dry THF (10 mL) was added and the resulting dark orange solution was then stirred for 20 min. Dry HMPA (4.7 mL) was added and after 15 min., Mel (238 µL, 544 mg, 3.83 mmol). The solution was then stirred for 5 min. then was warmed up to -30 °C and stirred for a further 1 h. The reaction was quenched with aqueous saturated NH₄Cl and extracted with BuOAc. The organic phase was washed with brine (6X), aqueous 1M Na₂SO₃, brine, dried (MgSO₄), filtered then rotary evaporated to give a crude liquid. Purification by flash chromatography on SiO₂ (1:9 → 2:8 BuOAc/Hexanes) provided EM-1125 (82 mg, 52%) as a yellow solid. M.p. 195-7 °C; [α]25D +72.8 ° (c 1.61, CDCl3); IR ν 3421 (br, OH), 3194, 2954, 2927, 2873, 1718 (vs, CO), 1631, 1578, 1523, 1476, 1458, 1438, 1386, 1312, 1298, 1271, 1204, 1151, 1118, 1059, 1032, 1016, 931, 898, 872, 855, 758, 663, 595 cm⁻¹; ¹H NMR δ 1.02 (3H, s), 1.28 (6H, s), 1.32-1.77 (10H, m), 1.85-2.15 (6H, m), 2.36 (1H, br s), 2.89 (2H, dd, J = 8.2 Hz, J = 4.9 Hz), 6.85 (1H, s), 7.97 (1H, s), 10.42 (H, s, OH); ¹³C NMR δ 177.7, 152.8, 149.0, 133.3, 131.7, 121.5, 118.9, 93.4, 48.6, 47.1, 43.1, 38.5, 37.8, 34.7, 31.6, 31.5, 29.7, 27.7(4), 27.6(8), 26.7, 25.9, 25.5, 23.3, 14.4.
**Example 19I**

2-Nitro-3-methylthioethyloxy-1,3,5(10)-estratrien-17(R)-spiro-2-(6'-oxo)tetrahydropyran (EM-1118). To a solution of EM-1124 (40 mg, 0.117 mmol) in anhydrous acetonitrile (20 mL) at room temperature under Ar(g) was added K₂CO₃ (16 mg, 0.117 mmol) and chloroethylmethyl sulfide (39 mg, 35 mL, 0.350 mmol). The solution was refluxing for 44 h. The acetonitrile solution was evaporated to dryness and ethyl acetate (40 mL) was then added. The organic phase was washed with water, brine and dried with MgSO₄ to gave crude product (49 mg). Purification by flash chromatography on silica gel (SiO₂, 3 g) using ethyl acetate/hexanes (2:8) as eluent gave EM-1118 (35 mg, 70%). IR (ν) 2921, 1727, 1608, 1576, 1499, 1466, 1438, 1382, 1348, 1330, 1310, 1280, 1236, 1187, 1159, 1100, 1067, 1036, 992, 931, 914, 860, 818, 731 cm⁻¹; ¹H NMR (CDCl₃) δ 1.01 (3H, s), 1.25-2.57 (19H, m), 2.20 (3H, s) 2.85 (4H, t, J=6.88 Hz), 4.12 (2H, t, J=6.74 Hz), 6.62 (1H, d, J=2.49 Hz), 6.71 (1H, dd, J₁=2.58 Hz J₂=5.89 Hz), 7.19 (1H, d, J=8.54 Hz); ¹³C NMR(CDCl₃) δ 14.8, 15.9, 16.2, 23.5, 26.1, 27.5, 28.0, 29.5, 29.7, 32.0, 33.1, 34.0, 39.1, 43.7, 47.3, 48.9, 67.4, 93.3, 112.1, 114.6, 126.3, 132.6, 137.9, 156.5, 172.1.
Example 20

Synthesis of 3-hydroxy-1,3,5(10)-estratrien-17(R)-spiro-2'-
(5',5'-dimethyl-6'-oxo) tetrahydropyran (89)

This synthesis is described in Scheme 17

Scheme 17
3-t-butyldimethylsilyloxy-1,3,5(10)-Estratriene-17-one (83). The ether was prepared from estrone (37) following the method described Pelletier et al. (Steroids 59: 536-547, 1994).

5 3-t-butyldimethylsilyloxy-17β-hydroxy-17α-[4'-(2"-tetrahydro-2"H-pyranyl)butyl-1'-yl]-1,3,5(10)-estratriene (84). To a solution of HCC=CH(2)OEt (18.3 mL, 117 mmol) in dry THF (600 mL) at 0 °C, was added dropwise n-butyllithium (43.7 mL, 109 mmol) and the mixture was stirred for 90 min. The mixture was cooled to -78 °C and a solution of TBDMS-estrone (15 g, 39 mmol) in THF (500 mL) was added dropwise. Then, the reaction mixture was allowed to come to room temperature and left stirring for a period of 15 h. Solvents were evaporated to the half volume and 200 mL of water was added. The mixture was extracted with EtOAc (3 x 200 mL), the organic layer was washed with brine, dried (MgSO4) and evaporated to dryness. The residue was purified over silica gel column chromatography with hexanes/EtOAc (9/1) as an eluent to furnish 15.1 g (72%) of the product; IR (NaCl cm⁻¹) 3432, 2934, 2858, 1607, 1495, 1287, 1256, 1033, 958, 839; ¹H NMR (300 MHz, CDCl₃) δ 7.12 (d, 1H, J = 8.4 Hz), 6.62 (dd, 1H, J = 2, 8.4 Hz), 6.54 (d, 1H, J = 2.2 Hz), 4.66 (br.s., 1H), 3.89-3.97 (m, 2H), 3.56-3.50 (m, 2H), 2.97 (br.s., 2H), 2.56 (t, 2H, J = 7.0 Hz), 2.35-2.17 (m, 3H), 2.07-1.23 (m, 17H), 0.98 (s, 9H), 0.87 (s, 3H, 18-Me), 0.19 (s, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 153.3, 137.8, 133.0, 126.1, 119.9, 117.1, 98.7, 84.7, 83.2, 80.0, 65.8, 62.1, 49.5, 47.2, 43.7, 39.4, 39.0, 32.9, 30.6, 29.7, 27.3, 26.4, 25.7, 25.4, 22.8, 20.3, 19.3, 18.1, 12.8, -4.4.

25 3-t-butyldimethylsilyloxy 17β-Hydroxy-17α-[4'-(2"-tetrahydro-2"H-pyranyl)butyl-1'-yl]-1,3,5(10)-estratriene (85). 5% Palladium on activated carbon (1.5 g, 10% wt) was added to a solution of the alkyne 84 (15.1 g, 28 mmol) in EtOAc (500 mL) at room temperature. The flask was purged with H₂ three times (vacuum followed by H₂) and left stirring under 1 atm pressure of H₂. The reaction was followed by TLC. After a period of 3 h, the mixture was filtered over a plug of celite and the solvent was removed under reduced pressure. The crude product was used in the next step without further purification; IR (NaCl cm⁻¹) 3474, 2935, 2858, 1607, 1570, 1496, 1471, 1286, 1257, 1156, 1137, 1119, 1033, 954, 839, 780; ¹H NMR (300 MHz, CDCl₃) δ 7.12 (d, 1H, J = 8.4 Hz), 6.62 (dd, 1H, J = 2.1, 8.4 Hz), 6.55 (s, 1H), 4.59 (br.s., 1H), 3.92-3.73 (m, 2H), 3.55-3.38 (m, 2H), 2.82-2.77 (m, 2H), 2.30-1.33 (m, 26H), 0.97 (s, 9H), 0.90 (s, 3H, 18-Me), 0.18 (s, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 153.27, 137.81, 133.08, 126.02, 119.87, 117.06, (98.90, 98.84), 83.38, 67.61, 62.33, 49.50, 46.67, 43.81, 39.58, 36.35, 34.28, 31.60, 30.75, 30.36, 29.62, 27.51, 26.26, 25.67, 25.47, 23.37, 20.45, 19.66, 18.12, 14.35, -4.43.
3,4-butyldimethylsilyloxy 17β-hydroxy-17α-(4'-hydroxybutan-1'-yl)-1,3,5(10)-estratriene (86). To a solution of the THF ether 85 (15.1 g, 28 mmol) in MeOH (400 mL), was added p-toluenesulfonic acid monohydrate (150 mg, 0.8 mmol) and the reaction was stirred over a period of 5 h. A saturated solution of NaHCO₃ (100 mL) was added and volume of solvent was reduced to half on a rotary evaporator. The mixture was extracted with CH₂Cl₂, the organic phase was washed with brine, dried (MgSO₄) and evaporated to dryness. The crude product was used in the next step without purification; IR (NaCl, cm⁻¹) 3356, 2931, 2858, 1608, 1496, 1471, 1286, 1256, 954, 839, 780; ¹H NMR (300 MHz, CDCl₃) δ 7.12 (d, 1H, J=8.5 Hz), 6.61 (dd, 1H, J=2.5, 8.5 Hz), 6.55 (s, 1H), 3.69 (br.d, 2H, J=5.2 Hz), 2.82-2.78 (m, 2H), 2.35-2.26 (m, 1H), 2.20-1.94 (m, 2H), 1.90-1.81 (m, 1H), 1.62-1.22 (m, 17H), 0.98 (s, 9H), 0.90 (s, 3H, 18-Me), 0.19 (s, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 153.27, 137.81, 133.05, 126.02, 119.89, 117.09, 83.59, 62.56, 49.50, 46.69, 48.81, 39.58, 35.98, 34.32, 33.20, 31.61, 29.62, 27.51, 26.26, 25.68, 23.37, 19.74, 18.14, 14.36, -4.40.

3,4-butyldimethylsilyloxy-1,3,5(10)-estratrien-17(R)-spiro-2'-(6'-oxo) tetrahydropyran (87). To a solution of the diol 86 (12.5 g, 27 mmol) in acetone (500 mL) at 0°C, was added dropwise of a 2.7 M solution of Jones's reagent (15.1 mL, 41 mmol). The reaction was stirred for 30 min. 2-Propanol (100 mL) was added, followed by of a saturated solution of NaHCO₃ (200 mL). The volume of solvents was reduced to half by evaporation and the mixture was extracted with EtOAc. The organic phase was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The residue was purified a silica gel column chromatography with hexanes/acetone (6/1) to afford 8.6 g of the lactone. (68% yield for 3 steps); IR (NaCl, cm⁻¹): 2960, 2930, 2857, 1732, 1607, 1496, 1284, 1264, 1037, 958, 840; ¹H NMR (300 MHz, CDCl₃) δ 7.11 (d, 1H, J=8.4 Hz), 6.61 (dd, 1H, J=2.3, 8.4Hz), 6.56 (s, 1H), 2.85-2.79 (m, 2H), 2.58-2.39 (m, 2H), 2.38-2.25 (m, 1H), 2.21-2.10 (m, 1H), 2.03-1.27 (m, 15H), 1.02 (s, 3H, 18-Me), 0.97 (s, 9H), 0.18 (s, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 172.00, 153.36, 137.63, 132.62, 126.02, 119.92, 117.19, 93.25, 48.88, 47.26, 43.68, 39.05, 33.98, 31.96, 29.50, 29.48, 27.94, 27.46, 25.98, 25.67, 23.48, 18.12, 15.87, 14.30, -4.43.

3,4-butyldimethylsilyloxy-1,3,5(10)-estratrien-17(R)-spiro-2'-(5'-5'-dimethyl-6'-oxo) tetrahydropyran(88). In a dry 1L flask under argon, was dissolved the lactone 87 (8.6 g, 19 mmol) in dry THF (300 mL), and cooled to 0°C. A 1M solution of LiHMDS (47.3 mL, 47.3 mmol) was added dropwise. The mixture was stirred 15 min at 0°C and cooled to -78°C and then methyl iodide (5.9 mL, 79 mmol) was added. The reaction was stirred 1 h at this temperature and then allowed to warm to room temperature over a period of 2 h. A saturated solution of NH₄Cl (200 mL) was added
and the mixture was extracted with EtOAc. The organic layer was washed with a saturated solution of Na₂SO₃, brine, dried (MgSO₄) and concentrated under reduced pressure. The residue was purified by column chromatography with hexanes/acetone (5/1) as an eluent to afford 7.4 g (81%) of the dimethyl compound; IR (NaCl, cm⁻¹) 2954, 2930, 2858, 1725, 1496, 1287, 1258, 1150, 1137, 956, 840; ¹H NMR (300 MHz, CDCl₃) δ 7.11 (d, 1H, J=8.5Hz), 6.62 (dd, 1H, J=2.4, 8.5Hz), 6.55 (d, 1H, J=2.1Hz), 2.81-2.78 (m, 2H), 2.36-2.28 (m, 1H), 2.20-1.38 (m, 16H), 1.28 (s, 3H), 1.27 (s, 3H), 1.02 (s, 3H, 18-Me), 0.97 (s, 9H), 0.18 (s, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 177.79, 153.33, 137.62, 132.62, 125.99, 119.90, 117.14, 93.66, 48.67, 47.24, 43.65, 39.06, 37.74, 34.79, 31.96, 31.56, 29.50, 27.73, 27.61, 27.42, 26.01, 25.65, 25.55, 23.26, 18.11, 14.42, -4.43.

1,3,5(10)-estratrien-3-ol-17(R)-spiro-2'-5',5'-dimethyl-6'-oxo) tetrahydropyrany (89): To a solution of the silyl ether 88 (7.1 g, 14.7 mmol) in THF (300 mL) at 0°C, was added dropwise a 1M solution of TBAF (17.6 mL, 17.6 mmol) and the reaction was stirred for 15 min. Ice water (200 mL) was added to precipitate the compound. The flask was placed on a retary evaporator to reduce the volume of THF, and then placed on an ice bath.

The precipitate was collected by filtration, washed with cold water and dried in an oven (30°C) over a period of 24 h to furnish 5.4 g (100%) of the 3-OH compound; IR (NaCl, cm⁻¹): 3357, 2932, 2871, 1695, 1287, 1158; ¹H NMR (300 MHz, CDCl₃) δ 7.14 (d, 1H, J=8.4Hz), 6.63 (dd, 1H, J=2.6, 8.4 Hz), 6.55 (d, 1H, J=2.6Hz), 4.62 (br.s, 1H, OH), 2.81-2.79 (m, 2H), 2.38-2.29 (m, 1H), 2.20-1.81 (m, 5H), 1.76-1.31 (m, 11H), 1.29 (s, 3H), 1.28 (s, 3H), 1.01 (s, 3H, 18-Me); ¹³C NMR (75 MHz, CDCl₃) δ 178.06, 153.52, 138.08, 132.19, 126.42, 115.26, 112.74, 93.80, 48.69, 47.29, 43.65, 39.14, 37.81, 34.84, 31.98, 31.61, 29.53, 27.76, 27.64, 27.39, 26.12, 25.59; 23.29, 14.43.
Example 21

Synthesis of 3-hydroxy-1,3,5(10)-estratrien-17(R)-spiro-2'-
(5',5'-dimethyl-6'-oxo)tetrahydropyran-3-derivatives

These syntheses are described in Scheme 18.

Scheme 18
Example 21A
Synthesis of 3-ethoxy-1,3,5(10)-estratrien-17(R)-spiro-2'-[(5',5'-dimethyl-6'-oxo)-tetrahydropyran (EM-1369). To a solution of lactone 89 (50 mg, 0.136 mmol) in anhydrous THF (10 ml) and under argon was added K$_2$CO$_3$ (26 mg, 0.190 mmol), 18-crown-6 (14 mg, 0.054 mmol) and bromoethane (303 µl, 4.08 mmol). The solution was refluxed for 24 h. Then water (10 ml) was added and the product was extracted with ethyl acetate (2 x 20 ml), washed with brine, and dried with MgSO$_4$. After evaporation of solvent, the crude product was purified by flash chromatography on silica gel using ethyl acetate / hexanes (1:9) as eluent to give the pure EM-1369. White solid; 59% yield; IR (film NaCl) ν 2930, 2872, 1732, 1608, 1573, 1500, 1477, 1455, 1385, 1309, 1236, 1149, 1137, 1114, 1051, 1017; $^1$H NMR (300 MHz, CDCl$_3$) δ 1.02 (s, 3H, 18-CH$_3$), 1.28 (2s, 6H), 2 x CH$_3$), 1.30 to 2.36 (m, 17H), 1.39 (t, J = 7.0 Hz, 3H, CH$_3$CH$_2$O), 2.84 (m, 2H, 6-CH$_2$), 4.00 (q, J = 7.0 Hz, 2H, CH$_3$CH$_2$O), 6.62 (d, J = 2.5 Hz, 1H, 4-CH), 6.70 (dd, J$_1$ = 2.5 Hz and J$_2$ = 8.5 Hz, 1H, 2-CH), 7.19 (d, J = 8.5 Hz, 1H, 1-CH); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 14.44, 14.91, 23.30, 25.61, 26.11, 27.48, 27.66, 27.77, 29.73, 31.63, 32.01, 34.85, 37.80, 39.17, 43.69, 47.30, 48.71, 63.31, 93.70, 112.05, 114.50, 126.21, 132.10, 137.76, 156.88, 177.85; HRMS calculated for C$_{25}$H$_{37}$O$_3$ (M$^+$ + H): 397.27426, found: 397.27520.

Example 21B
Typical procedure for the synthesis of EM-1368-CS, EM-1389-CS, and EM-1390-CS. To a solution of lactone 89 (0.20 mmol) in anhydrous acetonitrile (20 ml) and under argon was added K$_2$CO$_3$ (0.20 mmol). The appropriate electrophile (16.0 mmol) (chloroethylmethylether, 2-dimethylaminoethylchloride-HCl, and 1-(2-chloro-ethyl)-piperidine-HCl). The solution was refluxed for 56 h. The acetonitrile was evaporated to dryness and ethyl acetate (40 ml) was then added. The organic phase was washed with water, brine, and dried with MgSO$_4$ to give the crude products which were purified by flash chromatography on silica gel using, as eluent, ethyl acetate / hexanes (1:9) for EM-1368CS and CH$_2$Cl$_2$ / Et$_3$N (95:5:0.5) for EM-1389CS and EM-1390CS.

3-Methoxyethoxy-1,3,5(10)-estratrien-17(R)-spiro-2'-[(5',5'-dimethyl-6'-oxo)-tetrahydropyran (EM-1368-CS). White solid; 74% yield; IR (film NaCl) ν 2930, 2873, 1722, 1609, 1574, 1499, 1455, 1385, 1309, 1237, 1201, 1135, 1064, 1032, 1017; $^1$H NMR (300 MHz, CDCl$_3$) δ 1.02 (s, 3H, 18-CH$_3$), 1.28 (2s, 6H, 2 x CH$_3$), 1.30 to 2.35 (m, 17H), 2.85 (m, 2H, 6-CH$_2$), 3.44 (s, 3H, OCH$_3$), 3.73 (t, J = 4.7 Hz, 2H, CH$_3$OCH$_2$CH$_2$O), 4.09 (t, J = 4.7 Hz, 2H, CH$_3$OCH$_2$CH$_2$O), 6.66 (d, J = 2.5 Hz, 1H, 4-CH), 6.73 (dd, J$_1$ = 2.5 Hz
and \( J_2 = 8.6 \text{ Hz}, 1\text{H}, 2-\text{CH}) \), 7.19 (d, \( J = 8.6 \text{ Hz}, 1\text{H}, 1-\text{CH} \)); \(^{13}\text{C} \text{NMR (75 MHz, CDCl}_3 \)) \( \delta \) 14.44, 23.29, 25.61, 26.09, 27.45, 27.66, 27.76, 29.71, 31.62, 31.99, 34.85, 37.79, 39.14, 43.67, 47.29, 48.70, 59.16, 67.20, 71.12, 93.70, 112.16, 114.67, 126.21, 132.49, 137.78, 156.72, 177.85; HRMS calculated for \( \text{C}_2\text{H}_3\text{O}_4 (\text{M}^+ + \text{H}) \): 427.28482, found: 427.28690.

3-(N,N-dimethylaminoethyl)-1,3,5(10)-estratrien-17(R)-spiro-2'-(5',5'-dimethyl-6'-oxo)-tetrahydropyran (EM-1389-CS). White solid; 40% yield; IR (film NaCl) \( \nu \) 2936, 2871, 2819, 2770, 1723, 1609, 1575, 1499, 1456, 1385, 1309, 1290, 1256, 1238, 1202, 1149, 1032; \(^1\text{H} \text{NMR (300 MHz, CDCl}_3 \)) \( \delta \) 1.01 (s, 3H, 18-CH\(_3\)), 1.28 (2s, 6H, 2 x CH\(_3\)), 1.30 to 2.20 (m, 17H), 2.33 (s, 6H, (CH\(_3\))\(_2\)N), 2.71 (t, \( J = 5.8 \text{ Hz}, 2\text{H}, \text{NCH}_2\text{CH}_2\text{O} \)), 2.83 (m, 2H, 6-CH\(_2\)), 4.04 (t, \( J = 5.8 \text{ Hz}, 2\text{H}, \text{NCH}_2\text{CH}_2\text{O} \)), 6.65 (d, \( J = 2.4 \text{ Hz}, 1\text{H}, 4-\text{CH} \)), 6.73 (dd, \( J_1 = 2.7 \text{ Hz} \) and \( J_2 = 8.6 \text{ Hz}, 1\text{H}, 2-\text{CH} \)), 7.19 (d, \( J = 8.6 \text{ Hz}, 1\text{H}, 1-\text{CH} \)); \(^{13}\text{C} \text{NMR (75 MHz, CDCl}_3 \)) \( \delta \) 14.45, 23.30, 25.62, 26.11, 27.46, 27.67, 27.77, 29.73, 31.64, 32.01, 34.86, 37.79, 39.16, 43.70, 45.87, 47.30, 48.72, 58.35, 65.92, 93.71, 112.13, 114.59, 126.21, 132.34, 137.79, 156.79, 177.85; HRMS calculated for \( \text{C}_8\text{H}_4\text{O}_3\text{N} (\text{M}^+ + \text{H}) \): 440.31647, found: 440.31520.

3-(N-piperidyl-ethyl)- 1,3,5(10)-estratrien-17(R)-spiro-2'-(5',5'-dimethyl-6'-oxo)-tetrahydropyran (EM-1390-CS)
White solid; 69% yield; IR (film NaCl) \( \nu \) 2933, 2871, 2783, 1724, 1609, 1574, 1499, 1455, 1385, 1308, 1290, 1256, 1236, 1202, 1148, 1136, 1033; \(^1\text{H} \text{NMR (300 MHz, CDCl}_3 \)) \( \delta \) 1.01 (s, 3H, 18-CH\(_3\)), 1.28 (2s, 6H, 2 x CH\(_3\)), 1.30 to 2.40 (m, 17H), 2.50 (m, 4H, (CH\(_2\))\(_2\)N), 2.76 (t, \( J = 6.2 \text{ Hz}, 2\text{H}, \text{NCH}_2\text{CH}_2\text{O} \)), 2.84 (m, 2H, 6-CH\(_2\)), 4.08 (t, \( J = 6.2 \text{ Hz}, 2\text{H}, \text{NCH}_2\text{CH}_2\text{O} \)), 6.63 (d, \( J = 2.6 \text{ Hz}, 1\text{H}, 4-\text{CH} \)), 6.71 (dd, \( J_1 = 2.6 \text{ Hz} \) and \( J_2 = 8.6 \text{ Hz}, 1\text{H}, 2-\text{CH} \)), 7.18 (d, \( J = 8.6 \text{ Hz}, 1\text{H}, 1-\text{CH} \)); \(^{13}\text{C} \text{NMR (75 MHz, CDCl}_3 \)) \( \delta \) 14.46, 23.31, 24.18, 25.63, 25.92, 26.13, 27.47, 27.68, 27.79, 29.74, 31.65, 32.02, 34.87, 37.81, 39.17, 43.71, 47.32, 48.73, 55.00, 57.98, 65.83, 93.70, 112.14, 114.60, 126.23, 132.32, 137.81, 156.72, 177.87; HRMS calculated for \( \text{C}_{31}\text{H}_{46}\text{O}_3\text{N} (\text{M}^+ + \text{H}) \): 480.34778, found: 480.34550.
Example 22

Synthesis of 2-chloro-1,3,5(10)-estratrien-17-spiro-5-lactone derivatives

These syntheses are described in Scheme 19.

Scheme 19

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a. PhSeCl, CHCl₃  b. NCS, CHCl₃  c. Cs₂CO₃, CH₂OCH₂CH₂Cl, CH₂CN, NaI
Example 22A
3-Hydroxy-2-phenylselenenyl-1,3,5(10)-estratrien-17(R)-spiro-2'- (5',5'-dimethyl-6'-oxo) tetrahydropyran (90). A solution of 3-hydroxy-1,3,5(10)-
estratrien-17(R)-spiro-2'-(5',5'-dimethyl-6'-oxo) tetrahydropyran (89) (406 mg, 1.10 mmol) and phenylselenenyi chloride (253 mg, 1.32 mmol) in dry
CHCl₃ (24 mL) under Ar (g) was stirred at 0 °C for 1 h then at room
temperature overnight. The resulting yellow solution was poured onto
ice/H₂O then extracted with CH₂Cl₂ (3x). The combined organic phase
was dried (cotton plug) then rotary evaporated to give a crude foamy
solid. Purification by flash chromatography (SiO₂) using 1:9
EtOAc/Hexanes as eluent gave compound 90 (353 mg, 61%) with the 4-
isomer (86 mg, 15%). Compound 90: [α]²₅D +77.7 ° (c 1.14, CHCl₃); IR
v 3366, 3050, 2965, 2928, 2869, 1709, 1603, 1576, 1550, 1458, 1438, 1384, 1349,
1310, 1294, 1262, 1202, 1157, 1141, 1114, 1065, 1017, 984, 892, 845, 736, 689,
665, 593, 555, 498, 460 cm⁻¹; ¹H NMR(CDCl₃) δ 1.02 (3H, s), 1.27(9) (3H, s),
1.28(4) (3H, s), 1.27-1.80 (11H, m), 1.88-2.28 (6H, m), 2.87 (2H, t, J = 4.8 Hz),
6.24 (1H, s, OCH), 6.80 (1H, s), 7.21 (5H, br s), 7.52 (1H, s); ¹³C NMR(CDCl₃)
δ 14.4, 23.3, 25.5, 26.1, 27.2, 27.6, 27.7, 29.5, 31.5, 31.8, 34.7, 37.7, 38.9, 43.4,
47.2, 48.6, 93.6, 111.6, 114.7, 126.5, 129.2(6), 129.3(4), 131.2, 133.3, 134.7,
141.4, 154.4, 177.8.

Example 22B
2-Chloro-3-hydroxy-1,3,5(10)-estratrien-17(R)-spiro-2'-(5',5'-dimethyl-6'-
oxo) tetrahydropyran (91). A solution of compound 90 (347 mg, 0.66
mmol) and N-chlorosuccinimide (177 mg, 1.33 mmol) in dry CHCl₃ (30
mL) under Ar(g) at 0 °C were stirred for 30 min. The solution was poured
onto ice/H₂O then was extracted with CH₂Cl₂ (3x). The combined
organic phase was dried (cotton plug) then rotary evaporated to give a
 crude solid. Purification by flash chromatography (SiO₂) using 1:9
EtOAc/Hexanes as eluent gave compound 91 (104 mg, 39%) as a white
solid. ¹H NMR (CDCl₃) δ 1.01 (3H, s), 1.28 (6H, s), 1.25-1.75 (11H, m), 1.85-
2.28 (6H, m), 2.80 (2H, dd, J = 8.7 Hz, J = 3.9 Hz), 5.39 (1H, br s, OH), 6.73
(1H, s), 7.19 (1H, s); ¹³C NMR(CDCl₃) δ 14.4, 23.3, 25.6, 26.1, 27.2, 27.7,
27.8, 29.1, 31.6, 31.9, 34.8, 37.8, 38.8, 43.5, 47.3, 48.6, 93.6, 116.0, 117.1, 125.6,
133.5, 137.2, 149.0, 177.8.

Example 22C
2-Chloro-3-methoxyethoxyethoxy-1,3,5(10)-estratrien-17(R)-spiro-2'-(5',5'-
dimethyl-6'-oxo) tetrahydropyran (EM-1371). A mixture of compound 91
(95 mg, 0.24 mmol), Cs₂CO₃ (230 mg, 0.70 mmol) 2-chloroethylmethyl
ether (1.72 mL, 1.78 g, 18.86 mmol) and NaI (4 mg, 0.02 mmol) in
acetonitrile (45 mL) were refluxed for 4 h. The solvent was rotary
evaporated and the residue was taken into H₂O/CH₂Cl₂. The aqueous
phase was extracted with CH₂Cl₂ (3x). The combined organic phase was dried (MgSO₄), filtered then rotary evaporated. The crude solid was purified by flash chromatography (SiO₂) using 1:9 to 2:8 EtOAc/Hexanes as eluent to give EM-1371 (73 mg, 67%) as a white solid. IR ν 2982, 2963, 2927, 2880, 1718, 1654, 1598, 1499, 1458, 1397, 1387, 1364, 1323, 1307, 1286, 1259, 1247, 1210, 1151, 1125, 1059, 1032, 1018, 987, 928, 885, 866, 738, 669 cm⁻¹; ¹H NMR (CDCl₃) δ 1.02 (3H, s), 1.28 (6H, s), 1.29-1.75 (11H, m), 1.85-2.35 (6H, m), 2.80 (2H, br t, J=5.0 Hz), 3.48 (3H, s), 3.78 (2H, t, J=5.2 Hz), 4.14 (2H, t, J=5.2 Hz), 6.66 (1H, s), 7.25 (1H, s); ¹³C NMR(CDCl₃) δ 14.4, 23.2, 25.5, 26.0, 27.2, 27.6, 27.7, 29.3, 31.5, 31.9, 34.7, 37.7, 38.7, 43.4, 47.2, 48.5, 59.3, 68.9, 70.8, 93.5, 114.5, 120.3, 127.0, 133.7, 136.1, 152.1, 177.7.
Example 23

3-hydroxy-2,4-dihalo-1,3,5(10)-estratrien-17-spiro-(dimethyl-5-lactone) derivatives

These syntheses are described in Scheme 20.

Scheme 20
Example 23A
2,4-dihalo-3-hydroxy-1,3,5(10)-estraatrien-17(R)-spiro-2'-(5',5'-dimethyl-6'-oxo)tetrahydropyran (92). Under argon atmosphere, a solution of 3-hydroxy-1,3,5(10)-estraatriene-17(R)-spiro-2'-(5',5'-dimethyl-6'-oxo)tetrahydropyran (89) and N-halosuccinimide (2 equiv) in anhydrous chloroform (1.3% W/V) was stirred at room temperature for 1.5 h. The reaction mixture was diluted with dichloromethane, washed with brine, dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by flash chromatography (hexanes-ethyl acetate 32:1 to hexanes-ethyl acetate 9:1) to provide compound 92 (e.g., EM-1382-CS, X=Br, 62%): IR (NaCl) 3197, 2936, 2872, 1694, 1466, 1387, 1297, 1154 cm⁻¹; \(^1\)H NMR (300 MHz, CDCl₃) δ 1.01 (s, 3H), 1.29 (s, 6H), 1.35-2.35 (m, 17H), 2.65 (m, 1H), 2.88 (dd, J=6.1 and 18.0 Hz, 1H), 5.83 (s, 1H), 7.40 (s, 1H); \(^{13}\)C NMR (75 MHz, CDCl₃) δ 14.35, 23.25, 25.55, 26.29, 27.39, 27.69, 27.76, 30.98, 31.58, 31.77, 34.76, 37.80, 38.12, 43.59, 47.11, 48.50, 93.44, 106.36, 113.21, 128.45, 135.23, 136.51, 147.17, 177.75.

Example 23B
3-substituted-2,4-dihalo-1,3,5(10)-estraatrien-17(R)-spiro-2'-(5',5'-dimethyl-6'-oxo)tetrahydropyran (93)

Method A: Under argon atmosphere, a solution of compound 92, anhydrous potassium carbonate (1.2 equiv) and alkylidide (2.0 equiv) in dimethylformamide (3.3% W/V) was stirred at 30 °C for 1 h. The reaction mixture was cooled at room temperature, quenched with saturated ammonium chloride, and extracted with ethyl acetate. The organic phase was quenched with brine, dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by flash chromatography (hexanes-ethyl acetate 49:1 to hexanes-ethyl acetate 19:1) to provide compound 93 (e.g., EM-1385-CS, X=Br, R=Me, 78%): IR (NaCl) 2935, 2870, 1720, 1462, 1384, 1298, 1150 cm⁻¹; \(^1\)H NMR (300 MHz, CDCl₃) δ 1.01 (s, 3H), 1.29 (s, 6H), 1.30-2.35 (m, 17H), 2.68 (m, 1H), 2.88 (dd, J=5.6 and 18.2 Hz, 1H), 3.86 (s, 3H), 7.45 (s, 1H); \(^{13}\)C NMR (75 MHz, CDCl₃) δ 14.36, 23.28, 25.59, 26.24, 27.45, 27.72, 27.80, 31.07, 31.62, 31.84, 34.79, 37.84, 37.99, 43.87, 47.11, 48.63, 60.38, 93.45, 114.48, 121.45, 129.07, 137.31, 139.26, 151.96, 177.75.
Method B: Under argon atmosphere, a solution of compound 92, triphenylphosphine (6 equiv) and alcohol (6 equiv) in THF (1.5% W/V) was cooled at 0 °C and treated with diethyl azodicarboxylate (6 equiv). The solution was warmed at room temperature, stirred for 2 h, and evaporated. The crude mixture was purified by flash chromatography (hexanes-ethyl acetate 19-1) to provide compound 93 (e.g., EM-1387, X=Br, R=allyl, 72%): IR (neat) 2937, 2871, 1722, 1453, 1386 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 1.01 (s, 3H), 1.28 (s, 6H), 1.30-2.35 (m, 17H), 2.68 (m, 1H), 2.88 (dd, J=5.8 and 18.0 Hz, 1H), 4.50 (d, J=5.6 Hz, 2H), 5.30 (dd, J=1.3 and 10.3 Hz, 1H), 5.46 (dd, J=1.3 and 17.0 Hz, 1H), 6.18 (m, 1H), 7.45 (s, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 14.35, 23.26, 25.56, 26.20, 27.42, 27.69, 27.77, 31.09, 31.59, 31.81, 34.76, 37.81, 37.93, 43.84, 47.08, 48.60, 73.79, 93.42, 114.76, 118.41, 121.73, 129.04, 133.20, 137.24, 139.21, 150.86, 177.72.
Example 24
Synthesis of 2-fluoro-1,3,5(10)-estratrien-17-dimethyl-8-lactone derivatives

These syntheses are described in Scheme 21.

Scheme 21

a. K₂CO₃, NaI, CH₂OCH₂CH₂Cl, CH₃CN, Δ  
b. Na₂S₂O₄, NaOH, acetone, H₂O  
c. BF₃·OEt₂, t-BuONO,  
d. HCO(CH₂)₂OETHP, MeLi  
e. H₂, Pd/C, CO₂, MeOH  
f. PTSA, MeOH  
g. Jones' reagent  
h. LiHMDS,

10
Example 24A
2-Nitro-3-methylxyloxyethyloxy-1,3,5(10)-estratrien-17-one (94). A mixture of compound 79a (905 mg, 2.87 mmol), K₂CO₃ (793 mg, 5.74 mmol), 2-chloroethylmethyl ether (17 mL, 18 g, 189 mmol) and NaI (43 mg, 0.29 mmol) in acetonitrile (60 mL) were refluxed for 24 h. The solvent was rotary evaporated and the residue was taken into H₂O/EtOAc. The aqueous phase was extracted with EtOAc (3x). The combined organic phase was washed with brine, dried (MgSO₄), filtered then rotary evaporated. The crude solid was purified by flash chromatography (SiO₂) using 1:9 to 2:8 EtOAc/Hexanes as eluent to give recovery of the starting material (416 mg, 46%) and the title compound (520 mg, 49%) as a yellow solid. [α]₂₅D +135.8° (c 1.28, CHCl₃); IR ν 3448 (w), 2930, 2890, 1737 (CO), 1617, 1568, 1518, 1498, 1454, 1409, 1373, 1351, 1339, 1287, 1194, 1129, 1070, 1031, 1006, 960, 916, 895, 895, 833, 795, 758, 733, 669, 594, 588 cm⁻¹; ¹H NMR (CDCl₃) δ 0.91 (3H, s), 1.42-1.66 (6H, m), 1.95-2.42 (6H, m), 2.51 (1H, dd, J=18.9 Hz, J=8.9 Hz), 2.92 (2H, br t, J=9.5 Hz), 3.45 (3H, s), 3.78 (2H, t, J=5.1 Hz), 4.20 (2H, t, J=4.8 Hz), 6.80 (1H, s), 7.79 (1H, s); ¹³C NMR (CDCl₃) δ 13.8, 21.5, 25.7, 26.0, 29.7, 31.3, 35.7, 37.8, 43.5, 47.8, 50.2, 59.4, 69.7, 70.7, 115.4, 122.9, 132.9, 137.9, 144.1, 150.4, 220.1.

Example 24B
2-Amino-3-methylxyloxyethyloxy-1,3,5(10)-estratrien-17-one (95). To a refluxing solution of compound 94 (506 mg, 1.35 mmol) in acetone (120 mL), H₂O (24 mL) and aqueous 1 M NaOH (24 mL, 24.0 mmol) was portionwise added with care solid Na₂S₂O₄ (3.89 g, 18.97 mmol). The reflux was continued for 2 h then the acetone was removed under vacuum. More H₂O (50 mL) was added and the aqueous phase was extracted with CH₂Cl₂ (4x). The organic phase was dried (MgSO₄), filtered then rotary evaporated. The crude solid was purified by flash chromatography (SiO₂) using 3:7 to 1:1 EtOAc/Hexanes as eluent to give compound 95 (228 mg, 49%) as a foamy solid. ¹H NMR (CDCl₃) δ 0.88 (3H, s), 1.37-2.78 (14 H, m), 3.41 (3H, s), 3.71 (2H, t, J=5.0 Hz), 4.09 (2H, 2.9 Hz), 6.52 (1H, s), 6.65 (1H, s).

Example 24C
2-Fluoro-3-methylxyloxyethyloxy-1,3,5(10)-estratrien-17-one (96). To neat BF₃-Et₂O (126 µL, 141 mg, 1.00 mmol) at -15 °C under Ar (g) was added a solution of compound 95 (228 mg, 0.66 mmol) in dry CH₂Cl₂ (4 mL) followed by the dropwise addition tert-butylnitrile (95 µL, 82 mg, 0.80 mmol). This solution was stirred at -15 °C for 10 min then at 0 °C for 1.5 h. Pentane (16 mL) was added to the cold solution and a red gummy solid precipitated. The solvent was decanted and the residue was rinsed with cold Et₂O (5 mL). The residue was dried under vacuum to give a
foamy orange-red solid. This solid was heated under vacuum with a
heatgun for 5 min. The residue was purified by flash chromatography
(SiO₂) using 3:7 to 1:1 EtOAc/Hexanes as eluent to give compound 96 (32
mg, 14%) as a solid. ¹H NMR (CDCl₃) δ 0.90 (3H, s), 1.25-1.64 (m, 6H),
1.92-2.29 (6H, m), 2.50 (1H, dd, J=18.9 Hz, 8.9 Hz), 2.82 (2H, dd, J=8.4 Hz,
J'=3.5 Hz), 3.45 (3H, s), 3.75 (2H, t, J=4.7 Hz), 4.15 (2H, t, J=4.5 Hz), 6.71
(1H, d, J=8.7 Hz), 6.98 (1H, d, J=13.2 Hz); ¹³C NMR(CDCl₃) δ 13.8, 21.5,
25.9, 26.5, 29.0, 29.7, 31.5, 35.8, 38.0, 43.9, 47.9, 50.3, 59.2, 69.2, 71.0, 113.1
(d, J=18.7 Hz), 116.1, 132.6 (d, J=87.0 Hz), 144.5, 149.5, 152.7, 220.6.

Example 24D
2-Fluoro-17β-hydroxy-3-methoxyethyl-17α-(4'-(2''-tetrahydro-2''H-
pyranyloxy)-butynyl)-1,3,5(10)-estratriene (97). To a stirred solution of 2-
(3-butyne-1-yl)tetrahydro-2H-pyran (58 µL, 57 mg, 0.37 mmol) in dry THF
(4 mL) at -30 °C under Ar(g) was dropwise added MeLi (1.4 M in Et₂O,
5.39 mL, 7.55 mmol). The solution was stirred at room temperature for 25
min. then cooled down to -30 °C. A solution of compound 96 (32 mg, 0.09
mmol) in dry THF (4 mL) was added and the solution was stirred for a
further 1 h. The solution was quenched with ice and aqueous sat.
NaHCO₃ and extracted with EtOAc. The organic layer was washed with
brine, dried (MgSO₄), filtered then rotary evaporated to give compound
97 as a crude oil (73 mg). This compound was used without further
purification.

Example 24E
2-Fluoro-17β-hydroxy-3-methoxyethyl-17α-(4'-(2''-tetrahydro-2''H-
pyranyloxy)-butyl)-1,3,5(10)-estratriene (98). A mixture of crude
compound 97 (73 mg, ~0.09 mmol) and 5% Pd/CaCO₃ (35 mg) in MeOH
(10 mL) was stirred under H₂(g) (balloon) at room temperature for 2 h.
The mixture was filtered through celite and the solvent evaporated under
vacuum to give compound 98 (52 mg) as a crude oil. This compound was
used without further purification.

Example 24F
2-Fluoro-17β-hydroxy-3-methoxyethyl-17α-(4'-hydroxybutyl)-1,
3,5(10)-estratriene (99). Crude compound 98 (52 mg, ~0.09 mmol) and
PTSA monohydrate (7 mg, 0.04 mmol) were stirred in MeOH (5 mL) at
room temperature for 3 h. The solvent was reduced to about 1-2 mL,
EtOAc was added, and the organic phase was washed with cold aqueous
saturated NaHCO₃, H₂O, brine and was dried (MgSO₄), filtered and then
evaporated to give compound 99 (42 mg) as a crude solid. This
compound was used without further purification.
Example 24G
2-Fluoro-3-methoxyethylxyloxy-1,3,5(10)-estratrien-17(R)-spiro-2'-(6'-oxo) tetrahydropyran (100). To a stirred solution of crude compound 99 (42 mg, ~0.09 mmol) in acetone (6 mL) at 0 °C was added 1.25 M H₂CrO₄ (Jones' reagent, 326 µL, 0.4 mmol). The solution was stirred for 20 min. then more 1.25 M H₂CrO₄ (Jones' reagent, 326 µL, 0.4 mmol) was added. After 20 min, iso-propanol (1 mL) was added and the solution was stirred for a further 10 min. The precipitated formed was decanted and washed with acetone (4x). The acetone volume was reduced to about 2 mL and was then poured onto cold aqueous saturated NaHCO₃ with the help of EtOAc. The organic phase was washed with H₂O, brine, dried (MgSO₄), filtered and then evaporated to give compound 100 (33 mg) as a crude solid. This compound was used without further purification. ¹H NMR (CDCl₃) δ 1.01 (3H, s), 1.24-2.60 (19H, m), 2.78 (2H, br s), 3.45 (3H, s), 3.74 (2H, dd, J=5.0 Hz, J=4.5 Hz), 4.14 (2H, dd, J=5.0 Hz, J=4.5 Hz), 6.69 (1H, d, J=8.7 Hz), 6.97 (1H, d, J=13.2 Hz); ¹³C NMR (CDCl₃) δ 14.3, 15.9, 23.4, 26.0, 27.4, 27.9, 29.0, 29.5, 31.9, 34.0, 38.7, 43.6, 47.2, 48.8, 59.2, 69.2, 71.0, 93.2, 113.1 (d, J=18.6 Hz), 116.1, 132.0, 133.3, 144.4, 151.1 (d, Jcr=243.4 Hz), 172.0.

Example 24H
2-Fluoro-3-methoxyethylxyloxy-1,3,5(10)-estratrien-17(R)-spiro-2'-{(5',5',dimethyl-6'-oxo) tetrahydropyran (EM-1393). To a stirred solution of compound 100 (33 mg, ~0.08 mmol) in dry THF (3 mL) at 0 °C under Ar(g) was dropwise added HMDSLi (1.0 M in THF, 198 µL, 0.20 mmol). After 25 min, the solution was cooled down to -60 °C and Mel (99 µL, 225 mg, 1.58 mmol) was added. The solution temperature was allowed to slowly rise from -60 to 0 °C over 40 min. The reaction was quenched with aqueous NH₄Cl, extracted with EtOAc (4x), dried (MgSO₄), filtered and then evaporated. The crude compound was purified on silica gel using 1:9 EtOAc/Hexanes as eluent to give EM-1393 (8 mg, 19% yield from compound 96, 5 steps) as a solid. IR (v) 2924, 2871, 2832, 1722 (CO), 1620, 1586, 1513, 1454, 1384, 1357, 1306, 1290, 1268, 1201, 1149, 1133, 1116, 1032, 1018, 931, 874, 810, 789, 722 cm⁻¹; ¹H NMR (CDCl₃) δ 1.02 (3H, s), 1.28(0) (3H, s), 1.28(3) (3H, s), 1.29-2.30 (17H, m), 2.80 (2H, br s), 3.45 (3H, s), 3.75 (2H, t, J=4.8 Hz), 4.15 (2H, dd, J=5.0 Hz, J=4.6 Hz), 6.69 (1H, d, J=8.6 Hz), 6.98 (1H, d, J=15.2 Hz); ¹³C NMR (CDCl₃) δ 14.4, 23.3, 25.6, 26.1, 27.4, 27.6, 27.8, 29.1, 29.7, 31.6, 34.9, 37.8, 38.8, 43.6, 47.3, 48.6, 59.2, 69.2, 71.0, 93.6, 113.1 (d, J=17.7 Hz), 116.1, 132.1, 133.4, 144.4, 151.1 (d, J=248.6 Hz), 177.8.
Example 25

Synthesis of 3-methylthioethyloxy-1,3,5(10)-estratrien-5-lactone

These syntheses are described in Scheme 22.

Scheme 22

\[
\text{EM-919} \xrightarrow{a} \text{CS-242}
\]

\text{a. K}_2\text{CO}_3, \text{ClCH}_2\text{CH}_2\text{SCH}_3, \text{DMF, reflux}
Example 25A
3-Methylthioethyloxy-1,3,5(10)-estratrien-17(R)-spiro-2'-(6'-oxo)
tetrahydropyran (CS-242). To a solution of 1,3,5(10)-estratrien-3-ol-17(R)-
spiro-2'(6'-oxo)tetrahydropyran (EM-919, 40 mg, 0.117 mmol) in
anhydrous acetonitrile (20 mL) at room temperature under Ar(g) was
added K₂CO₃ (16 mg, 0.117 mmol) and chloroethylmethyl sulfide (39 mg,
35 mL, 0.350 mmol). The solution was refluxing for 44 h. The acetonitrile
solution was evaporated to dryness and ethyl acetate (40 mL) was then
added. The organic phase was washed with water, brine and dried with
MgSO₄ to give crude product (49 mg). Purification by flash
chromatography on silica gel (SiO₂, 3 g) using ethyl acetate/hexanes (2:8)
as eluent gave desired product (35 mg, 70%). IR (v) 2921, 1727, 1608, 1576,
1499, 1466, 1438, 1382, 1348, 1330, 1310, 1280, 1236, 1187, 1159, 1100, 1067,
1036, 992, 931, 914, 860, 818, 731 cm⁻¹; ¹H NMR(CDCl₃) δ 1.01 (3H, s), 1.25-
2.57 (19H, m), 2.20 (3H, s) 2.85 (4H, t, J=6.88 Hz), 4.12 (2H, t, J=6.74 Hz),
6.62 (1H, d, J=2.49 Hz), 6.71 (1H, dd, J1=2.58 Hz J2=5.89 Hz), 7.19 (1H, d,
J=8.54 Hz); ¹³C NMR(CDCl₃) δ 14.8, 15.9, 16.2, 23.5, 26.1, 27.5, 28.0, 29.5,
29.7, 32.0, 33.1, 34.0, 39.1, 43.7, 47.3, 48.9, 67.4, 93.3, 112.1, 114.6, 126.3,
132.6, 137.9, 156.5, 172.1 ppm. HRMS: FAB M/S [M]+ calculated for
C₂₈H₃₅O₃S: 415.23068, found 415.23250.
Example 26

Synthesis of 3-fluoro-1,3,5(10) estratrien-17-spiro(dimethyl-δ-lactone) derivatives

These syntheses are described in Scheme 22.

Scheme 22

\[ \text{Scheme 22} \]

\[ \text{EM-1157} \]

\[ \text{EM-1170} \]

\[ \text{101} \]

\[ \text{102} \]

\[ \text{103} \]

\[ \text{104} \]

\[ \text{105} \]

\[ \text{EM-1170} \]

- a. BF₃·OEt₂, glcONHO, CH₂Cl₂ then b. HOCO(CH₂)₃OTHP, MeLi
- b. H₂, Pd/C, MeOH
- c. H₂, Na/C, MeOH
- d. PTSA, MeOH
- e. benzoyl acetate f. LDA, MeI
Example 26A
2-Amino-1,3,5(10)-estratriene (101). The title compound was synthesized from estrone according to the procedure reported by Morrow and Hofer (J. Med. Chem. 9, 249-51, 1966).

Example 26B
3-Fluoro-1,3,5(10)-estratriene (102). 3-Fluoroestrone was synthesized from 2-aminoestrone by adapting the procedure reported by Doyle and Bryker (J. Org. Chem. 44, 1572-1574, 1979) for the synthesis of arenediazonium tetrafluoroborate salts from aromatic amines. The procedure is as follows: To neat stirred boron trifluoride etherate (642 µL, 719 mg, 5.07 mmol) at -15 °C under Ar (g) was added a solution of 3-aminoestrone (101) (910 mg, 3.38 mmol) in dry CH₂Cl₂ (10 mL). After 15 min, a solution of t-butyl nitrite (482 µL, 418 mg, 4.05 mmol) in dry CH₂Cl₂ (5 mL) was dropwise added. The now dark brown solution was stirred at -15 °C for 15 min, then at 0 °C for 30 min. Pentane was added to the solution and a gummy solid precipitated. The solvent was decanted and the residue was dried under vacuum to give a crude light brown solid. The neat solid was heated under vacuum at 70-80 °C in an oil bath for 15 min to give a crude orange solid. Purification by flash chromatography on SiO₂ using 1:9 EtOAc/Hexanes gave 3-fluoroestrone (102) as a white solid (437 mg, 47 %). M.p. 178 °C; [α]D25 +152.0 ° (c 1.03, CDCl₃); IR (ν) 3044, 3039, 2928, 1740 (CO), 1611, 1585, 1495, 1474, 1458, 1428, 1405, 1377, 1340, 1272, 1245, 1230, 1212, 1148, 1094, 1084, 1053, 1008, 908, 889, 817, 784, 718, 704, 642, 620, 580, 564, 467 cm⁻¹; ¹H NMR (CDCl₃) δ 0.92 (3H, s), 1.37-1.70 (6H, m), 1.93-2.44 (6H, m), 2.52 (1H, dd, J=18.8 Hz, J=9.0 Hz), 2.91 (2H, d, J=13.8 Hz), 3.8 (3H, q, J=7.8 Hz), 6.82 (2H, d, J=8.3 Hz, J=2.8 Hz), 7.23 (1H, dd, J=8.5 Hz, J=5.8 Hz); ¹³C NMR (CDCl₃) δ 13.8, 21.6, 25.9, 26.3, 29.5, 31.5, 35.9, 38.1, 44.0, 47.9, 50.4, 112.5 (d, J=21.6 Hz), 115.1 (d, J=19.7 Hz), 126.8 (d, J=7.5 Hz), 135.3, 138.7, 161.0 (d, J=244.3 Hz), 220.7.

Example 26C
3-Fluoro-17β-hydroxy-17α-(4'-(2''-tetrahydro-2''H-pyranlyoxy-1''-butynyl)-1,3,5(10)-estratriene (103). To a stirred solution of 2-(3-butynlyoxy)tetrahydro-2H-pyran (1.21 mL, 1.199 g, 7.77 mmol) in dry THF (45 mL) at -30 °C under Ar (g) was dropwise added MeLi (1.4 M in Et₂O, 5.39 mL, 7.55 mmol). After 30 min., a solution of 3-fluoroestrone (102) in dry THF was added and the solution was stirred for a further 2 h. The solution was quenched with ice and aqueous sat NaHCO₃ and extracted with EtOAc. The organic layer was washed with H₂O, brine, dried (MgSO₄), filtered then rotary evaporated to give a crude orange oil. Purification by flash chromatography on silica gel using EtOAc/Hexanes (1:9 --> 2:8 --> 3:7) gave pure compound 103 (789 mg, 83 %) as a solid.
[α]_{D}^{25} = -4.7 ° (c 1.14, CDCl₃); IR (ν) 3432 (br, OH), 2937, 2870, 1611, 1589, 1495, 1458, 1438, 1420, 1380, 1354, 1285, 1233, 1201, 1183, 1122, 1073, 1032, 970, 911, 870, 846, 815, 783, 728, 692, 563, 466 cm⁻¹; ¹H NMR (CDCl₃) δ 0.87 (3H, s), 1.25-2.05 (17H, m), 2.22-2.36 (3H, m), 2.56 (2H, t, J=7.0 Hz), 2.84 (2H, br t, J=4.8 Hz), 3.49-3.59 (2H, m), 3.79-3.88 (2H, m), 4.66 (1H, br s), 6.80 (2H, dq, J=9.8 Hz, J=2.8 Hz), 7.24 (1H, dd, J=8.4 Hz, J=5.8 Hz); ¹³C NMR (CDCl₃) δ 12.7, 19.1, 20.2, 22.7, 25.3, 26.3, 26.9, 29.5, 30.4, 32.7, 38.9, 39.1, 43.5, 46.9, 49.3, 61.8, 65.7, 79.6, 82.9, 84.6, 98.4, 112.1 (d, J=20.9 Hz), 114.9 (d, J=20.6 Hz), 126.6 (d, J=8.5 Hz), 135.7, 138.7 (d, J=6.7 Hz), 160.7 (d, J=244.8 Hz).

**Example 26D**

3-Fluoro-17β-hydroxyl-17α-(4'-2''-tetrahydro-2''H-pyranloxy-1'-butyl)-1,3,5(10)-estratriene. A suspension of compound 103 (720 mg, 1.69 mmol) and 5% Pd/CaCO₃ (123 mg) in MeOH (40 mL) was stirred under H₂(g) atmosphere (balloon) at room temperature for 2h. The mixture was filtered through celite and the solvent rotary evaporated to give compound 104 (692 mg, 95%) as a solid. This compound was used without further purification for the next step. [α]_{D}^{25} = +28.8 ° (c 0.59, CDCl₃); IR (ν) 3464 (br w, OH), 2938, 2870, 1611, 1588, 1494, 1453, 1440, 1380, 1271, 1252, 1234, 1200, 1142, 1119, 1076, 1024, 989, 971, 930, 910, 868, 815, 782, 731 cm⁻¹; ¹H NMR (CDCl₃) δ 0.91 (3H, s), 1.31-2.35 (20H, m), 2.84 (2H, br t, J=4.8 Hz), 3.43-3.52 (2H, m), 3.78-3.88 (2H, m), 6.80 (2H, dq, J=8.3 Hz, J=2.7 Hz). 7.23 (1H, dd, J=8.4 Hz, J=6.1 Hz) ppm; ¹³C NMR (CDCl₃) δ 14.4, 19.7, 20.5, 23.4, 25.5, 26.3, 27.3, 29.7, 30.4, 30.8, 31.6, 34.3, 36.4, 39.2, 39.4, 43.8, 46.7, 62.4, 67.7, 83.4, 98.4 (d, J=4.5 Hz), 112.3 (d, J=21.0 Hz), 115.0 (d, J=20.3 Hz), 126.7 (d, J=8.1 Hz), 136.0, 138.9 (d, J=6.6 Hz), 169.9 (d, J=244.1 Hz).

**Example 26E**

3-Fluoro-17β-hydroxyl-17α-(4'-hydroxybutyl)-1,3,5(10)-estratriene (105). A solution of compound 104 (692 mg, 1.61 mmol), PTSA monohydrate (31 mg, 0.16 mmol) in MeOH was stirred overnight at room temperature. The volume of solvent was reduced to about 10 mL and EtOAc (125 mL) was added. This solution was washed with aqueous saturated NaHCO₃, H₂O, brine, dried (MgSO₄), filtered then rotary evaporated to give a crude solid. Purification on silica gel (3:7:1 EtOAc/Hexanes) gave compound 105 (469 mg, 84%) as a solid. [α]_{D}^{25} = +42.6 ° (c 1.07, CDCl₃); IR (ν) 3362 (br s, OH), 2933, 2866, 1740 (w), 1611, 1589, 1495, 1458, 1420, 1376, 1303, 1271, 1236, 1144, 1036, 1008, 931, 912, 870, 816, 783, 728, 562, 468 cm⁻¹; ¹H NMR (CDCl₃) δ 0.91 (3H, s), 1.25-1.65 (17H, m), 1.85-2.35 (4H, m), 2.85 (2H, br t, J=4.6 Hz), 3.70 (2H, br t, J=5.9 Hz), 6.81(2H, dq, J=8.4 Hz,
Example 26F

3-Fluoro-1,3,5(10)-estratrien-17(R)-spiro-2'-6'-oxo)tetrahydropyran (EM-1170). To a stirred solution of compound 105 (410 mg, 1.18 mmol) in acetone (40 mL) at 0°C was added Jones' reagent (1.25 M, 1.04 mL, 1.30 mmol). The solution was stirred for 15 min. then more Jones' reagent (1.25 M, 1.04 mL, 1.30 mmol) was added. After 50 min., the excess reagent was quenched with isopropanol and the solution was stirred for a further 10 min. The precipitate was filtered through celite and the acetone rotary evaporated. The residue was taken in EtOAc and the solution was washed with aqueous saturated NaHCO₃, H₂O, brine, dried (MgSO₄), filtered, then rotary evaporated to give a crude solid. Purification on silica gel (2:8 → 3:7 EtOAc/Hexanes) gave EM-1170 (298 mg, 72 %) as a solid. [α]D²⁰ +47.0° (c 1.09, CDCl₃); IR (v) 3435, 3040, 2955, 2933, 2906, 2879, 2836, 2815, 1885, 1736 (CO), 1611, 1586, 1494, 1467, 1435, 1419, 1383, 1366, 1356, 1328, 1312, 1290, 1263, 1232, 1182, 1140, 1103, 1067, 1026, 990, 964, 933, 912, 861, 822, 782, 718, 670, 636, 584, 563, 536, 508 cm⁻¹; ¹H NMR (CDCl₃) δ 1.02 (3H, s), 1.23-2.58 (19H, m), 2.85 (2H, br t, J=5.2 Hz), 6.80 (2H, dq, J=8.4 Hz, J’=2.7 Hz), 7.23 (1H, dd, J=8.4 Hz, J’=6.0 Hz); ¹³C NMR (CDCl₃) δ 14.2, 15.7, 23.4, 25.9, 27.1, 27.8, 29.4, 31.8, 33.8, 38.7, 43.5, 47.1, 48.7, 93.1, 112.2 (d, J=20.5 Hz), 114.9 (d, J=20.1 Hz), 126.6 (d, J=8.5 Hz), 135.4, 138.6 (d, J=6.7 Hz), 160.8 (d, J=243.8 Hz), 171.9.

Example 26G

3-Fluoro-1,3,5(10)-estratrien-17(R)-spiro-2-(5',5'-dimethyl-6'-oxo)tetrahydropyran (EM-1157). LDA was prepared as follows. To a stirred solution of disopropylamine (501 µL, 386 mg, 3.82 mmol) in dry THF (20 mL) at -78 °C under Ar(g) was dropwise added n-BuLi (1.6 M in Hexane, 2.38 mL, 3.82 mmol). The solution was stirred at room temperature for 30 min then was cooled down to -78 °C for the addition of a solution of EM-1170 (218 mg, 0.64 mmol) in dry THF (15 mL). The solution was then stirred at room temperature for 1h. then cooled down to -78 °C. Mel (476 µL, 1.084g, 0.64 mmol) was added and the solution was stirred at -78 °C for 5 h and was allowed to rise to room temperature overnight. The reaction was quenched with ice/H₂O, extracted with EtOAc, washed with aqueous saturated NH₄Cl, 1M aqueous Na₂SO₃, H₂O, brine, dried (MgSO₄), filtered then rotary evaporated to give a crude solid. Purification on silica gel (1.9 EtOAc/Hexanes) gave EM-1157 (40 mg, 17 %) as a solid. [α]D²⁰ +43.3° (c 1.09, CHCl₃); IR (v) 3430, 2969, 2941,
DEMANDES OU BREVETS VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D’UN TOME.

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JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME _ | OF _

NOTE: For additional volumes please contact the Canadian Patent Office
What is claimed is:

1. A method of inhibiting activity of type 5 17β-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

![Chemical Structure]

wherein the dotted line is optional pi bond;

wherein A is selected from the group consisting of straight or branched C₁₋₅ alkyl, -ORₖ (Rₖ being a C₁₋₅ alkyl radical), and -N(R₆)R₇ (R₆ and R₇ being independently hydrogen or C₁₋₅ alkyl or aryl), and unsaturated analogs of any of the foregoing definitions for substituent A;

wherein R¹ is selected from the group consisting of hydrogen and methyl and ethyl;

wherein R₆ is selected from the group consisting of hydrogen, and halogen, and C₁₋₅ alkyl;

wherein R₇ is selected from the group consisting of straight or branched C₁₋₅ alkyne, C₂₋₇ cycloalkylene; and

R₈ is selected from the group consisting of hydrogen, substituted or
unsubstituted phenyl, C₂-C₁₀ acyl, C₂-C₁₀ acyloxy, C₂-C₁₀ alkoxy carbonyl, and halogen.

2. The method of Claim 1 wherein the optional pi bond at 6 is present.

3. The method of Claim 1 wherein R⁴ is methyl.

4. The method of Claim 1 wherein R⁴ is C₁-C₆ alkylene.

5. The method of Claim 1 where A is either methyl or -N(R²)R⁴.

6. The method of Claim 1 where A is -N(R²)R⁴.

7. The method of Claim 6 wherein R⁴ is methyl.

8. The method of Claim 6 wherein R⁴ is C₁-C₆ alkyl or C₇-C₁₂ phenyl alkyl.

9. A method of inhibiting activity of type 5 17β-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:
wherein the dotted line is an optional pi bond;

wherein R^{16b} is selected from the group consisting of hydrogen, C_1-C_4 alkyl, fluoro, chloro, C_1-C_4 haloalkyl, a moiety which together with R^{16a} is

C_4-C_spirocycloalkyl, C_7-C_spirocycloalkyl, or \_\_\_\_\_\underscore R^{16} (R^{16} being C_1-C_3 alkyl) and unsaturated analogs of any of the foregoing definitions of R^{16b};

wherein R^{16a} is selected from the group consisting of hydrogen, C_1-C_4 alkyl, C_1-C_spirocycloalkyl, C_7-C_spirocycloalkyl, or \_\_\_\_\_\underscore R^{16} (R^{16} being C_1-C_spirocycloalkyl, C_7-C_spirocycloalkyl, or \_\_\_\_\_\underscore R^{16} (R^{16} being C_1-C_3 alkyl) and unsaturated analogs of any of the foregoing;

wherein R^{15a} is selected from the group consisting of hydrogen, C_1-C_4 alkyl, C_1-C_4 alkenyl and C_1-C_4 alkynyl;

wherein R^{15} is either 'H or 'CH_3; and

wherein R^6 is selected from the group consisting of 'H, 'CH_3, and halo; provided that R^{14b}, R^{16a}, and R^{15a} are not simultaneously hydrogen.
10. The method of Claim 9 wherein \( R^{16\alpha} \) is a larger substituent than \( R^{14\beta} \).

11. The method of Claim 9 wherein \( R^6 \) is hydrogen.

12. The method of Claim 9 wherein the optional pi bond at position 1 is not present.

13. The method of Claim 9 wherein \( R^{16\alpha} \) is a \( C_2-C_5 \) alkyl chain.

14. A method of inhibiting activity of type 5 \( 17\beta \)-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 \( 17\beta \)-hydroxysteroid dehydrogenase having the molecular structure:

\[
\begin{align*}
\text{wherein the dotted line is optional pi bond} \\
\text{wherein } X \text{ is } C_1-C_9 \text{ alkyl;} \\
\text{wherein } Y \text{ is hydrogen or an acyloxy moiety;} \\
\text{wherein } R^6 \text{ is } \cdot H \text{ or } \cdot CH_3; \\
\text{wherein } R^{16} \text{ is } \cdot H \text{ or halo;}
\end{align*}
\]
wherein $R^1$ is $'H$ or $'CH_3$.

15. The method of Claim 14 wherein $R^4$ is methyl.

16. The method of Claim 14 wherein the optional pi bond at position 1 is present.

17. The method of Claim 14 wherein $Y$ is a $C_2-C_6$ fluoroacyloxy.

18. The method of Claim 14 wherein $X$ is methyl.

19. A method of inhibiting activity of type 5 17β-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

wherein $n$ is an integer from 1-2;

wherein the dotted lines are optional double bonds;
wherein X and Y are independently selected from the group consisting of \( \cdot H \), \((C_1-C_9)\)alkyl, \((C_2-C_7)\) alkenyl, and methoxycarbonyl;

wherein Z is selected from the group consisting of \(-\cdot H\) and \((C_1-C_9)\)alkyl;

wherein \( R^2 \) is selected from the group consisting of hydrogen, acyl, carboxyl, alkoxy carbonyl, substituted or unsubstituted carboxamide, cyano, alkoxy, alkoxyalkoxy, alkylthioalkoxy, acyloxy; hydroxy, halo, -O-SO\(_2\)R\(^8\) (R\(^8\) being selected from the group consisting of \(C_1-C_9\) alkyl and \(C_6-C_{10}\) aryl), and a moiety which, together with \( R^2 \), is a 5-6 member ring containing at least one oxygen and one nitrogen atom;

wherein \( R^2 \) is selected from the group consisting of hydrogen, amido, acyloxy, carboxyl, carboxamide, alkoxy carbonyl, cyano, halo, nitro, \(C_1-C_9\) alkyl, and \(CF_3\) and a moiety which, together with \( R^3 \), is a 5-6 member ring containing at least one oxygen and one nitrogen atom;

wherein \( R^4 \) is hydrogen or halo;

wherein \( R^4 \) is selected from the group consisting of hydrogen and oxo;

wherein \( R^9 \) is \'H or \'OH,

provided that X, Y, and Z are not all hydrogen when \( R^3 \) is methoxy.

20. The method of Claim 19 wherein \( R^3 \) is alkoxyalkoxy.

21. The method of Claim 19 wherein \( R^3 \) is carboxyl or alkoxyl;

22. The method of Claim 19 wherein at least one of X, Y or Z is methyl.

23. The method of Claim 19 wherein \( R^3 \) is carboxamide.

24. The method of Claim 19 wherein both X and Y are methyl.
25. The method of Claim 19 wherein \( n = 1 \) or 2.

26. The method of Claim 19 wherein \( R^6 \) is oxo.

27. A method of inhibiting activity of type 5 17\(^\beta\)-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 17\(^\beta\)-hydroxysteroid dehydrogenase having the molecular structure:

![Molecular Structure](image)

wherein the dotted lines are optional pi bonds;
wherein \( n = 1 \) or 2; and
wherein \( a \) is either \(^1\text{H} \) or \(^1\text{CH}_3\);
wherein \( b \) and \( c \) are independently hydrogen or methyl;
wherein \( Z \) is oxygen or sulfur.

28. The pharmaceutical composition of Claim 27 wherein \( n = 1 \).
29. The method of Claim 27 wherein at least one of b or c is methyl.

30. The method of Claim 27 wherein both b and c are methyl.

31. The method of Claim 27 wherein Z is oxygen.

32. The method of claim 1, wherein the inhibitor of type 5 17β-hydroxysteroid dehydrogenase selected from the group consisting of:

EM-1291

\[ \text{EM-1291} \]

EM-1195-CS

\[ \text{EM-1195-CS} \]
EM-1181

EM-1159 and

EM-1165

AMENDED SHEET
33. The method of Claim 32 wherein an inhibitor of 17β-hydroxysteroid dehydrogenase selected from the group consisting of:

EM-1404

and

EM-1394

is administered.
34. The method of claim 1, wherein the inhibitor of type 5 17β-hydroxysteroid dehydrogenase selected from the group consisting of:

- Medroxyprogesterone acetate
- Megestrol acetate
- Chlormadinone acetate
- 1-dehydromegestrol acetate
- Melengestrol acetate
- Nomegestrol acetate
and compounds having the molecular structure:

wherein the dotted lines are optional pi bonds;

wherein A is selected from the group consisting of straight or branched C₁₋₄ alkyl, -OR⁺ (R⁺ being a C₁₋₄ alkyl radical), and -N(R⁺)R⁺ (R⁺ and R⁺ being independently hydrogen or C₁₋₄ alkyl or aryl), and unsaturated analogs of any of the foregoing definitions for substituent A;

wherein R¹ is selected from the group consisting of hydrogens, and methyl;

wherein R⁴ is selected from the group consisting of hydrogen, halogen, and C₁₋₄ alkyl;

wherein R₁₆ is selected from the group consisting of H, H and CH₂;

wherein R⁴ is selected from the group consisting of straight or branched C₁₋₄ alkyne, C₂₋₅ cycloalkylene; and R⁴ is selected from the group consisting of
of hydrogen, substituted or unsubstituted phenyl, C_{2-10} acyl, C_{2-10} acyloxy, C_{2-10} alkoxy carbonyl, and halogen.

35. A method of inhibiting activity of type 5 17β-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

![Molecular Structure Diagram]

wherein the dotted line is an optional pi bond;

wherein R^{16α} is selected from the group consisting of hydrogen, C_{1-6} alkyl fluoro, chloro, C_{1-6} haloalkyl, a moiety which together with R^{16α} forms C_{4-7} spirocycloalkyl, C_{4-7} halospiro cycloalkyl, or \( \text{---R}^{16} \) (\( R^{16} \) being C_{1-6} alkyl) and unsaturated analogs of any of the foregoing definitions of R^{16α};

wherein R^{16α} is selected from the group consisting of hydrogen, C_{1-6} alkyl, C_{1-6} haloalkyl, a moiety which together with R^{16α} forms C_{4-7} spirocycloalkyl C_{4-7} halospiro cycloalkyl, or \( \text{---R}^{16} \) (\( R^{16} \) being C_{1-6} alkyl) and unsaturated analogs of any of the foregoing;
wherein R₁⁵α is selected from the group consisting of hydrogen, C₁-C₄ alkyl, C₁-C₄ alkenyl and C₁-C₄ alkynyl;

wherein R¹⁹ is either 'H or 'CH₃; and

wherein R⁶ is selected from the group consisting of 'H, 'CH₃, and halo;

provided that R¹⁸α, R₁⁵α, and R₁⁵α are not simultaneously hydrogen.

36. A method of inhibiting activity of type 5 17β-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

![Molecular Structure](image)

wherein n = 1 or 2;

wherein the dotted lines are optional pi bonds;

wherein X and Y are independently selected from the group consisting of 'H, (C₁-C₃)alkyl, (C₁-C₃) alkenyl, and methoxycarbonyl;

wherein Z is selected from the group consisting of -H and (C₁-C₃)alkyl;

wherein R¹ is selected from the group consisting of acyl, carboxyl, alkoxy, alkoxy carbonyl, substituted or unsubstituted carboxamide, cyano, alkoxy,
alkoxyalkoxy, alkythioalkoxy, acyloxy, hydroxy, halo, -O-SO₂R¹ (R¹ being selected from the group consisting of C₁-C₆ alkyl and C₆-C₁₀ aryl), and a moiety which, together with R₂, is a 5-6 member ring containing at least one oxygen and one nitrogen atom;

wherein R² is selected from the group consisting of hydrogen, amido, acyloxy, carboxyl, carboxamide, alkoxy carbonyl, cyano, halo, nitro, C₁-C₄ alkyl and CF₃ and a moiety which, together with R₃, is a 5-6 member ring containing at least one oxygen and one nitrogen atom;

wherein R⁴ is hydrogen or halo;

wherein R⁵ is selected from the group consisting of hydrogen and oxo;

wherein R⁶ is 'H or 'OH;

provided that X, Y and Z are not all hydrogen.

37. A method of inhibiting activity of type 5 17β-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:
wherein the dotted lines are optional pi bonds;
wherein n = 1 or 2; and
wherein a is either 'H or 'CH₃;
wherein b and c are independently hydrogen or methyl;
wherein Z is oxygen or sulfur.

38. A method of inhibiting activity of type 5 17β-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

![Molecular Structure Diagram]

wherein the dotted line is an optional pi bond;
wherein A is selected from the group consisting of straight or branched C₁-C₄ alkyl, -OR' (R' being a C₁-C₄ alkyl radical), and -N(R⁴)R⁵ (R⁴ and R⁵ being independently hydrogen or C₁-C₄ alkyl or aryl), and unsaturated analogs of any of the foregoing definitions for substituent A;
wherein R¹ is selected from the group consisting of hydrogen and methyl;
wherein R⁴ is selected from the group consisting of hydrogen, halogen, and C₁-C₄ alkyl;
wherein \( R^1 \) is selected from the group consisting of straight or branched \( C_1-C_9 \) alkylene, \( C_1-C_9 \) cycloalkylene; and

\( R^b \) is selected from the group consisting of hydrogen, substituted or unsubstituted phenyl, \( C_1-C_{16} \) acyl, \( C_1-C_{16} \) acyloxy, \( C_1-C_{16} \) alkoxy carbonyl, and halogen;

provided that wherein \( A \) is methyl, \( R^a \) and \( R^b \) together have at least two carbon atoms and \( R^1 \) is methyl.

39. A method of inhibiting activity of type 5 17\( \beta \)-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 17\( \beta \)-hydroxysteroid dehydrogenase having the molecular structure:

![Chemical Structure](image)

wherein the dotted lines are optional pi bonds

wherein \( X \) is \( C_1-C_5 \) alkyl;

wherein \( Y \) is hydrogen or an acyloxy moiety;

wherein \( R^6 \) is 'H or 'CH\(_2\);

wherein \( R^{16} \) is 'H or halo;

wherein \( R^1 \) is 'H or 'CH\(_3\).
40. A pharmaceutical composition comprising a pharmaceutically acceptable excipient, diluent or carrier and a therapeutically acceptable amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

wherein the dotted lines are optional pi bonds;

wherein A is selected from the group consisting of straight or branched C₁-C₄ alkyl, -N(R⁴)R⁵ (R⁴ and R⁵ being independently hydrogen or C₁-C₄ alkyl or aryl), and unsaturated analogs of any of the foregoing definitions for substituent A;

wherein R¹ is selected from the group consisting of hydrogen and methyl;

wherein R⁰ is selected from the group consisting of hydrogen, halogen, and C₁-C₄ alkyl;

wherein R⁶ is selected from the group consisting of straight or branched C₁-C₄ alkyne, C₂-C₇ cycloalkylene; and

R⁸ is selected from the group consisting of hydrogen, substituted or unsubstituted phenyl, C₁-C₁₀ acyl, C₂-C₁₀ acyloxy, C₁-C₁₀ alkoxy carbonyl, and halogen;

provided that when A is methyl, R⁶ and R⁸ together have at least two carbon atoms, and R¹ is methyl.
41. The pharmaceutical composition of Claim 40 wherein the optional pi bond at 6 is present.

42. The method of Claim 40 wherein R^6 is methyl.

43. The pharmaceutical composition of Claim 40 wherein R^8 is C_1-C_6 alkylene.

44. The pharmaceutical composition of Claim 40 where A is either methyl or -N(R^4)R^4.

45. The pharmaceutical composition of Claim 40 where A is -N(R^4)R^4.

46. The pharmaceutical composition of Claim 45 wherein R^d is methyl.

47. The pharmaceutical composition of Claim 45 wherein R^e is C_1-C_6 alkyl or phenyl C_1-C_6 alkyl.

48. A pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and a therapeutically acceptable amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:
wherein the dotted line is an optional pi bond;

wherein $R^{16b}$ is selected from the group consisting of hydrogen, $C_1-C_4$ alkyl, fluoro, chloro, $C_1-C_4$ haloalkyl, a moiety which together with $R^{16a}$ is $C_4-C_7$ spirocycloalkyl, $C_4-C_7$ halospirocycloalkyl, or $R^{16b}$ (R'16 being $C_1-C_3$ alkyl) and unsaturated analogs of any of the foregoing definitions of $R^{16b}$;

wherein $R^{16a}$ is selected from the group consisting of hydrogen, $C_1-C_4$ alkyl, $C_1-C_4$ haloalkyl, a moiety which together with $R^{16b}$ forms $C_4-C_7$ spirocycloalkyl, $C_4-C_7$ halospirocycloalkyl, or $R^{16a}$ (R'16 being $C_1-C_3$ alkyl) and unsaturated analogs of any of the foregoings;

wherein $R^{15a}$ is selected from the group consisting of hydrogen, $C_1-C_4$ alkyl, $C_1-C_4$ alkenyl and $C_1-C_4$ alkynyl;

wherein $R^{19}$ is either 'H or 'CH$_3$; and

wherein $R^{6}$ is selected from the group consisting of 'H, 'CH$_3$, and halo; provided that $R^{16b}$, $R^{16a}$, and $R^{15a}$ are not simultaneously hydrogen.
49. The pharmaceutical composition of Claim 48 wherein $R^{16\alpha}$ is a large substituent than $R^{16\beta}$.

50. The pharmaceutical composition of Claim 48 wherein $R^6$ is hydrogen.

51. The pharmaceutical composition of Claim 48 wherein the optional pi bond at position 1 is not present.

52. The pharmaceutical composition of Claim 48 wherein $R^{16\alpha}$ is a $C_3-C_5$ alkyl chain.

53. A pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and a therapeutically acceptable amount of an inhibitor of type 5 17$\beta$-hydroxysteroid dehydrogenase having the molecular structure:

![Chemical Structure](image)

wherein the dotted line is an optional pi bond

wherein $X$ is $C_1$-$C_3$ alkyl;

wherein $Y$ is hydrogen or an acyloxy moiety;
wherein R^6 is 'H or 'CH₃;
wherein R^1₅ is 'H or halo;
wherein R^1 is 'H or 'CH₃.

54. The pharmaceutical composition of Claim 53 wherein R^6 is methyl.

55. The pharmaceutical composition of Claim 53 wherein the optional pi bond at position 1 is present.

56. The pharmaceutical composition of Claim 53 wherein Y is a C₃-C₆ fluoroacyloxy.

57. The pharmaceutical composition of Claim 53 wherein X is methyl.

58. A pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and a therapeutically acceptable amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:
wherein \( n \) is an integer from 1-2;
wherein the dotted lines are optional pi bonds;
wherein \( X \) and \( Y \) are independently selected from the group consisting of \( H, (C_1-C_3) \text{alkyl,} (C_2-C_3) \text{alkenyl,} \) and methoxycarbonyl;

wherein \( Z \) is selected from the group consisting of \( -H \) and \((C_1-C_3) \text{alkyl;}
wherein \( R^4 \) is selected from the group consisting of hydrogen, acyl, carboxyl, alkoxy carbonyl, substituted or unsubstituted carboxamide, cyano, alkoxy, alkoxyalkoxy, alkylthioalkoxy, acyloxy; hydroxy, halo, \(-O-SO_2R^8 \) (\( R^8 \) being selected from the group consisting of \( C_1-C_6 \) alkyl and \( C_6-C_{10} \) aryl), and

a moiety which, together with \( R^7 \), is a 5-6 member ring containing at least one oxygen and one nitrogen atom;

wherein \( R^2 \) is selected from the group consisting of hydrogen, amido, acyloxy, carboxyl, carboxamide, alkoxy carbonyl, cyano, halo, nitro, \( C_1-C_6 \) alkyl, and \( CF_3 \) and a moiety which, together with \( R^7 \), is a 5-6 member ring containing at least one oxygen and one nitrogen atom;

wherein \( R^4 \) is hydrogen or halo;

wherein \( R^8 \) is selected from the group consisting of hydrogen and oxo;
wherein $R^3$ is $'H$ or $'OH$,

provided that $X$, $Y$, and $Z$ are not all hydrogen.

59. The pharmaceutical composition of Claim 58 wherein $R^3$ is alkoxyalkoxy.

60. The pharmaceutical composition of Claim 58 wherein $R^3$ is carboxyl or alkoxyl;

61. The pharmaceutical composition of Claim 58 wherein at least one of $X$, $Y$ or $Z$ is methyl.

62. The pharmaceutical composition of Claim 58 wherein both $X$ and $Y$ are methyl.

63. The pharmaceutical composition of Claim 58 wherein $n = 1$ or 2.

64. The pharmaceutical composition of Claim 58 wherein $R^4$ is oxo.

65. The pharmaceutical composition of Claim 58 wherein $R^3$ is carboxamide.

66. A pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and a therapeutically acceptable amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:
wherein the dotted lines are optional pi bonds;
wherein $n = 1$ or $2$; and
wherein $a$ is either $\text{H}$ or $\text{CH}_3$;
wherein $b$ and $c$ are independently hydrogen or methyl;
wherein $Z$ is oxygen or sulfur.

67. The pharmaceutical composition of Claim 66 wherein $n = 1$.

68. The pharmaceutical composition of Claim 66 wherein at least one of $b$ or $c$ is methyl.

69. The pharmaceutical composition of Claim 66 wherein both $b$ and $c$ are methyl.

70. The pharmaceutical composition of Claim 66 wherein $Z$ is oxygen.
71. The pharmaceutical composition of claim 40, wherein the inhibitor of type 5 17β-hydroxysteroid dehydrogenase has a molecular structure selected from the group consisting of:

EM-1291

EM-1195-CS

CS-243
EM-1181

EM-1159 and

EM-1165
72. The pharmaceutical composition of Claim 71 wherein said inhibitor of 17β-hydroxysteroid dehydrogenase is selected from the group consisting of:

![Chemical Structure Image]

EM-1404 and

![Another Chemical Structure Image]

and EM-1394

73. A pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and a therapeutically acceptable amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:
wherein the dotted lines are optional pi bonds
wherein X is C₁-C₃ alkyl;
wherein Y is hydrogen or an acyloxy moiety;
wherein R₅ is 'H or 'CH₃;
wherein R¹₆ is 'H or halo;
wherein R¹ is 'H or 'CH₃.

74. An inhibitor of type 5 17β-hydroxysteroid dehydrogenase
having the molecular structure:

wherein the dotted line is optional pi bond;
wherein A is selected from the group consisting of straight or branched C₆₋C₁₄ alkyl, -OR (R being a C₁₋C₄ alkyl radical), and -N(R₄)R₅ (R₄ and R₅ being independently hydrogen or C₁₋C₄ alkyl or aryl), and unsaturated analogs of any of the foregoing definitions for substituent A;

wherein R¹ is selected from the group consisting of hydrogen and methyl;

wherein R₄ is selected from the group consisting of halogen and C₁₋C₄ alkyl;

wherein R₅ is selected from the group consisting of straight or branched C₁₋C₄ alkyne, C₂₋C₇ cycloalkylene; and

R₆ is selected from the group consisting of hydrogen, substituted or unsubstituted phenyl, C₂₋C₁₀ acyl, C₂₋C₁₀ acyloxy, C₁₋C₁₀ alkoxy-carbonyl, and halogen;

provided that when A is methyl, R₄ and R₅ together have at least two carbon atoms, and R¹ is methyl.

75. The inhibitor of Claim 74 wherein the optional pi bond at 6 is present.

76. The inhibitor of Claim 74 wherein R₄ is methyl.

77. The inhibitor of Claim 74 wherein R₄ is C₁₋C₄ alkyne.

78. The inhibitor of Claim 74 where A is either methyl or -N(R₄)R₅.

79. The inhibitor of Claim 74 where A is -N(R₄)R₅.

80. The inhibitor of Claim 79 wherein R₄ is methyl.
81. The inhibitor of Claim 79 wherein R⁴ is C₁⁻C₆ alkyl or phenyl C₁⁻C₆ alkyl.

82. An inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

![Molecular Structure Diagram]

wherein the dotted line is an optional pi bond;
wherein R¹⁶⁸ is selected from the group consisting of hydrogen, C₁⁻C₆ alkyl, fluoro, chloro, C₁⁻C₆ haloalkyl, a moiety which together with R¹⁶α is C₄⁻C₇ spirocycloalkyl, C₄⁻C₇ halospirocyloalkyl, or =R¹⁶⁺ (R¹⁶⁺ being C₁⁻C₆ alkyl) and unsaturated analogs of any of the foregoing definitions for R¹⁶⁺;

wherein R¹⁶α is selected from the group consisting of hydrogen, C₁⁻C₆ alkyl, C₁⁻C₆ haloalkyl, a moiety which together with R¹⁶θ is C₄⁻C₇ spirocycloalkyl, C₄⁻C₇ halospirocyloalkyl, or =R¹⁶⁺ (R¹⁶⁺ being C₁⁻C₆ alkyl) and unsaturated analogs of any of the foregoing;

wherein R¹⁵α is selected from the group consisting of hydrogen, C₁⁻C₆ alkyl, C₁⁻C₆ alkenyl and C₁⁻C₆ alkynyl;
wherein $R^{19}$ is either 'H or 'CH$_3$; and
wherein $R^6$ is selected from the group consisting of 'H, 'CH$_3$, and halo;
provided that $R^{16b}$, $R^{16a}$, and $R^{15a}$ are not simultaneously hydrogen.

83. The inhibitor of Claim 82 wherein $R^{16a}$ is a larger substituent than $R^{16b}$.

84. The inhibitor of Claim 82 wherein $R^6$ is hydrogen.

85. The inhibitor of Claim 82 wherein the optional pi bond at position 1 is not present.

86. The inhibitor of Claim 82 wherein $R^{16a}$ is a C$_2$-C$_3$ alkyl.

87. An inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

wherein the dotted line is optional pi bond
wherein $X$ is C$_1$-C$_2$ alkyl;
wherein $Y$ is hydrogen or an acyloxy moiety;
wherein $R^6$ is 'H or 'CH$_3$;
wherein $R^{16}$ is 'H or halo;
wherein $R^1$ is 'H or 'CH$_3$.

88. The inhibitor of Claim 87 wherein $R^6$ is methyl.

89. The inhibitor of Claim 87 wherein the optional pi bond at position 1 is present.

90. The inhibitor of Claim 87 wherein Y is a C$_2$-C$_6$ fluoroacyloxy.

91. The inhibitor of Claim 87 wherein X is methyl.

92. An inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

![Molecular Structure Diagram]

wherein n is an integer from 1-2;
wherein the dotted lines are optional pi bonds;
wherein X and Y are independently selected from the group consisting of 'H, (C$_1$-C$_5$) alkyl, (C$_2$-C$_4$) alkenyl, and methoxycarbonyl;

AMENDED SHEET
wherein Z is selected from the group consisting of -H and (C₁-C₅)alkyl;
wherein R₁ is selected from the group consisting of hydrogen, acyl,
carboxyl, alkoxy carbonyl, substituted or unsubstituted carboxamide, cyano,
alkoxy, alkoxyalkoxy, alkythioalkoxy, acyloxy; hydroxy, halo, -O-SO₂R⁵ (R⁵
being selected from the group consisting of C₁-C₅ alkyl and C₆-C₁₅ aryl), and
a moiety which, together with R₂, is a 5-6 member ring containing at least one
oxygen and one nitrogen atom;

wherein R₁ is selected from the group consisting of hydrogen, amido,
acyloxy, carboxyl, carboxamide, alkoxy carbonyl, cyano, halo, nitro, C₁-C₅
alkyl, and CF₃ and a moiety which, together with R₂, is a 5-6 member ring
containing at least one oxygen and one nitrogen atom;

wherein R₂ is hydrogen or halo;
wherein R₂ is selected from the group consisting of hydrogen and oxo;
wherein R₉ is 'H or 'OH,

provided that X, Y, and Z are not all hydrogen.

93. The inhibitor of Claim 92 wherein R₁ is alkoxyalkoxy.

94. The inhibitor of Claim 92 wherein R₁ is carboxyl unsubstituted
   carboxamide or alkoxyl;

95. The inhibitor of Claim 92 wherein at least one of X, Y or Z is
   methyl.

96. The inhibitor of Claim 92 wherein both X and Y are methyl.

97. The inhibitor of Claim 92 wherein n = 1.

98. The inhibitor of Claim 92 wherein R₉ is oxo.
99. The inhibitor of Claim 92 wherein R¹ is carboxamide.

100. An inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

![Molecular Structure](image)

wherein the dotted lines are optional pi bonds;
wherein \( n = 1 \) or 2; and
wherein \( a \) is either \( \cdot H \) or \( \cdot CH_3 \);
wherein \( b \) and \( c \) are independently hydrogen or methyl;
wherein \( Z \) is oxygen or sulfur.

101. The inhibitor of Claim 100 wherein \( n = 1 \).

102. The inhibitor of Claim 100 wherein at least one of \( b \) or \( c \) is methyl.

103. The inhibitor of Claim 100 wherein both \( b \) and \( c \) are methyl.

104. The inhibitor of Claim 100 wherein \( Z \) is oxygen.

AMENDED SHEET
105. The inhibitor of claim 74 having a molecular structure selected from the group consisting of:

EM-1291

EM-1195-CS

CS-243
CS-245

EM-1401

EM-1396
EM-1181

EM-1159 and

EM-1165
106. The inhibitor of Claim 105 wherein said inhibitor of 17\(\beta\)-hydroxysteroid dehydrogenase selected from the group consisting of:

\[
\text{EM-1404 and EM-1394}
\]

107. A method of inhibiting type 3 17\(\beta\)-hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of type 3 17\(\beta\)-hydroxysteroid dehydrogenase having the molecular structure:
wherein R is selected from the group consisting of alkoxy, alkylthio, alkoxyalkoxy, alkoxyalkylthio, alkylthioalkoxy, and alkylthioalkylthio,

or

wherein n is an integer from 1 to 4.

108. The method of Claim 107 wherein said inhibitor is selected from the group consisting of:
109. A pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and a therapeutically acceptable amount of an
inhibitor of type 3 17β-hydroxysteroid dehydrogenase having the molecular structure:

\[
\begin{align*}
\text{R} & \quad \text{R} \\
\end{align*}
\]

wherein \( R \) is selected from the group consisting of alkoxy, alkylthio, alkoxyalkoxy, alkoxyalkylthio, alkylthioalkoxy, and alkylthioalkylthio,

or

\[
\begin{align*}
\text{(CH}_2\text{n)} & \quad \text{OH} \\
\end{align*}
\]

wherein \( n \) is an integer from 1 to 4.
110. The pharmaceutical composition of Claim 109 wherein said inhibitor is selected from the group consisting of:

- EM-1071
- EM-1065
- EM-1066
- EM-1064
- EM-1074
- EM-1073
- EM-1070
- CS-213 and
- EM-1324-CS
111. An inhibitor of type 3 17β-hydroxysteroid dehydrogenase having the molecular structure:

wherein R is selected from the group consisting of alkoxyethoxy, alkoxyalkylthio, alkylthioalkoxy, and alkylthioalkylthio, or

wherein n is an integer from 1 to 4.

112. The inhibitor of Claim 111 wherein said inhibitor is selected from the group consisting of:
113. A method of treating, or reducing the risk of developing, prostate cancer, comprising administering to a patient in need of such
treatment or reduction a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase, and an amount of an LHRH agonist or antagonist effective to reduce testicular secretion of sex steroids, wherein the inhibitor of type 5 17β-hydroxysteroid dehydrogenase is not chlormadinone acetate.

114. The method of claim 113, further comprising administering a therapeutically effective amount of an antiandrogen.

115. The method of claim 114, further comprising administering a therapeutically effective amount of a 5α-reductase inhibitor.

116. The method of claim 113, further comprising administering a therapeutically effective amount of a 5α-reductase inhibitor.

117. A method of treating, or reducing the risk of developing prostate cancer, comprising administering to a patient in need of such treatment or reduction a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase and of an inhibitor of type 3 17β-hydroxysteroid dehydrogenase.

118. The method of claim 117 further comprising a therapeutically effective amount of a 5α-reductase inhibitor.

119. The method of claim 117 further comprising a therapeutically effective amount of an LHRH agonist or antagonist.

120. The method of claim 117 further comprising a therapeutically effective amount of an antiandrogen.
121. The method of claim 117 further comprising a therapeutically effective amount of an LHRH agonist or antagonist and an antiandrogen.

122. The method of claim 117 further comprising a therapeutically effective amount of an LHRH agonist or antagonist, an antiandrogen, and a 5α-reductase inhibitor.

123. The method of claim 117 further comprising a therapeutically effective amount of an antiandrogen and a 5α-reductase inhibitor.

124. The method of claim 117 further comprising a therapeutically effective amount of an LHRH agonist (or antagonist) and a 5α-reductase inhibitor.

125. A method of treating, or reducing the risk of developing, benign prostatic hyperplasia comprising administering to a patient in need of such treatment or reduction, a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase, wherein the inhibitor of type 5 17β-hydroxysteroid dehydrogenase is not chlormadinone acetate.

126. The method of claim 125, further comprising administering to said patient a therapeutically effective amount of an agent selected from the group consisting of an antiestrogen or an aromatase inhibitor.

127. The method of claim 125, further comprising administering to said patient a therapeutically effective amount of an antiandrogen.
128. The method of claim 125, further comprising administering to said patient a therapeutically effective amount of an antiandrogen and a 5α-reductase inhibitor.

129. The method of claim 125, further comprising administering to said patient a therapeutically effective amount of a 5α-reductase inhibitor.

130. The method of claim 125, further comprising administering to said patient a therapeutically effective amount of a 5α-reductase inhibitor and an antiestrogen or an aromatase inhibitor.

131. The method of claim 125, further comprising administering to said patient a therapeutically effective amount of a 5α-reductase inhibitor, an antiandrogen, an antiestrogen or an aromatase inhibitor.

132. A method of treating or reducing the risk of developing, acne, seborrhea, hirsutism or androgenic alopecia, comprising administering to a patient in need of such treatment or reduction, a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase and a therapeutically effective amount of an antiandrogen, wherein the inhibitor of type 5 17β-hydroxysteroid dehydrogenase is not medroxyprogesterone acetate, megestrol acetate, chlormadinone acetate, 1-dehydromegestrol acetate, melengestrol acetate, nomegestrol acetate, 1-dehydromelengestrol acetate, and cyproterone acetate.

133. A method of treating or reducing the risk of developing, acne, seborrhea, hirsutism or androgenic alopecia, comprising administering to a patient in need of such treatment or reduction, a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase and a
therapeutically effective amount of a 5α-reductase inhibitor, wherein the
inhibitor of type 5 17β-hydroxysteroid dehydrogenase is not
medroxyprogesterone acetate, megestrol acetate, chlormadinone acetate, 1-
dehydromegestrol acetate, melengestrol acetate, nomegestrol acetate,
1-dehydromelengestrol acetate, and cyproterone acetate.

134. A method of treating or reducing the risk of developing, acne,
seborrhea, hirsutism or androgenic alopecia, comprising administering to
a patient in need of such treatment or reduction, a therapeutically effective
amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase and a
therapeutically effective amount of a 5α-reductase inhibitor and a
therapeutically effective amount of an antiandrogen, wherein the inhibitor
of type 5 17β-hydroxysteroid dehydrogenase is not medroxyprogesterone
acetate, megestrol acetate, chlormadinone acetate, 1-dehydromegestrol
acetate, melengestrol acetate, nomegestrol acetate, 1-dehydromelengestrol
acetate, and cyproterone acetate.

135. A method of treating, or reducing the risk of developing,
polycystic ovarian syndrome comprising administering to a patient in need
of such treatment or reduction a therapeutically effective amount of an
inhibitor of type 5 17β-hydroxysteroid dehydrogenase and an inhibitor of
type 3 17β-hydroxysteroid dehydrogenase.

136. A method of treating, or reducing the risk of developing,
polycystic ovarian syndrome comprising administering to a patient in need
of such treatment or reduction, a therapeutically effective amount of an
inhibitor of type 3 17β-hydroxysteroid dehydrogenase.
137. A method of treating, or reducing the risk of developing, polycystic ovarian syndrome comprising administering to a patient in need of such treatment or reduction a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase and a therapeutically effective amount of an antiandrogen.

138. A method of treating, or reducing the risk of developing, polycystic ovarian syndrome comprising administering to a patient in need of such treatment or reduction a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase, an inhibitor of type 3 17β-hydroxysteroid dehydrogenase, and an antiandrogen.

139. The method of claim 136 further comprising administering a therapeutically effective amount of an antiandrogen.

140. A method of treating, or reducing the risk of developing, breast cancer comprising administering to a patient in need of such treatment or reduction, a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase and a therapeutically effective amount of an antiestrogen, wherein the inhibitor of type 5 17β-hydroxysteroid dehydrogenase is not medroxyprogesterone acetate.

141. The method of claim 140 further comprising administering a therapeutically effective amount of an LHRH agonist or antagonist.

142. The method of claim 140, further comprising administering a therapeutically effective amount of an androgenic compound.
143. The method of claim 142, further comprising administering an amount of an LHRH agonist effective to reduce ovarian secretion of sex steroids.

144. A method of treating, or reducing the risk of developing, endometriosis or leiomyoma comprising administering to a patient in need of such treatment or reduction, a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase and a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase wherein the inhibitor of type 5 17β-hydroxysteroid dehydrogenase is not medroxyprogesterone acetate, megestrol acetate, chlormadinone acetate, 1-dehydromegestrol acetate, melengestrol acetate, nomegestrol acetate, 1-dehydromelengestrol acetate, cyproterone acetate and 6-methyl-3-oxo-Δ⁴⁶-steroid compounds.

145. The method of claim 144, further comprising administering to said patient a therapeutically effective amount of an antiestrogen or of an inhibitor of aromatase.

146. The method of claim 144, further comprising administering to said patient an amount of an LHRH agonist (or antagonist) effective to reduce ovarian secretion of sex steroids.

147. The method of claim 146, further comprising administering a therapeutically effective amount of an antiestrogen or of an aromatase inhibitor.

148. A method of inhibiting testicular hormonal secretions comprising administering to a patient in need of such inhibition an amount
of an inhibitor of type 3 17β-hydroxysteroid dehydrogenase effective to reduce testicular production of sex steroids.

149. The method of claim 148 further comprising administering a therapeutically effective amount of an antiandrogen.

150. The method of claim 149 further comprising administering a therapeutically effective amount of a 5α-reductase inhibitor.

151. The method of claim 148, further comprising administering an LHRH agonist.

152. The method of claim 151, further comprising administering an antiandrogen.

153. The method of claim 151, further comprising administering an antiandrogen and a 5α-reductase inhibitor.

154. The method of claim 148, further comprising administering an LHRH antagonist.

155. The method of claim 154, further comprising administering an antiandrogen.

156. The method of claim 154, further comprising administering an antiandrogen and a 5α-reductase inhibitor.

157. A method of treating precocious puberty comprising administering to a male or female patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid
dehydrogenase, wherein the inhibitor of type 5 17\(\beta\)-hydroxysteroid dehydrogenase is not medroxyprogesterone acetate.

158. The method of claim 157 comprising administering to a male patient a therapeutically effective amount of an LHRH agonist or antagonist.

159. The method of claim 157 comprising administering to a male or female patient a therapeutically effective amount of an antiandrogen.

160. The method of claim 157 comprising administering to a male patient a therapeutically effective amount of an antiandrogen and an LHRH agonist or antagonist.

161. The method of claim 157 comprising administering to a male patient a therapeutically effective amount of an inhibitor of type 3 17\(\beta\)-hydroxysteroid dehydrogenase.

162. A method of treating precocious puberty comprising administering to a male or female patient in need of such treatment a therapeutically effective amount of an inhibitor of type 3 17\(\beta\)-hydroxysteroid dehydrogenase.

163. The method of claim 162 further comprising administering to a male patient a therapeutically effective amount of an LHRH agonist or antagonist.

164. The method of claim 162 further comprising administering to a male or female patient a therapeutically effective amount of an antiandrogen.
165. The method of claim 162 further comprising administering to a male patient a therapeutically effective amount of an antiandrogen and an LHRH agonist or antagonist.

166. A pharmaceutical composition comprising:
   a) a pharmaceutically acceptable excipient, diluent or carrier;
   b) a therapeutically effective amount of at least one inhibitor of type 5 17β-hydroxysteroid dehydrogenase; and
   c) a therapeutically effective amount of at least one antiandrogen.

167. A pharmaceutical composition of claim 166 wherein said composition further comprising a therapeutically effective amount of a 5α-reductase inhibitor.

168. A pharmaceutical composition of claim 166 wherein said composition further comprising a therapeutically effective amount of at least one antiestrogen or one aromatase inhibitor.

169. A pharmaceutical composition of claim 164 wherein said composition further comprising a therapeutically effective amount of at least one antiestrogen or one aromatase inhibitor.

170. A pharmaceutical composition comprising:
   a) a pharmaceutically acceptable excipient, diluent or carrier;
b) a therapeutically effective amount of at least one
inhibitor of type 5 17β-hydroxysteroid dehydrogenase;
and

c) a therapeutically effective amount of at least one inhibitor
of type 3 17β-hydroxysteroid dehydrogenase

171. A pharmaceutical composition comprising:
   a) a pharmaceutically acceptable excipient, diluent or
      carrier;
   b) a therapeutically effective amount of at least one
      inhibitor of type 3 17β-hydroxysteroid dehydrogenase;
      and
   c) a therapeutically effective amount of at least one
      antiandrogen.

172. A pharmaceutical composition comprising:
   a) a pharmaceutically acceptable excipient, diluent or
      carrier;
   b) a therapeutically effective amount of at least one
      inhibitor of type 3 17β-hydroxysteroid dehydrogenase;
      and
   c) a therapeutically effective amount of at least one
      5α-reductase inhibitor.

173. A pharmaceutical composition of claim 171 wherein said
composition further comprising a therapeutically effective amount of at least
one 5α-reductase inhibitor.
174. A pharmaceutical composition of claim 170 wherein said composition further comprising a therapeutically effective amount of at least one antiandrogen.

175. A pharmaceutical composition of claim 170 wherein said composition further comprising a therapeutically effective amount of at least one 5α-reductase inhibitor.

176. A pharmaceutical composition of claim 174 wherein said composition further comprising a therapeutically effective amount of at least one 5α-reductase inhibitor.

177. A pharmaceutical composition comprising:
   a) a pharmaceutically acceptable excipient, diluent or carrier;
   b) a therapeutically effective amount of at least one inhibitor of type 5 17β-hydroxysteroid dehydrogenase; and
   c) a therapeutically effective amount of at least one 5α-reductase inhibitor.

178. A pharmaceutical composition of claim 177 wherein said composition further comprising a therapeutically effective amount of at least one antiestrogen or one aromatase inhibitor.

179. A pharmaceutical composition comprising:
   a) a pharmaceutically acceptable excipient, diluent or carrier;
b) a therapeutically effective amount of at least one
inhibitor of type 5 17β-hydroxysteroid dehydrogenase;
and

c) a therapeutically effective amount of at least one
antiestrogen or one aromatase inhibitor,

wherein the inhibitor of type 5 17β-hydroxysteroid dehydrogenase is
not medroxyprogesterone acetate, megestrol acetate, chlormadinone acetate,
1-dehydromegestrol acetate, melengestrol acetate, nonmegestrol acetate, l-
dehydromelengestrol acetate, and cyproterone acetate.

180. A kit having a plurality of containers wherein contents of each
container differ in whole or in part from content of another container, said
kit comprising a therapeutically effective amount of at least one inhibitor of
type 5 17β-hydroxysteroid dehydrogenase and a therapeutically effective
amount of at least one antiandrogen.

181. A kit having a plurality of containers wherein contents of each
container differ in whole or in part from content of another container, said
kit comprising a therapeutically effective amount of at least one inhibitor of
type 5 17β-hydroxysteroid dehydrogenase and a therapeutically effective
amount of at least one inhibitor of type 3 17β-hydroxysteroid dehydrogenase

182. A kit having a plurality of containers wherein contents of each
container differ in whole or in part from content of another container, said
kit comprising a therapeutically effective amount of at least one inhibitor of
type 3 17β-hydroxysteroid dehydrogenase and a therapeutically effective
amount of at least one antiandrogen.

183. A kit having a plurality of containers wherein contents of each
container differ in whole or in part from content of another container, said
kit comprising a therapeutically effective amount of at least one inhibitor of
type 3 17β-hydroxysteroid dehydrogenase and a therapeutically effective
amount of at least one 5α-reductase inhibitor.

184. A kit having a plurality of containers wherein contents of each
container differ in whole or in part from content of another container, said
kit comprising a therapeutically effective amount of at least one inhibitor of
type 5 17β-hydroxysteroid dehydrogenase and a therapeutically effective
amount of at least one 5α-reductase inhibitor.

185. A kit having a plurality of containers wherein contents of each
container differ in whole or in part from content of another container, said
kit comprising a therapeutically effective amount of at least one inhibitor of
type 5 17β-hydroxysteroid dehydrogenase and a therapeutically effective
amount of at least one antiestrogen or one aromatase inhibitor, wherein the
inhibitor of type 5 17β-hydroxysteroid dehydrogenase is not
medroxyprogesterone acetate, megestrol acetate, chlormadinone acetate, 1-
dehydromegestrol acetate, melengestrol acetate, nonmegestrol acetate, 1-
dehydromelengestrol acetate, and cyproterone acetate.

186. A kit having a plurality of containers wherein contents of each
container differ in whole or in part from content of another container, said
kit comprising a therapeutic effective amount of at least one inhibitor of type
5 17β-hydroxysteroid dehydrogenase and an amount of at least one LHRH
agonist or antagonist, wherein the inhibitor of type 5 17β-hydroxysteroid
dehydrogenase is not medroxyprogesterone acetate, megestrol acetate,
chlormadinone acetate, 1-dehydromegestrol acetate, melengestrol acetate,
nonmegestrol acetate, 1-dehydromelengestrol acetate, and cyproterone acetate.
187. A kit having a plurality of containers wherein contents of each
container differ in whole or in part from content of another container, said
kit comprising a therapeutically effective amount of at least one inhibitor of type
3 17β-hydroxysteroid dehydrogenase and an amount of at least one LHRH
agonist or antagonist.

188. A method of treating, or reducing the risk of developing,
prostate cancer, comprising administering to a patient in need of such
treatment or reduction a therapeutically effective amount of an inhibitor of
type 5 17β-hydroxysteroid dehydrogenase, and an amount of an LHRH
agonist or antagonist effective to reduce testicular secretion of sex steroids
wherein the inhibitor of type 5 17β-hydroxysteroid dehydrogenase is not
medroxyprogesterone acetate, megestrol acetate, chlormadinone acetate, 1-
dehydromegetrol acetate, melengestrol acetate, nomegestrol acetate,
1-dehydromelengestrol acetate, and cyproterone acetate.

189. A method of treating, or reducing the risk of developing,
benign prostatic hyperplasia comprising administering to a patient in need
of such treatment or reduction, a therapeutically effective amount of an
inhibitor of type 5 17β-hydroxysteroid dehydrogenase wherein the inhibitor
of type 5 17β-hydroxysteroid dehydrogenase is not medroxyprogesterone
acetate, megestrol acetate, chlormadinone acetate, 1-dehydromegetrol
acetate, melengestrol acetate, nomegestrol acetate, 1-dehydromelengestrol
acetate, and cyproterone acetate.

190. A method of treating or reducing the risk of developing, acne,
seborrhea, hirsutism or androgenic alopecia, comprising administering to
a patient in need of such treatment or reduction, a therapeutically effective
amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase wherein
the inhibitor of type 5 17β-hydroxysteroid dehydrogenase is not
medroxyprogesterone acetate, megestrol acetate, chlormadinone acetate, 1-
dehydromegestrol acetate, melengestrol acetate, nomegestrol acetate,
1-dehydromelengestrol acetate, and cyproterone acetate.

191. A method of treating, or reducing the risk of developing,
polycystic ovarian syndrome comprising administering to a patient in need
of such treatment or reduction a therapeutically effective amount of an
inhibitor of type 5 17β-hydroxysteroid dehydrogenase wherein the inhibitor
of type 5 17β-hydroxysteroid dehydrogenase is not medroxyprogesterone
acetate, megestrol acetate, chlormadinone acetate, 1-dehydromegestrol
acetate, melengestrol acetate, nomegestrol acetate, 1-dehydromelengestrol
acetate, and cyproterone acetate.

192. A method of treating, or reducing the risk of developing,
breast cancer comprising administering to a patient in need of such
treatment or reduction, a therapeutically effective amount of an inhibitor of
type 5 17β-hydroxysteroid dehydrogenase and a therapeutically effective
amount of an antiestrogen wherein the inhibitor of type 5 17β-
hydroxysteroid dehydrogenase is not medroxyprogesterone acetate,
megestrol acetate, chlormadinone acetate, 1-dehydromegestrol acetate,
melengestrol acetate, nomegestrol acetate, 1-dehydromelengestrol acetate,
and cyproterone acetate.

193. A method of treating, or reducing the risk of developing,
endometriosis or leiomyoma comprising administering to a patient in need
of such treatment or reduction, a therapeutically effective amount of an
inhibitor of type 5 17β-hydroxysteroid dehydrogenase wherein the inhibitor
of type 5 17β-hydroxysteroid dehydrogenase is not medroxyprogesterone acetate, megestrol acetate, chlormadinone acetate, 1-dehydromegestrol acetate, melengestrol acetate, nomegestrol acetate, 1-dehydromelengestrol acetate, cyproterone acetate, and exclude the use of the "6-methyl-3-oxo-Δ^4^-steroid compounds.

194. A method of treating precocious puberty comprising administering to a male or female patient in need of such treatment a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase, wherein the inhibitor of type 5 17β-hydroxysteroid dehydrogenase is not medroxyprogesterone acetate, megestrol acetate, chlormadinone acetate, 1-dehydromegestrol acetate, melengestrol acetate, nomegestrol acetate, 1-dehydromelengestrol acetate, and cyproterone acetate.

195. The method of claim 1 wherein the inhibitor of type 5 17β-hydroxysteroid dehydrogenase is not medroxyprogesterone acetate, megestrol acetate, chlormadinone acetate, 1-dehydromegestrol acetate, melengestrol acetate, nomegestrol acetate, 1-dehydromelengestrol acetate, and cyproterone acetate.

196. The method of claim 38 wherein the inhibitor of type 5 17β-hydroxysteroid dehydrogenase is not medroxyprogesterone acetate, megestrol acetate, chlormadinone acetate, 1-dehydromegestrol acetate, melengestrol acetate, nomegestrol acetate, 1-dehydromelengestrol acetate, and cyproterone acetate.

197. The pharmaceutical composition of claim 40 wherein the inhibitor of type 5 17β-hydroxysteroid dehydrogenase is not medroxyprogesterone acetate, megestrol acetate, chlormadinone acetate, 1-
dehydromegestrol acetate, melengestrol acetate, nomegestrol acetate, 1-dehydromelengestrol acetate, and cyproterone acetate.

198. A pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and a therapeutically effective amount of an inhibitor of type 5 17β-hydroxysteroid dehydrogenase having the molecular structure:

![Molecular Structure](image)

wherein the dotted line is optional pi bond;

wherein A is selected from the group consisting of straight or branched C₁-C₆ alkyl, -OR¹ (R¹ being a C₁-C₆ alkyl radical), and -N(R⁴)R⁵ (R⁴ and R⁵ being independently hydrogen or C₁-C₆ alkyl or aryl), and unsaturated analogs of any of the foregoing definitions for substituent A;

wherein R¹ is selected from the group consisting of hydrogen and methyl and ethyl;

wherein R⁶ is selected from the group consisting of hydrogen, and halogen, and C₁-C₆ alkyl;

wherein R⁷ is selected from the group consisting of straight or branched C₁-C₆ alkyne, C₅-C₆ cycloalkylene; and

R⁸ is selected from the group consisting of hydrogen, substituted or unsubstituted phenyl, C₂-C₁₀ acyl, C₂-C₁₀ acyloxy, C₂-C₁₀ alkoxy carbonyl, and
halogen;

provided that when A is methyl, R¹ is methyl; and

wherein the inhibitor of type 5 17β-hydroxysteroid dehydrogenase is not medroxyprogesterone acetate, megestrol acetate, chlormadinone acetate, 1-dehydromegestrol acetate, melengestrol acetate, nomegestrol acetate, 1-dehydromelengestrol acetate, and cyproterone acetate.

199. The method of claim 136 wherein the inhibitor of type 3 17β-hydroxysteroid dehydrogenase is not androsterone.

200. The method of claim 148 wherein the inhibitor of type 3 17β-hydroxysteroid dehydrogenase is not androsterone.

201. The method of claim 162 wherein the inhibitor of type 3 17β-hydroxysteroid dehydrogenase is not androsterone.

202. The method of claim 188, further comprising administering a therapeutically effective amount of an antiandrogen.

203. The method of claim 202, further comprising administering a therapeutically effective amount of a 5α-reductase inhibitor.

204. The method of claim 188, further comprising administering a therapeutically effective amount of a 5α-reductase inhibitor.

205. The method of claim 189, further comprising administering to said patient a therapeutically effective amount of an agent selected from the group consisting of an antiestrogen or an aromatase inhibitor.
206. The method of claim 189, further comprising administering to said patient a therapeutically effective amount of an antiandrogen.

207. The method of claim 189, further comprising administering to said patient a therapeutically effective amount of an antiandrogen and a 5α-reductase inhibitor.

208. The method of claim 189, further comprising administering to said patient a therapeutically effective amount of a 5α-reductase inhibitor.

209. The method of claim 189, further comprising administering to said patient a therapeutically effective amount of a 5α-reductase inhibitor and an antiestrogen or an aromatase inhibitor.

210. The method of claim 189, further comprising administering to said patient a therapeutically effective amount of a 5α-reductase inhibitor, an antiandrogen, an antiestrogen or an aromatase inhibitor.

211. The method of claim 192 further comprising administering a therapeutically effective amount of an LHRH agonist or antagonist.

212. The method of claim 192, further comprising administering a therapeutically effective amount of an androgenic compound.

213. The method of claim 212, further comprising administering an amount of an LHRH agonist effective to reduce ovarian secretion of sex steroids.
UNSCANABLE ITEM

RECEIVED WITH THIS APPLICATION

(ITEM ON THE 10TH FLOOR ZONE 5 IN THE FILE PREPARATION SECTION)

DOCUMENT REÇU AVEC CETTE DEMANDE
NE POUVANT ÊTRE BALAYÉ

(DOCUMENT AU 10 ÈME ÉTAGE AIRE 5 DANS LA SECTION DE LA
PRÉPARATION DES DOSSIERS)
Biosynthetic pathway of active androgens in the prostate.

\( \times \), inhibition target.

Fig. 8