# New Progesterone Esters as 5α-Reductase Inhibitors

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**The pharmacological activity of four new progesterone derivatives: 4-bromo-17α-(*p*-fluorobenzoyloxy)-4-pregnene-3,20-dione (7), 4-bromo-17α-(*p*-bromobenzoyloxy)-4-pregnene-3,20-dione (8), 4-bromo-17α-(*p*-chlorobenzoyloxy)-pregnene-3,20-dione (9) and 4-bromo-17α-(*p*-toluoyloxy)-4-pregnene-3,20-dione (10) was determined. These compounds were evaluated as 5α-reductase inhibitors on gonadectomized hamster seminal vesicles and flank organs. The pharmacological data of this study indicate that compounds 7 and 9 having at C-17 *p*-fluorobenzoyloxy and *p*-chlorobenzoyloxy ester functions respectively showed the highest antiandrogenic effect as measured by the reduction of the weight of the seminal vesicles. In the flank organ model, the same compounds 7 and 9 exhibited a smaller diameter, 1.8 and 1.0 mm, respectively, than the commercially available finasteride 3 (2.3 mm), thus indicating a higher inhibitory effect on 5α-reductase enzyme. Steroid 7 showed a higher inhibitory activity on the conversion of T to DHT (Fig. 3) than the presently used finasteride, thus indicating a higher antiandrogenic effect. The nonsubstituted benzoyloxy ester (compound 15) showed a lower antiandrogenic activity as measured in the seminal vesicles model than the *p*-substituted benzoyloxy compounds.**

**Key words** flank organ; seminal vesicle; 5α-reduction; T-conversion; C-16 substituent

Prostate cancer is now the most common malignancy and the second leading cause of cancer deaths in North American males. Androgen antagonists offer a potentially useful treatment for androgen mediated diseases such as: prostate cancer, hirsutism, acne, seborrhea, androgenic alopecia and benign prostatic hyperplasia.[1)] Although surgery is presently the usual treatment for prostatic cancer, there are several other alternative modalities currently available for the treatment of these androgen dependent afflictions.[2)] Dihydrotestosterone **2** (DHT) (Fig. 1), the 5α-reductase metabolite of testosterone **1** (T) has been implicated as a causative factor in the progression of these diseases.[3—5)] It has also been observed that DHT interacts more efficiently with the androgen receptors than testosterone. This fact indicates that the logical step in the treatment of these diseases should be the blocking of the enzyme 5α-reductase[6)] for the inhibition of DHT-receptor complex formation.[6)]

The most extensively studied class of 5α-reductase inhibitors is the 4-azasteroids[7,8)] which includes the drug finasteride **3**. This compound is the first 5α-reductase inhibitor approved in the U.S.A. for the treatment of BPH. This drug has approximately a 100-fold greater affinity for type 2-5α-reductase than for the type 1 enzyme. In humans, finasteride **3** decreases prostatic DHT **2** levels by 70—90% and reduces prostate size, while T tissue levels remain constant.[9)] The use of finasteride **3** demonstrated a sustained improvement in the treatment of androgen dependent diseases and reduction in prostate specific antigen (PSA) levels.[10)] Related analogs **4**, **5** and **6** (Fig. 1) have also shown effectiveness *in vitro* and *in vivo*.[11—14)]

In previous studies,[15)] we found that the C-4 chloro analog combined with a C-17 benzoyloxy group on the progesterone skeleton exhibited a higher antiandrogenic activity than the C-4 bromo compound. This phenomenon could probably be explained by taking into consideration the higher electronegativity of C-4 chlorine atom as compared to the C-4 bromo compound.

On the basis of this hypothesis, that an electronegative group in the steroidal skeleton increases the antiandrogenic activity, we synthesized several new C-4 bromo analogs (lower electronegativity at C-4) having more electronegative substituents at C-17 position (*p*-halo substituted phenyl groups) with the idea of ascertaining if higher electronegativity of C-17 side chain increases the antiandrogenic activity. This activity was tested on the 5α-reductase inhibition in flank organs and seminal vesicle models.

Flank organs are pilosebaceous structures which are androgen dependent. In males, these glands measure 8 mm and shrink upon castration,[16)] until they look like those of females; however, daily injections of T or DHT restores them to their original size.[16)] The presence of 5α-reductase in flank organs has been demonstrated before[17,18)] as well as the inhibition of this enzyme by finasteride.[19)] Many steroidal and non-steroidal compounds have been evaluated as antiandrogens using flank organs as a model.[13)]

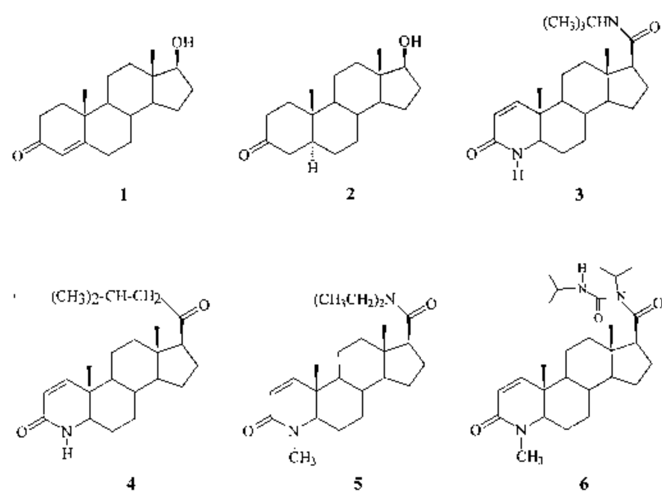


Fig. 1. Steroids Structures

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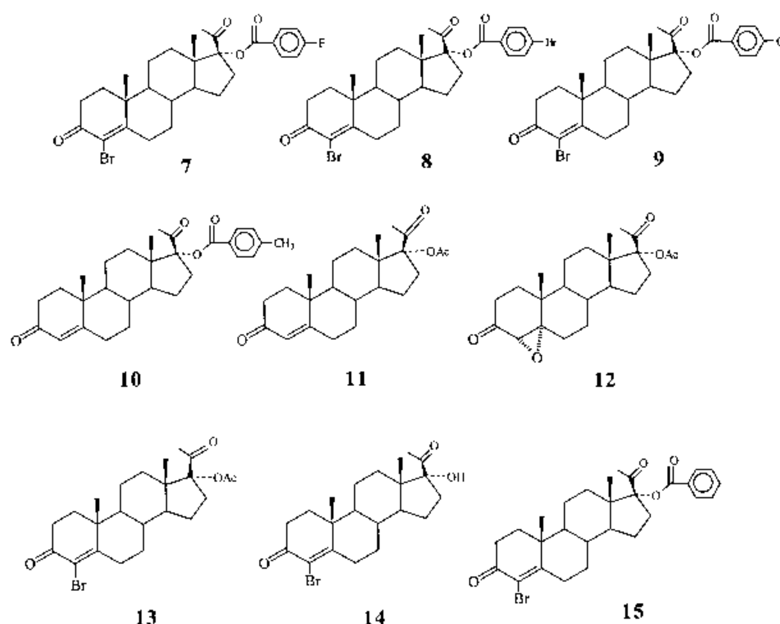

Fig. 2. Synthesis of New Steroids

Seminal vesicles are male accessory glands that are also 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.[14,16)]

In this study we evaluated the following compounds: 4-bromo-17$\alpha$-(*p*-fluorobenzoyloxy)-4-pregnene-3,20-dione **7**, 4-bromo-17$\alpha$-(*p*-bromobenzoyloxy)-4-pregnene-3,20-dione **8**, 4-bromo-17$\alpha$-(*p*-chlorobenzoyloxy)-4-pregnene-3,20-dione **9**, 4-bromo-17$\alpha$-(*p*-toluoyloxy)-4-pregnene-3,20-dione **10** and 4-bromo-17$\alpha$-benzoyloxy-4-pregnene-3,20-dione **15**. These compounds were prepared from the commercially available 17$\alpha$-acetoxyprogesterone **11** (Fig. 2).

## Chemistry

As shown in Fig. 2, the active compounds **7**—**10** and **15** were synthesized from the commercially available 17$\alpha$-acetoxyprogesterone **11**. In the first step in **11**, the C-4 double bond was epoxydized with hydrogen peroxide in basic medium, and the desired compound **12** underwent a ring opening reaction with hydrogen bromide to form the bromo derivative **13**. Treatment of **13** with aqueous sodium hydroxide solution afforded the corresponding alcohol **14**. The preparation of the intermediate compounds **12**—**15** is described in reference 15. The new progesterone esters **7**—**10** were synthesized when the alcohol **14** was esterified with the corresponding *p*-substituted benzoic acid and trifluoroacetic anhydride.[20)]

## Methods and Results

To test the antiandrogenic effect of the new steroids **7**—**10** and **15**, we used the flank organ model,[13,16,17)] the seminal vesicles[14)] and the effect of the new steroids on the *in vitro* metabolism of [$^3$H]T to [$^3$H]DHT in castrated male hamster seminal vesicle homogenates of gonadectomized male hamsters.[5,17)]

**Pigmented Spot** Table 1 shows the diameter of the pigmented spot of the control (vehicle), testosterone and steroids (**3**, **7**—**10** and **15**) treated hamsters. Testosterone increases significantly ($p<0.005$) the diameter of the pigmented spot whereas **3** decreases the size of the spot ($p<0.005$) of the animals previously treated with testosterone. The most effective compounds in diminishing the diameter of the pigmented spot were steroids **7** and **9**, whereas **10** and **15** showed a lower biological activity as 5$\alpha$-reductase inhibitor. Compound **8** exhibited the same inhibitory activity as **3**.

Table 1. Diameter of the Pigmented Spot and Weight of Seminal Vesicles±S.D. from Animals Receiving Different s.c. Treatments

| Treatment (mg) | Diameter of the pigmented spot (mm) | Weight of seminal vesicles (mg) |
|---|---|---|
| CONTROL | 2.0±0.89 | 152.3±15.6 |
| T (0.2) | 4.0±0.90 | 321.4±79.0 |
| T+**3** | 2.3±0.89 | 199.0±14.0 |
| T+**7** | 1.8±0.90 | 180.3±42.9 |
| T+**8** | 2.3±0.50 | 213.4±18.5 |
| T+**9** | 1.0±0.00 | 199.3± 2.5 |
| T+**10** | 2.8±0.50 | 290.0±65.3 |
| T+**15** | 2.5±0.50 | 230.0±20.0 |

Significant differences ($p<0.05$) were observed between T and control or T plus each one of the different steroid treatments.

**Seminal Vesicles** When **1** (T) and **3** were injected together, the weight of the seminal vesicles decreased significantly ($p<0.005$) as compared to testosterone-treated animals (entry 2, Table 1). However, when the same experiment was carried out with compound **9**, the weight of the seminal vesicles decreased to the level of finasteride **3**, thus showing a comparable antiandrogenic effect.

Since the weight of the seminal vesicles depends on the 5$\alpha$-reduced androgens,[5)] it was important to determine the effect of the new steroidal compounds (**7**—**10**) on the conversion of **1** (T) to **2** (DHT). The dichloromethane extract from castrated male hamster seminal vesicles was subjected to TLC analysis. The zone corresponding to DHT (**2**) standard (*Rf* value of 0.33) of each experimental chromatogram

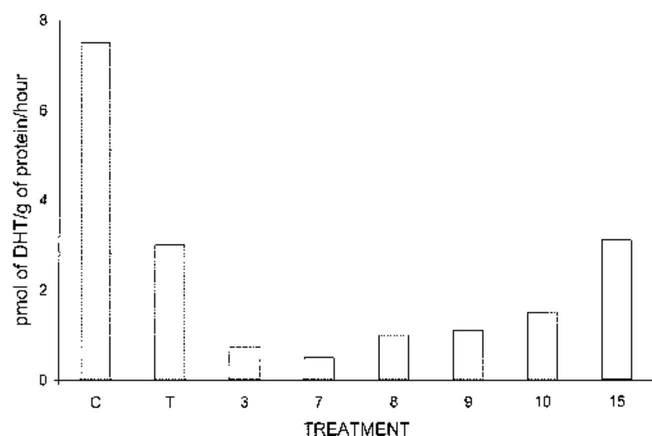


Fig. 3. Effect of Different Synthetic Steroids on *in Vitro* Conversion of [$^3$H]T to [$^3$H]DHT in Castrated Male Hamster Flank Organs

Significant differences ($p<0.05$) were observed between T and control or T plus each one of the different steroid treatments.

was eluted and the radioactivity determined.

The results (Fig. 3) 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, thus indicating that finasteride **3** is a good inhibitor for the conversion of T to DHT at pH of 6. Figure 3 also shows the effect of the new steroids **7**—**10** and **15** on the rate of DHT formation expressed as pmoles of DHT/g of protein/h. The four compounds (**7**—**10**) produced a much lower concentration of DHT as compared to testosterone, however, only compound **7** exhibited a higher antiandrogenic activity than the commercially available **3**.

## Discussion

This study reports the effect of several new progesterone derivatives **7**—**10** and **15**[15] having a bromine atom at C-4 and a different benzoyloxy moiety at C-17. Apparently the bromine atom at C-4 enhances the biological activity as previously demonstrated by our group.[15] In the flank organ model, compounds **7** and **9** exhibited a smaller diameter of the pigmented spot than that of finasteride **3**, thus indicating a higher inhibitory activity to the enzyme 5$\alpha$-reductase. All four compounds **7**—**10** (Fig. 3) inhibited the conversion of T to DHT as compared to T, although compound **7** having a fluorine atom in the *p*-position of the benzoyloxy group showed a higher antiandrogenic effect than finasteride **3**, as demonstrated by the lower values of pmoles of DHT/g of protein/h. Compound **9**, however, (a chlorine substituent in the benzoyloxy moiety) has a lower activity than **3** in the conversion of T to DHT contrary to that observed in flank organs. This phenomenon could probably be explained by taking into consideration the presence of androgen receptors in flank organs.[17] In this model, compound **15** failed to show any appreciable activity, probably due to the absence of a *p*-substituent in the benzoyloxy moiety (Fig. 2).

In the seminal vesicle model (Table 1), all four compounds **7**—**10** showed a lower weight of the seminal vesicles as compared to the testosterone treated hamsters, however, steroid **7** exhibited a lower weight than that of the control finasteride thus demonstrating a higher antiandrogenic activity. This enhancement of the biological activity probably results from the presence of the strongly electronegative fluorine atom in the *p*-position of the benzoyloxy group at C-17 as compared to compounds **8** and **9**, which have a less electronegative halogen atom. On the other hand, compound **15** with a non-substituted phenyl group at C-17 side chain (lower electronegativity) showed in both experiments (flank organs and seminal vesicles) consistently lower antiandrogenic activity (Table 1).

## 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 $Me_4Si$ ($\delta=0$) 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, was provided by New England Nuclear Corp. (Boston, MA, U.S.A.). Radioniert T and 5$\alpha$-DHT were supplied by Steraloids (Wilton, NH, U.S.A.). D($\alpha$)-Glucose, was purchased from Merck (Mexico). Sigma Chemical Co. (St. Louis, MO, U.S.A.) supplied $NADH^+$ and the finasteride was obtained by extraction from Proscar (Merck, Sharp & Dohme).

**Synthesis of the Steroid Compounds** The preparation of the intermediate compounds **12**—**15** is described in ref. 15.

4-Bromo17$\alpha$-(*p*-fluorobenzoyloxy)-4-pregnene-3,20-dione **7**: A solution of trifluoroacetic anhydride (5.18 g; 0.024 mmol), *p*-fluorobenzoic acid (3.46 ; 0.0024 mmol) and *p*-toluenesulfonic acid (0.3 g; 0.0017 mmol) was stirred under nitrogen for 3 h at room temperature. Compound **14** (1.0 g; 0.0024 mmol) dissolved in chloroform (5 ml) was added and the resulting solution was allowed to stir for 3 h at room temperature. Water (5 ml) was added and the mixture was neutralized with an aqueous solution of sodium bicarbonate to a pH of 7. The reaction mixture was extracted with chloroform, the combined organic extracts were washed with water, dried over anhydrous sodium sulfate and the solvent evaporated in vacuum. The crude product was purified by silica gel column chromatography (hexane : AcOEt=7 : 3) eluted 0.58 g; 0.001 mmol (44.57%) of the pure compound **7**, mp 188—190 °C. UV: 253 nm (log $\varepsilon$, 4.12). IR (KBr): 1268, 1702, 1718 cm$^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 0.77 (3H, s), 1.24 (3H, s), 2.15 (3H, s), 7.2 (2H, m), 8.1 (2H, m). $^{13}$C-NMR, $\delta$: 14.5 (C-18), 18.2 (C-19), 21.3 (C-21), 128.5 (C=C–Br), 170.3 (PhCOO), 190.6 (C-3) and 203.4 (C-20). MS (*m/z*) 531 ($M^+$).

4-Bromo-17$\alpha$-(*p*-bromobenzoyloxy)-4-pregnene-3,20-dione **8**: A solution of trifluoroacetic anhydride (7.74 g; 0.035 mmol), *p*-bromobenzoic acid (4.94 g; 0.024 mmol) and *p*-toluenesulfonic acid (0.3 g; 0.0017 mmol) was stirred under nitrogen for 3 h at room temperature. Compound **14** (1.0 g; 0.0024 mmol) dissolved in chloroform (5 ml) was added and the resulting solution was allowed to stir for 5 h at room temperature. Water (5 ml) was added and the reaction mixture was neutralized with an aqueous solution of sodium bicarbonate to a pH of 7. The mixture was extracted with chloroform, the combined organic extracts were washed with water, dried over anhydrous sodium sulfate and the solvent evaporated in vacuum. The crude product was purified by silica gel column chromatography (hexane : AcOEt=7 : 3) eluted 0.48 g; 0.0008 mmol (32.6%) of the pure compound **8**, mp 210—212 °C. UV: 254 nm (log $\varepsilon$ 4.12) IR (KBr): 610, 1705, 1725 cm$^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 0.85 (3H, s), 1.15 (3H, s), 2.0 (3H, s), 7.6 (2H, m), 7.95 (2H, m). $^{13}$C-NMR, $\delta$: 16.5 (C-18), 18.1 (C-19), 22.2 (C-21), 129.4 (C=C–Br), 175.6 (PhCOO), 191.2 (C-3), 205.0 (C-20). MS (*m/z*): 592 ($M^+$).

4-Bromo-17$\alpha$-(*p*-chlorobenzoyloxy)-4-pregnene-3,20-dione **9**: A solution of trifluoroacetic anhydride (5.18 g; 0.024 mmol), *p*-chlorobenzoic acid (3.85 g; 0.024 mmol) and *p*-toluenesulfonic acid (0.3 g; 0.0017 mmol) was stirred under nitrogen for 3 h at room temperature. Compound **14** (1.0 g; 0.0024 mmol) dissolved in chloroform (6 ml) was added and the resulting solution was stirred for 2 h at room temperature. Water (6 ml) was added and the mixture was neutralized with an aqueous solution of sodium bicarbonate to a pH of 7. The reaction mixture was extracted with chloroform, the combined organic extracts were washed with water, dried over anhydrous sodium sulfate and the solvent evaporated in vacuum. The crude product was purified by silica gel column chromatography (hexane : AcOEt=7 : 3) eluted 0.85 g; 0.0015 mmol (63%) of the pure product **9**, mp 190—192 °C. UV: 253 nm (log $\varepsilon$, 4.12). IR: 740, 1700 cm$^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 0.7 (3H,

s),1.05 (3H, s), 2.3 (3H, s), 7.2 (2H, m), 8.0 (2H, m). $^{13}$C-NMR, $\delta$: 12.3 (C-18), 16.2 (C-19), 25.3 (C-21), 134.5 (C=C–Br), 172.6 (PhCOO), 194.8 (C-3), 202.6 (C-20). MS (*m/z*): 547 ($M^+$).

4-Bromo-17$\alpha$-(*p*-toluoyloxy)-pregnene-3,20-dione **10**: A solution of trifluoroacetic anhydride (5.18 g; 0.024 mmol), *p*-toluic acid (3.36 g; 0.024 mmol) and *p*-toluenesulfonic acid (0.3 g; 0.0017 mmol) was stirred under nitrogen for 3 h at room temperature. Compound **14** (1.0 g; 0.0024 mmol) dissolved in chloroform (5 ml) was added and the reaction mixture was stirred for 30 min at room temperature. Water (5 ml) was added and the mixture was neutralized with an aqueous solution of sodium bicarbonate to a pH of 7. The reaction mixture was extracted with chloroform, the combined organic extracts were washed with water, dried over anhydrous sodium sulfate and the solvent evaporated in vacuum. The crude product was purified by silica gel column chromatography (hexane : AcOEt=7 : 3) eluted 0.95 g; 0.0018 mmol (73.4%) of the pure compound **10**, mp 196—198 °C. UV: 252 nm (log $\varepsilon$, 4.12). IR: (KBr) 1695, 1718 cm$^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 0.85 (3H, s), 1.2 (3H, s), 2.2 (3H, s), 7.4 (2H, m), 8.1 (2H, m). $^{13}$C-NMR, $\delta$: 14.5 (C-18), 18.3 (C-19), 23.6 (C-21), 130.8 (C=C–Br), 170.4 (PhCOO), 190.8 (C-3), 204.6 (C-20). MS (*m/z*): 527 ($M^+$).

**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.).

**Flank Organ Test** The flank organ test was performed as previously reported.[14] The effect of the new steroids on the flank organs of male hamsters, which had been gonadectomized 30 d prior to the experiment was determined on 8 groups of 4 animals/experiment which were selected at random. 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*.

Daily subcutaneous injections of 200 $\mu$g of the steroids **7—10** and **15** (Fig. 2) dissolved in 200 $\mu$l of sesame oil were administered for 6 d together with 200 $\mu$g of T. Three groups of animals were kept as control, one was injected with 200 $\mu$l of sesame oil, the second with 200 $\mu$g of testosterone and the third with T with finasteride for 6 d. After the treatment, the animals were sacrificed by ether anesthesia. Both flank organs of the animals were shaven and the diameter of the pigmented spot was measured. The results were analyzed using one-way analysis of variance with EPISTAT software. The results of this study are described in Table 1.

**Seminal Vesicles Test** In this experiment, we determined the effect of the new steroids **7—10** and **15** on the seminal vesicles from castrated male hamsters. After the sacrifice, the seminal vesicles were dissected and weighed. Two separate experiments were performed for each group of steroid treated animals. The results were analyzed using one-way analysis of variance with EPISTAT software. The results of this experiment are tabulated in Table 1.

***In Vitro* Metabolic Studies with Seminal Vesicles** Homogenates from male hamster seminal vesicles (19—70 mg of protein) were prepared from intact adult male animals, using Krebs-Ringer phosphate buffer solution at pH of 6. Tissue preparations were incubated[14] in duplicate with 3 $\mu$Ci of [$^3$H]T (specific activity 95 Ci/mmol) in the presence of 1 mM $NADPH^+$ and 8.7 $\mu$M of finasteride or steroids **7—10** and **15** 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[21] using bovine serum albumin (BSA) as the standard. Isolation and purity assessment of radioactive dihydrotestosterone (DHT) was carried out by the reverse isotope dilution technique. 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 3255 liquid scintillation spectrometer, using standard solution as the counting vehicle. The counting efficiency of [$^3$H] was 47%. 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 pmol of DHT/g protein/h.

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