5α-Reductase Inhibitory and Antiandrogenic Activities of Novel Steroids in Hamster Seminal Vesicles

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The pharmacological activity of several 16-bromosubstituted trienediones 4 and 5, 16-methyl substituted dienediones 6 and 7 and the 16-methyl substituted trienedione 8 was determined on gonadectomized hamster seminal vesicles by measuring the *in vitro* conversion of testosterone (T) to dihydrotestosterone (DHT) as 5α-reductase inhibitors and also the ability of these steroids to bind to the androgen receptor. Steroids 6 and 7 when injected together with T decreased the weight of the seminal vesicles thus showing an antiandrogenic effect. Compounds 5 and 6 reduced substantially the conversion of T to DHT and therefore can be considered good inhibitors for the enzyme 5α-reductase; however both steroids failed to form a complex with the androgen receptor. On the other hand compound 7 which showed a very small inhibitory activity for the enzyme 5α-reductase, exhibited a very high affinity for the androgen receptor and thus can be considered an effective antiandrogen. This compound also reduced substantially the weight of the seminal vesicles. Steroids 4 and 8 did not reduce the weight of the seminal vesicles and exhibited a low affinity for the androgen receptor; 8 showed a weak 5α-reductase inhibitory activity, whereas 4 exhibited a weak androgenic effect.

Key words seminal vesicle; 5α-reduction; 5α-reductase; androgen receptor; T to DHT conversion; C-16 substituent

Steroid 5α-reductase (EC 1-3-99-5) is a NADPH dependent enzyme which converts testosterone (T) **1** into dihydrotestosterone (DHT) **2**.[1)] This enzyme is located in the androgen-dependent tissue such as prostate, seminal vesicles, epididymis and the skin. It is believed that DHT plays a role in pathological conditions such as benign prostatic hyperplasia (BHP), prostatic cancer, acne, female hirsutism and male pattern baldness.[2)]

The essential role of 5α-reductase in prostate growth has been determined by the discovery of 5α-reductase deficiency in male pseudohermaphroditism patients who had atrophic prostate glands despite normal testosterone level.[3,4)] DHT is the most active agonist for the androgen receptor[5)] although T also mediates the androgenic action in the tissues. Various steroidal compounds have been developed as 5α-reductase inhibitors for the medical therapy of BHP.[5)] Finasteride **3**[6)] (Fig. 1) a 4-aza steroid is the most extensively studied class of 5α-reductase inhibitors.[7)] This compound is the first approved 5α-reductase inhibitor in the U.S.A. for the treatment of BHP. This drug has approximately a 100-fold greater affinity for type 2—5α-reductase than for type 1. In humans finasteride decreases prostatic DHT level and reduces prostate size, while T tissue concentration remains constant.[8)]

In this study, we determined the antiandrogenic activity of five new steroidal compounds: **4**—**8** in gonadectomized hamster seminal vesicles.

Seminal vesicles are male accessory glands that are androgen-dependent. These organs are capable of reducing T to DHT in both intact and gonadectomized animals and have also been used for evaluation of steroidal and non steroidal compounds as antiandrogens.[9,10)]

Chemistry Several years ago we synthesized several new 17α-acyloyloxy-16β-methyl substituted pregnadiene-3,20-dione derivatives.[15,16)] Some of these compounds showed a high inhibitory effect for the enzyme 5α-reductase as well as high affinity for the androgen receptor. On the basis of these results and in order to expand the scope of this study, recently we synthesized several new 17α-acyloyloxy-16β-substituted (methyl, bromine) pregnadiene-3,20-diones and pregnatriene-3,20-triones (**4**—**8**) which are described in this paper.

Synthesis of Steroids 4—8 Compounds **4**—**8** (Fig. 1) were prepared from the commercially available 16-dehydropregnenolone acetate (Fig. 2). Epoxidation of the double bond at C-16 with hydrogen peroxide and sodium hydroxide afforded the epoxy derivative **9**. The bromohydrin **10** was obtained when the intermediate **9** was treated with hydrobromic acid (32%) in dioxane for 6 h. When compound **10** was allowed to reflux with dichloro-dicyanobenzoquinone (DDQ) in dioxane for 4 d, the trienedione **4** was obtained. Esterification of the hydroxyl group in **4** with trifluoroacetic anhydride, *p*-toluenesulfonic acid and cyclopentanecarboxylic acid yielded the desired ester **5**.

Compounds **6**—**8** (Fig. 1) were prepared from the epoxy derivative **9** (Fig. 3). Acetylation of **9** in the usual manner yielded the acetoxy compound **11**. The protection of the carbonyl group in **11** was effected with ethylene glycol, trimethyl orthoformate and *p*-toluenesulfonic acid (PTSA). The resulting dioxolane derivative **12** was allowed to reflux with methylmagnesium chloride in tetrahydrofuran (THF); this reaction afforded the 16-methyl substituted dioxolane derivative **13**. Hydrolysis of the dioxolane ring in **13** to recover the carbonyl moiety was carried out with PTSA in acetone thus forming the carbonyl derivative **14**. The oxidation of the hydroxyl group at C-3 in **14** was effected with lithium carbonate, lithium bromide and bromine in *N*,*N*-dimethylformamide (DMF). This reaction afforded the 4,6-diene-3,20-dione moiety **15**. Esterification of the hydroxyl group in **15** with trifluoroacetic anhydride, cyclopentylacetic acid and PTSA yielded the desired ester **6**. When alcohol **15** was treated in the same manner with cyclopentane carboxylic acid, the corresponding cyclopentanecarboxylate ester **7** was

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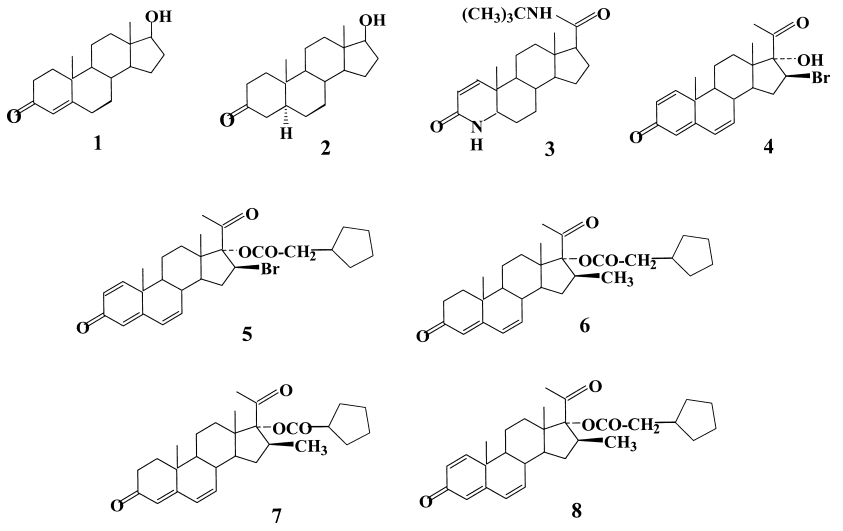

Fig. 1. Steroid Structures

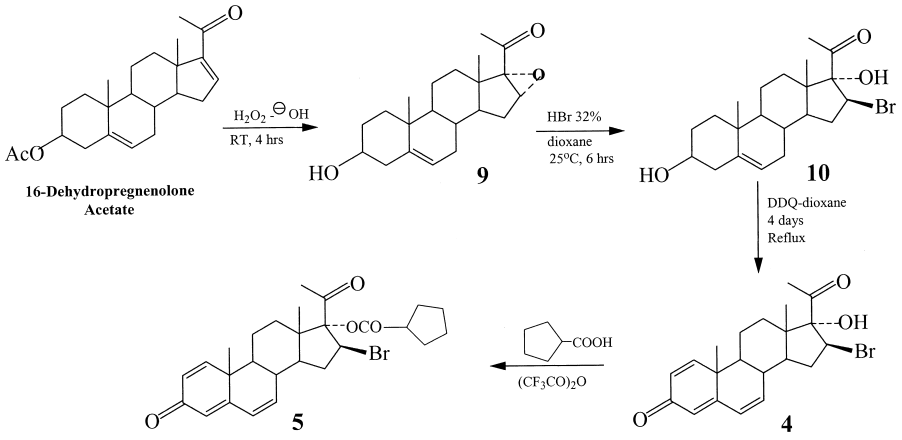

Fig. 2. Synthesis of New Steroids

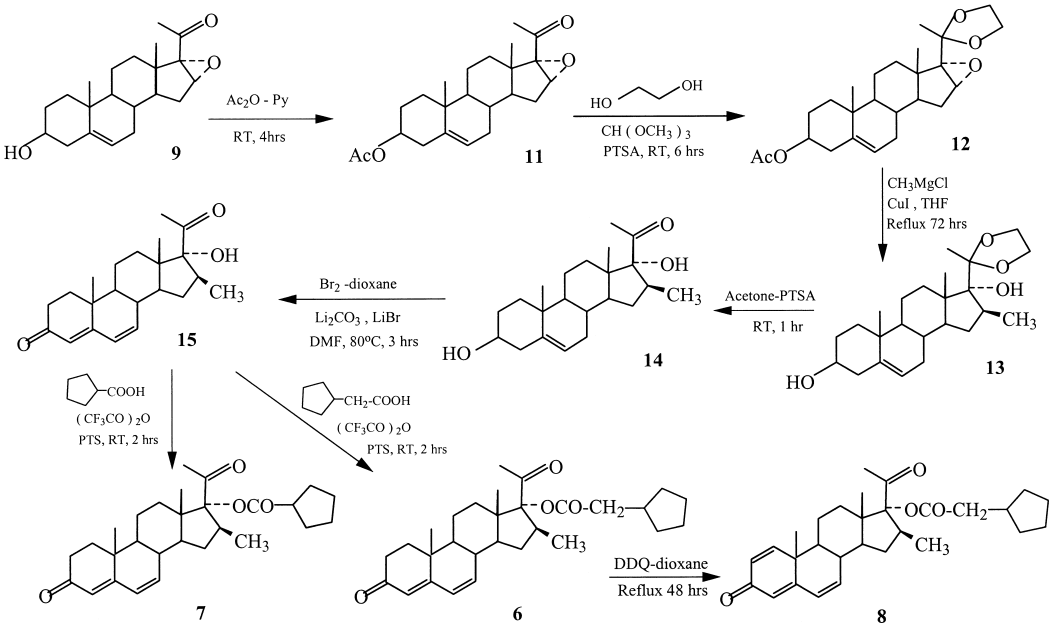

Fig. 3. Synthesis of New Steroids

obtained.

Treatment of **6** with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) in dioxane afforded the trienedione compound **8**.

Biological Activity The biological activity of steroids **4** to **8** were determined in gonadectomized male hamsters divided in several groups. The animals in the control group were injected with the vehicle (sesame oil); in the other groups they were treated with T,[11] a combination of T with finasteride or T with the new steroids. After 6 d of treatment, the animals were anesthetized with ether and sacrificed. The seminal vesicles were removed weighed, homogenized and used for the *in vitro* experiments.

Seminal Vesicles After castration the weight of the seminal vesicles of the male hamsters significantly decreased ($p<0.05$) as compared to that of the normal glands. Treatment with vehicle alone (control) did not change this condition whereas subcutaneous injections of 200 μg of **1** for 6 d significantly increased ($p<0.05$) the weight of the seminal vesicles in castrated male hamsters (141.7 mg) (Table 1). When **1** and finasteride **3** were injected together, the weight of the seminal vesicles decreased (120 mg). The injection of steroids **6** and **7** together with T decreased the weight of the seminal vesicles as compared to the T treated hamsters thus suggesting an inhibitory effect on 5α-reductase enzyme type 2. The most effective compounds showing the highest inhibitory effect for 5α-reductase enzyme were steroids **6** and **7** having the lowest value of the weight of the seminal vesicles, 118 and 115 mg respectively.

***In Vitro* Metabolism of T to DHT** Since the weight of the seminal vesicles depends on the 5α-reduced androgens,[1,12,13] it was important to determine the effect of the new steroids **4**—**8** on the conversion of **1** to **2** *in vitro* metabolism of [^{3}H]T to [^{3}H]DHT in seminal vesicles homogenates of castrated male hamsters at pH of 6 in the presence of NADPH. The extracts from castrated male hamster seminal vesicles were subjected to TLC analysis. The zone corresponding to DHT (**2**) standard (*Rf* value of 0.33) of each experimental chromatogram was eluted and the radioactivity determined. The results (Fig. 4) obtained from two separate experiments performed in duplicate demonstrated a lower [^{3}H]T to [^{3}H]DHT conversion in the T with finasteride treated animals than testosterone alone thus indicating that finasteride **3** is a good inhibitor for the conversion of T to DHT at pH of 6. Figure 4 also shows the effect of the new steroids **4**—**8** on the rate of DHT formation expressed as fmoles of DHT/mg of protein/h. The four compounds (**5**—**8**) produced a much lower concentration of DHT as compared to testosterone. On the other hand, compounds **5**, **6** and **8** exhibited a lower DHT conversion than finasteride **3** thus showing a high 5α-reductase inhibitory effect. Steroid **4** gave a higher *in vitro* conversion of **1** to **2** ($p<0.05$) than the other steroids thus showing a weak androgenic effect.

Compound **7** which is structurally very similar to steroid **6** (one carbon atom less) showed a higher *in vitro* conversion of **1** to **2** ($p<0.05$) than steroids **3**, **5**, **6** and **8** however it still can be considered a 5α-reductase inhibitor due to the lower production of DHT as compared to testosterone.

Scatchard Analysis of Receptor Androgen Binding When cytosol aliquots (2.64 mg protein) obtained from gonadectomized male hamster seminal vesicles homogenates were incubated with increasing concentrations of [^{3}H]DHT, a typical saturation curve was obtained. The levels of the nonspecific bound hormone increase progressively with the rise of radiolabeled compound concentration. Data were analyzed according to the Scatchard plot method.[14] A linear relationship indicates the presence of only one single class of high affinity and low capacity of binding sites with K_d and B_{max} values of 1.26 and 0.615 nM, respectively, for this tissue (Fig. 5).

Competition Analysis of Synthesized Steroids for Androgen Receptors (ARs) The effect of increasing concentrations of nonradioactive synthetic steroids upon [^{3}H]DHT binding to androgen receptors from male hamster seminal vesicles in two different experiments are presented in Fig. 6. The K_i values for the synthesized steroids showed the follow-

Table 1

Treatment (mg)	Weight of seminal vesicles (mg)
Control	109.7±15
Testosterone	141.7±10
T+**3**	120.0±05
T+**4**	130.0±10
T+**5**	150.0±13
T+**6**	118.0±08
T+**7**	115.0±05
T+**8**	135.0±10

The weight of seminal vesicles were measured from animals that received sc treatment of C-16 substituted steroids.

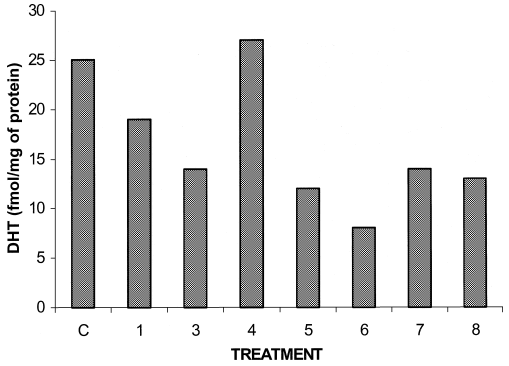

Fig. 4. Effect of New Steroids on *in Vitro* Conversion of [^{3}H]T to [^{3}H]DHT in Castrated Male Hamsters Flank Organs

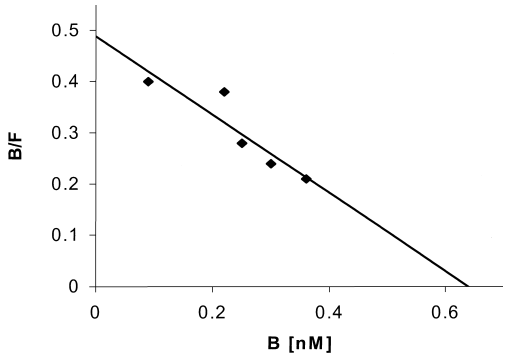

Fig. 5. Scatchard Plot of Receptor Androgen Binding

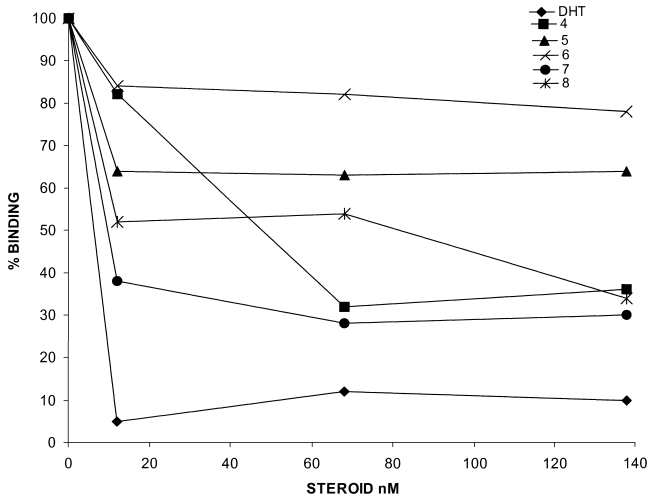

Fig. 6. Inhibition Constants (K_i) of Different Steroids

ing order of affinity to ARs: DHT>**7**>**4**>**8** with values of 8.29, 11.79, 45.38 and 73.04 respectively. Compounds **5** and **6** did not show any affinity for the androgen receptors.

Discussion

This study reports the 5α-reductase inhibitory activity and the antiandrogenic effect of novel 16-bromo substituted trienediones **4**, **5**, the 16β methyl substituted dienediones **6**, **7** and the trienedione **8** (Fig. 1). Compound **4** (Fig. 4) did not decrease the weight of the seminal vesicles and did not inhibit the conversion of T to DHT (it has a weak androgenic effect) however it competed for the ARs (K_i 45.38 nM). On the other hand, steroids **5**, **6** and **8** showed a 5α-reductase inhibitory effect slightly higher than the commercially available finasteride **3** presently used for the treatment of benign prostatic hyperplasia however compounds **5** and **8** did not decrease the weight of the seminal vesicles compounds **5** and **6** did not show any affinity for the androgen receptor as indicated previously, however they strongly inhibited the enzyme 5α-reductase. Steroid **8** showed a slight affinity for the ARs, a moderate 5α-reductase inhibitory activity and did not decrease the weight of the seminal vesicles. Compound **7** which has a low inhibitory activity for the conversion of T to DHT and is structurally similar to **6** binds with a high affinity to the ARs and reduces the weight of the seminal vesicles, thus showing high antiandrogenic and also an inhibitory effect for the enzyme 5α-reductase.

In addition, we demonstrated that radio-inert DHT in increasing concentrations, inhibited the binding of [^{3}H]DHT to the ARs present in cytosolic fraction as shown by the K_i value of 8.29 nM (Fig. 6). Furthermore, some radio-inert synthetic steroids (**4**, **8**, **7**) also inhibited the binding of [^{3}H]DHT to the cytosolic ARs, as indicated by the respective K_i values. In contrast steroids **5** and **6** did not have any inhibitory effect on cytosolic ARs, as shown in Fig. 5.

Experimental

Chemical and Radioactive Material Solvents were laboratory grade or better. Melting points were determined on a Fisher Johns melting point apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR were taken on Varian gemini 200 and VRX-300 respectively. Chemical shifts are given in ppm relative to that of Me_4Si (δ=0) in $CDCl_3$. The abbreviations of signal patterns are as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Mass spectra were obtained with a HP5985-B spectrometer. IR spectra were recorded on a Perkin-Elmer 200s spectrometer. (1, 2, 6, 7-^{3}H) Testosterone [^{3}H]T, specific activity: 95 Ci/mmol and (1, 2, 4, 5, 6, 7-^{3}H) dihydrotestosterone [^{3}H]DHT, specific activity 110 Ci/mmol, were provided by New England Nuclear Corp. (Boston, MA, U.S.A.). Radioniert T and 5-DHT were supplied by Steraloids (Wilton, NH, U.S.A.). Sigma Chemical Co. (ST. Louis, MO, U.S.A.) supplied $NADPH^+$, glucose-6-phosphate and glucose-6-phosphate dehydrogenase. The finasteride was obtained by extraction from Proscar (Merck, Sharp & Dohme).

Synthesis of the Steroidal Compounds The synthesis of the intermediates **9**, **11**—**15** (Fig. 3) is given in refs. 15 and 16. The preparation of the new compounds **4**, **5**, **10**, **6**—**8** is briefly described below.

16β-Bromo-17α-hydroxypregna-1,4,6-triene-3,20-dione 4 A solution of steroid **10** (1 g, 2.43 mmol) and DDQ (1 g, 13.22 mmol) in dioxane (50 ml) was allowed to reflux for 3 d. Upon cooling, the precipitated 2,3-dichloro-5,6-dicyanohydroquinone was filtered off. To the filtrate was added 3% aqueous sodium hydroxide solution (100 ml) and chloroform (100 ml); the mixture was stirred for 5 min. The organic phase was separated, washed 3 times with 3% aqueous sodium hydroxide solution and water. It was dried over anhydrous sodium sulfate and the solvent was removed in vacuum. The crude product was purified by silica gel column chromatography. Hexane–ethyl acetate (6 : 4) eluted 404 mg, 0.99 mmol (41% of the pure product **4**, mp 195—196°C. UV (nm) 219, 255, 298 (ε=15000, 12800, 17950 respectively). IR (KBr) cm^{-1}: 3378, 1715, 1646, 1600. ^{1}H-NMR ($CDCl_3$) δ: 1.2 (3H, s), 1.4 (3H, s), 2.4(3H, s), 3.6 (1H, s), 6.0 (1H, d, J=2 Hz), 6.3 (1H, d, J=2 Hz), 6.5 (1H, d, J=2 Hz), 6.7 (1H, d, J=3 Hz), 7.0 (1H, d, J=3 Hz). ^{13}C-NMR ($CDCl_3$) δ: 15.8 (C-18), 20.7 (C-19), 28.6 (C-21), 128.0 (C-6), 162.2 (C-5), 186.4 (C-3), 205.4 (C-20). MS (*m/z*) 404 (M^+).

16β-Bromo-17α-cyclopentylacetoxy-1,4,6-pregnatriene-3,20-dione 5 A solution containing steroid **4** (1 g, 2.47 mmol), PTSA (100 mg), trifluoroacetic anhydride (2 ml, 9.53 mmol) and cyclopentylacetic acid (0.8 ml, 4.99 mmol) was stirred for 2 h at room temperature (nitrogen atmosphere). The reaction mixture was neutralized with an aqueous sodium bicarbonate solution to a pH of 7 and diluted with chloroform (10 ml). The organic phase was separated, washed with water and dried over anhydrous sodium sulfate; the solvent was eliminated in vacuum. The crude product was purified by silica gel column chromatography. Hexane–ethyl acetate (8 : 2) eluted 369 mg, 0.74 mmol (30%) of the pure product **5**, mp 134—136°C. UV (nm) 217, 253, 291 (ε=14600, 12400, 17500 respectively). IR (KBr) cm^{-1}: 1731, 1710, 1656, 1603. ^{1}H-NMR ($CDCl_3$) δ: 1.1 (3H, s), 1.3 (3H, s), 2.1 (3H, s), 5.8 (1H, d, J=2 Hz), 6.3 (1H, d, J=2 Hz), 6.5 (1H, d, J=2 Hz), 6.8 (1H, d, J=3 Hz), 7.1 (1H, d, J=3 Hz). ^{13}C-NMR ($CDCl_3$) δ: 15.5 (C-18), 20.2 (C-19), 33.3 (C-21), 128.2 (C-6), 161.8 (C-5), 175.4 (ester carbonyl), 186.1 (C-3), 199.5 (C-20). MS (*m/z*) 500 (M^+).

16β-Bromo-3β,17α-dihydroxypregn-5-ene-20-one 10 A solution containing steroid **9** (1 g, 3.03 mmol) and hydrobromic acid (5.8 ml) in dioxane (12.2 ml) was stirred for 6 h at room temperature. The reaction mixture was poured into a beaker containing 30 ml of water. The resulting precipitate was filtered off and washed with water. The crude product was recrystallized from ethyl acetate–hexane; yield 0.72 g, 1.77 mmol (58%) of the pure product **10**, mp 196—198°C. IR (KBr) cm^{-1}: 3375, 1700, 1048. ^{1}H-NMR ($CDCl_3$) δ: 1.0 (3H, s), 1.2 (3H, s), 2.4 (3H, s), 3.5 (1H, m), 4.0 (1H, d, J=2 Hz), 5.3 (1H, d, J=2 Hz). ^{13}C-NMR ($CDCl_3$) δ: 16.2 (C-18), 19.3 (C-19), 28.2 (C-21), 71.6 (C-3), 140.8 (C-5), 120.9 (C-6), 51.7 (C-16), 206.4 (C-20). MS (*m/z*) 410 (M^+).

17α-Cyclopentylacetoxy-16β-methylpregna-4,6-diene-3,20-dione 6 A solution containing steroid **15** (1 g, 2.8 mmol), PTSA (200 mg), trifluoroacetic anhydride (0.2 g, 1.04 mmol) and cyclopentylacetic acid (1.2 ml, 8.7 mmol) was stirred for 2 h at room temperature (nitrogen atmosphere). The reaction mixture was neutralized with an aqueous sodium bicarbonate solution to pH of 7 and diluted with chloroform (10 ml). The organic phase was separated, washed with water and drier over sodium sulfate; the solvent was eliminated in vacuum. The crude product was purified by silica gel column chromatography. Hexane–ethyl acetate (8 : 2) eluted 850 mg, 1.74 mmol (62%) of pure product **6**, mp 182—183°C. UV (nm) 283 (ε=22900). IR (KBr) cm^{-1}: 1724, 1710, 1666, 1625. ^{1}H-NMR ($CDCl_3$) δ: 0.81 (3H, s), 1.1 (3H, s), 2.0 (3H, s), 1.4 (3H, d, J=2 Hz), 5.7 (1H, s), 6.1 (2H, s). ^{13}C-NMR ($CDCl_3$) δ: 15.4 (CH_3 at C-16), 16.5 (C-18), 20.0 (C-19), 28.0 (C-21), 124.0 (C-4), 128.0 (C-6), 173.0 (ester carbonyl), 199.0 (C-3), 204.0 (C-20). MS (*m/z*) 452 (M^+).

17α-Cyclopentylcarbonyloxy-16β-methylpregna-4,6-diene-3,20-dione 7 A solution containing steroid **15** (1 g, 2.21 mmol), PTSA (200 mg), trifluoroacetic anhydride (2 g, 1.04 mmol) and cyclopentanecarboxylic acid

(1.0 ml, 8.7 mmol) was stirred for 2 h at room temperature (nitrogen atmosphere). The reaction mixture was neutralized with an aqueous sodium bicarbonate solution to a pH of 7 and diluted with chloroform (10 ml). The organic phase was separated, washed with water and drier over anhydrous sodium sulfate; the solvent was eliminated in vacuum. The crude product was purified by silica gel column chromatography. Hexane–ethyl acetate (8 : 2) eluted 674 mg, 1.54 mmol (69%) of pure product **7**, mp 160—165°C. UV (nm) 284 (ε=23100). IR (KBr) cm^{-1} 1723, 1705, 1668, 1618. ^{1}H-NMR ($CDCl_3$) δ: 0.8 (3H, s), 1.1 (3H, s), 1.4 (3H, d, J=2 Hz), 1.98 (3H, s), 5.8 (1H, s), 6.1 (2H, d, J=3 Hz). ^{13}C-NMR ($CDCl_3$) δ: 16.0 (CH3 at C-16), 17.0 (C-18), 19.0 (C-19), 29.0 (C-21), 122.0 (C-4), 130.0 (C-6), 179.0 (ester carbonyl), 195.0 (C-3), 206.0 (C-20). MS (*m/z*) 438 (M^+).

17α-Cyclopentylacetoxy-16β-methylpregna-1—4,6-triene-3,20-dione 8 A solution of steroid **6** (1 g, 2.21 mmol) and DDQ (2.21 g, 26.44 mmol) in dioxane (90 ml) was allowed to reflux for 2 d. Upon cooling, the precipitated 2,3-dichloro-5,6-dicyanohydroquinone was filtered off. To the filtrate was added 3% aqueous sodium hydroxide solution (100 ml) and chloroform (100 ml); the mixture was stirred for 5 min. The organic phase was separated, washed 3 times with 3% aqueous sodium hydroxide solution and water. It was dried over anhydrous sodium sulfate and the solvent was removed in vacuum. The crude product was purified by silica gel column chromatography. Hexane–ethyl acetate (6 : 4) eluted 850 mg, 1.74 mmol (70%) of pure product **8**, mp 163—164°C. UV (nm) 222, 256, 299 (ε=15200, 13000, 18000 respectively). IR (KBr) cm^{-1}: 1724, 1710, 1668, 1649. ^{1}H-NMR ($CDCl_3$) δ: 0.8 (3H, s), 1.1 (3H, s), 1.41H, (3H, d, J=2 Hz), 2.0 (3H, s), 2.4 (2H, d, J=3 Hz), 6.1 (1H, d, J=2 Hz), 6.4 (1H, d, J=2 Hz), 6.6 (1H, d, J=2 Hz), 6.8 (1H, d, J=3 Hz). ^{13}C-NMR ($CDCl_3$) δ: 15.0 (C-18), 19.8 (C-19), 28.0 (C-21), 130.0 (C-6), 164.0 (C-5), 173.0 (ester carbonyl), 186.0 (C-3), 204.0 (C-20). MS (*m/z*) 450 (M^+).

Animal and Tissues Adult male Syrian Golden hamsters (150—200 g) were obtained from the Metropolitan University-Xochimilco of Mexico. Gonadectomies were performed under light ether anesthesia 30 d before treatment. Animals were sacrificed by ether anesthesia. The seminal vesicles were immediately removed, blotted and weighed prior to their use. Tissues used in the metabolic experiment were homogenized with a tissue homogenizer (model 985-370; variable speed 5000—30000 rpm, Biospec Products, Inc.).

Animal Treatment The biological activity of steroids **4** to **8** (Fig. 1) were determined in gonadectomized male hamsters divided in several groups. Daily subcutaneous injections of 400 μg of the steroids **4**—**8** (Fig. 1) dissolved in 200 μl of sesame oil were administered for 6 d together with 200 μg of T. Three groups of animals were kept as a control, one was injected with 200 μl of sesame oil, the second with 200 mg of testosterone and the third with T together with finasteride for 6 d. After the treatment, the animals were sacrificed by ether anesthesia and the seminal vesicles were dissected and weighed.

***In Vitro* Metabolic Studies with Seminal Vesicles** Homogenates from male hamster seminal vesicles (35.34 mg of protein) were prepared from intact adult male animals, using Krebs–Ringer phosphate buffer solution at pH of 6. Tissue preparations were incubated[11] in duplicate with 3.4 μCi of [^{3}H]T (specific activity 95 Ci/mmol) in the presence of 0.5 mM $NADPH^+$ and 12.5 mM glucose-6-phosphate plus 0.8×10^{-6} g of glucose-6-phosphate dehydrogenase[1] and 8.7 μM of finasteride or steroids **4**—**8** in a Dubnoff metabolic incubator at 37 °C for 60 min in the presence of air. The final incubation volume was 3 ml. The same experiment was performed with each one of the new steroidal compounds. Incubation without tissues was used as a control. The incubation was terminated by addition of dichloromethane and the [^{3}H] steroid was extracted (4×) using 10 ml of dichloromethane. The protein content of the homogenates was determined by Bradford's dye-binding method[12] using bovine serum albumin (BSA) as the standard. Isolation and purity assessment of radioactive DHT was carried out. The isolated compound was purified with steroid carriers (T and DHT) by a thin layer chromatographic system (chloroform : acetone=9 : 1). The steroid carriers were detected using phosphomolibdic acid reagent and an ultraviolet lamp (254 nm). Radioactivity was determined in a Packard Tri carb 2100 TR liquid scintillation Analyzer, using Ultima Gold (Packard Downers Grove, IL) as counting vehicle. The counting efficiency of [^{3}H] was 67%. The losses of radioactivity during the procedure were calculated in agreement with the results obtained from the control experiment without tissue. The formation of DHT was calculated and expressed as fmol of DHT/mg protein/h.

Cytosol Preparation for Androgen Receptor Competition Analysis Tissue homogenization was performed as described above[12] using TEDAM 1:3 20 mM Tris–HCl, pH 7.4 at 4 °C, 1.5 mM EDTA, 0.25 mM dithiothreitol, and 10 mM sodium molibdate containing 10% glycerol (v/v). The homogenate was centrifuged at 140000×***g*** for 1 h at 4 °C in a SW 60 Ti rotor (Beckman Instruments, Palo Alto, California). Cytosol protein content was determined by Bradford's dye binding method[12] using bovine serum albumin (BSA) as the standard.

Androgen Receptor Binding Assay Assays were performed essentially as described by Cabeza *et al.*[13]

Saturation Curves and Determination of the Binding Parameters Binding sites were saturated in the presence of increasing concentrations (0.5—2.5) of [^{3}H]DHT. Results were analyzed with Scatchard plots,[14] the calculation of affinity (K_d) and the total number of sites (B_{max}) were determined by linear regression.

Aliquots of 120 μl of cytosol (2.64 mg of protein) were incubated (in duplicate) for 18—20 h at 4 °C in tubes containing 0.5—2.5 nM [^{3}H]DHT. Parallel sets of tubes containing identical concentrations of [^{3}H]DHT plus 100-fold excess of radioinert DHT were also incubated. Dextran coated charcoal (800 μl) in TEDAM buffer was added and the mixture was incubated for 45 min at 4 °C. The tubes were vortexed and immediately centrifuged at 800×***g*** for 10 min.

Competition Analysis of Synthesized Steroids for ARs The competition analysis of steroids **4**—**8** for the androgen receptors of castrated male hamster seminal vesicles was determined on 58 animals (150—200 g), selected at random and gonadectomized 30 d before the experiments. The animals were kept in a room with controlled temperature (22 °C) and light–dark periods of 12 h. Food and water were provided *ad libitum*.

Androgen receptor competition studies were performed essentially as described by Cabeza *et al.*[13] and are briefly summarized below.

Competition Studies For competition experiments, tubes contained 1.26 nM [^{3}H]DHT (specific activity 110 Ci/mmol) plus a range of increasing concentrations (50—500 μM) of the following unlabeled steroid hormones: 5-α DHT, and **4**—**8** were prepared.

Aliquots 120 μl of cytosol (2.64 μg of protein) were added and incubated (in duplicate) for 18—20 h at 4 °C in tubes mentioned above. Parallel sets of tubes containing identical concentrations of [^{3}H]DHT plus a 100-fold excess of radio inert DHT were also incubated; 800 μl of dextran-coated charcoal in TEDAM buffer was added and the mixture was incubated for 45 min at 4 °C. The dextran was agitated during 30 min before the addition of charcoal to the mixture. The tubes were vortexed and incubated for 45 min at 4 °C. After the tubes were centrifuged at 800×***g*** for 10 min; aliquot (200 μl) were subsequently submitted to radioactive counting. Specific binding was determined by subtracting the mean disintegration per minute (dpm) in the presence of excess unlabeled steroids from the mean dpm of corresponding tubes containing only [^{3}H]DHT. Results were analyzed using one-way analysis of variance with EPISTAT software. The inhibition constant (K_i) of each compound was calculated according to the procedures described by Cheng and Prusoff.[17]

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