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RESEARCH ARTICLE

Synthesis and biological activity of progesterone derivatives as 5α-reductase inhibitors, and their effect on hamster prostate weight

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Abstract

In this study, we report the synthesis and biological evaluation of four 6- and 17-substituted progesterone derivatives (**7–10**). These compounds were prepared from the commercially available 17α-acetoxyprogesterone. The biological effect of these steroids was demonstrated in *in vivo* as well as *in vitro* experiments. In the *in vivo* experiments, we measured the activity of **6–10** on the weight of the prostate glands of gonadectomized hamsters treated with testosterone (T). For the studies *in vitro*, we determined the IC_{50} value by measuring the concentration of steroidal derivative that inhibited 50% of the activity of 5α-reductase present in the human prostate. The results from this work indicated that compounds **6–9** significantly decreased the weight of the prostate as compared to testosterone-treated animals and this reduction of prostate weight was comparable to that produced by finasteride. Steroid **8** was the most effective of the tested compounds. However, compound **10** did not exhibit this capacity. On the other hand, **6–9** exhibited a high inhibitory activity for the human 5α-reductase enzyme with IC_{50} values of 10, 70, 22, and 19 nM, respectively. However, **10** was not effective for the inhibition of 5α-reductase activity. In conclusion, the compounds that contained the acetate ester moiety in the molecule (**6**, **7**, **8**, and **9**) inhibited the activity of 5α-reductase and decreased the weight of the prostate. Nevertheless, the double bond in ring B seems to diminish the inhibitory potency (**7** and **9**), since **6**, which does not possess a double bond at **C-**6, had the highest inhibitory activity (the lowest IC_{50} value).

Keywords: *Human prostate; hamster prostate; 5α-reductase; androgen receptor; synthesis of 6- and 17-substituted progesterone derivatives*

Introduction

The androgen metabolism of target tissues is regulated by a variety of androgen metabolizing enzymes[1]. Among these, the enzyme 5α-reductase possesses the highest potential activity[2]. 5α-Reductase (EC 1.399.5) is the NADPH (reduced nicotinamide adenine dinucleotide phosphate)-dependent enzyme capable of reducing testosterone (T) **1** to dihydrotestosterone (DHT) **2** (Figure 1)[3]. The product of this enzyme is accumulated into the nuclei of responsive cells, such as those of the animal prostate[3,4]. There exist two isozymes[5] of 5α-reductase, type 1 and type 2, that are differentially expressed in various tissues such as skin, liver, prostate, seminal vesicles, and epididymides[5]. The role of DHT in benign prostate hyperplasia, the most common affliction in aging men, is well established, and DHT is also important in the viability of androgen-responsive cancer cells. These physiological roles of DHT have contributed to the interest in finding potent 5α-reductase inhibitors.

Various steroidal and non-steroidal compounds had been developed for the treatment of benign prostatic hyperplasia and prostate cancer. The most extensively studied 5α-reductase inhibitors are the 4-aza-steroids, with

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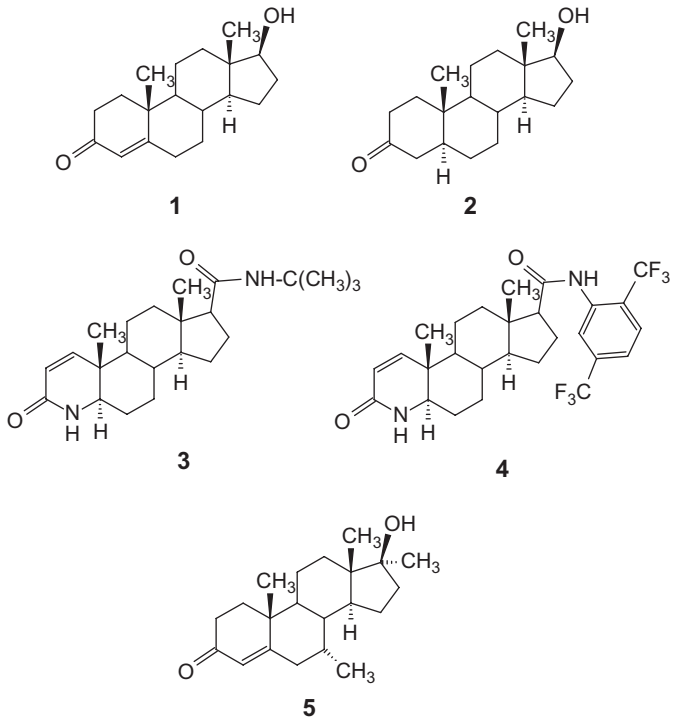


Figure 1. Reference compounds and their structures: **1**, testosterone; **2**, dihydrotestosterone; **3**, finasteride; **4**; dutasteride; **5**, mibolerone.

finasteride **3** and dutasteride[6] **4** (Figure 1) being the most active 5α-reductase inhibitors currently used. Finasteride **3** (Figure 1) is a potent inhibitor of both rat and human 5α-reductase enzyme activities in an irreversible way. This compound is a time-dependent inhibitor of type 2 5α-reductase enzyme activity, and, together with dutasteride **4**, is the only steroidal inhibitor approved for the treatment of benign prostatic hyperplasia in the United States[6].

Previously, the synthesis of several non-steroidal compounds that showed activity as inhibitors of 5α-reductase have been reported. These are derivates of phenanthrene or butyric acids having an indole group[6].

Previous studies with 16β-methyl-4,6-pregnadiene-3,20-dione derivatives having a halogen-containing ester side chain at C-17[7] indicated that these compounds exhibit higher *in vivo* and *in vitro* activities as compared to the commercially available finasteride **3**. In view of the fact that these compounds show a high 5α-reductase inhibitory activity, in this article we describe the synthesis of four progesterone derivatives having a similar carbon skeleton (**7–10**), as well as the pharmacological effect and their mechanism of action.

Materials and methods

Chemicals and radioactive materials

Solvents were laboratory grade or better. Melting points were determined on a Fisher Johns melting point apparatus and are uncorrected. ^{1}H-nuclear magnetic resonance (NMR) and ^{13}C-NMR spectra were taken on Varian Gemini 200 and VRX-300 spectrometers, 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. Infrared (IR) spectra were recorded on a PerkinElmer 200s spectrometer.

(1,2,6,7-^{3}H) Testosterone ([1,2,6,7-^{3}H]T) specific activity (95 Ci/mmol) and (17α-methyl-^{3}H) mibolerone ([^{3}H]MIB) **5** (Figure 1) specific activity (70–87 Ci/mmol) were provided by PerkinElmer Life and Analytical Sciences (Boston, MA). Radioinert T, 5α-dihydrotestosterone, and MIB were supplied by Steraloids (Wilton, NH). Sigma Chemical Co. (St. Louis, MO) provided NADPH. Finasteride was obtained by extraction from Proscar (Merck, Sharp & Dohme). The tablets were crushed and extracted with chloroform, and the solvent was eliminated under vacuum; the crude product was purified by silica gel column chromatography.

Synthesis of the steroidal derivatives

Synthesis of the biologically active compounds **7–10** has previously been reported[8]. In view of the fact that we made several changes to the reported synthesis, we describe below the modified synthesis of compounds **7–10**. These compounds were prepared from the commercially available 17α-acetoxyprogesterone (**6**), which also exhibits biological activity (Figure 2).

3,20-Dioxopregna-4,6-dien-17α-yl acetate 7

A solution of steroid **6** (1 g, 2.7 mmol), chloranil (0.7 g, 2.8 mmol), and glacial acetic acid (8 mL) in toluene (2 mL) was allowed to reflux for 4 h. The solution was cooled to room temperature and the resulting tetrachlorohydroquinone was filtered off. Water (10 mL) was added to the filtrate and it was extracted with chloroform (three times). The organic phase was washed with 11% aqueous sodium hydroxide solution (12.5 mL) and water and dried over anhydrous sodium sulfate; the solvent was eliminated under vacuum. The resulting crude product was recrystallized from ethyl acetate. Yield 700 mg, 1.89 mmol (70.3%) of pure product **7**, mp 224–226°C. UV (nm) 282 (ε = 27,400). IR (KBr) cm^{-1}: 2978, 1730, 1700, 1675, 1620. ^{1}H-NMR ($CDCl_3$) δ: 0.7 (3H, s, H-18), 1.1 (3H, s, H-19), 2.0 (3H, s, ester), 2.1 (3H, s, H-21), 5.7 (1H, q, J = 2 Hz, H-7), 6.0 (1H, s, H-4), 6.2 (1H, d, J = 10 Hz, H-6). ^{13}C-NMR ($CDCl_3$) δ: 14.3 (C-18), 16.3 (C-19), 21.2 (C-21), 123.9 (C-4), 128.3 (C-6), 130.3 (C-7), 170.6 (ester carbonyl), 199.9 (C-3), 203.8 (C-20). FAB-MS (m/z), calcd for $C_{23}H_{32}O_4$ 371.2195 (M + H), found 371.2135.

3,20-Dioxo-6,7-epoxypregn-4-ene-7α-yl acetate 8

A solution of steroid **7** (1 g, 2.7 mmol) and *m*-chloroperbenzoic acid (1.25 g, 2.7 mmol) in benzene (15 mL) was allowed to reflux for 4 h. The solvent was eliminated under vacuum and the resulting crude product was dissolved in chloroform (12.5 mL); a saturated aqueous sodium bicarbonate solution (15 mL) containing sodium bisulfate (1 g) was added and the mixture was stirred for 30 min at room temperature. The organic phase was separated, washed with water, and dried over anhydrous sodium sulfate; the solvent was eliminated under vacuum. The resulting crude product was

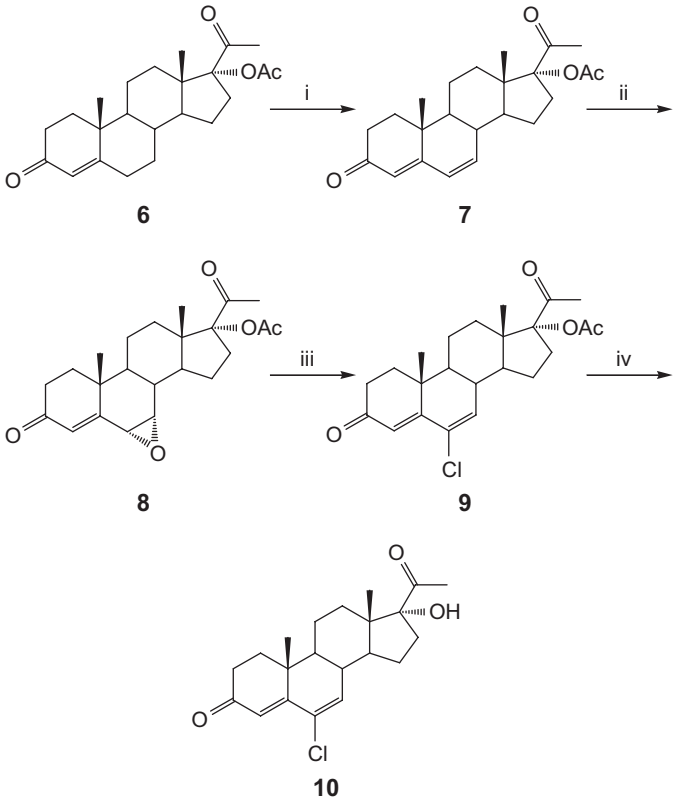


Figure 2. Synthesis of compounds **7–10**. Reagents and conditions: (i) chloranil, AcOH, toluene, reflux 4 h; (ii) *m*-chloroperbenzoic acid (mCPBA), benzene, reflux 4 h; (iii) HCl, Ac_2O, 24 h, room temperature; (iv) NaOH, MeOH, reflux 2 h.

recrystallized from methanol. Yield 450 mg, 1.17 mmol (43%) of pure product **8**, mp 245–246°C. UV (nm) 238 (ε = 15,600). IR (KBr) cm^{-1}: 2968, 1726, 1716, 1678. ^{1}H-NMR ($CDCl_3$) δ: 0.7 (3H, s, H-18), 1.1 (3H, s, H-19), 2.1 (3H, s, ester), 2.2 (3H, s, H-21), 2.8 (1H, d, *J* = 4 Hz, H-7), 3.5 (1H, d, *J* = 4 Hz, H-6), 6.2 (1H, d, *J* = 4 Hz, H-4). ^{13}C-NMR ($CDCl_3$) δ: 14.2 (C-18), 17.2 (C-19), 26.3 (C-21), 59.6 (C-7), 62.3 (C-6), 131.3 (C-4), 170.7 (ester carbonyl), 198.0 (C-3), 203.8 (C-20). FAB-MS (*m/z*) calcd for $C_{23}H_{30}O_5$ 387.2457 (M + H), found 387.2489.

6-Chloro-3,20-dioxopregna-4,6-diene-17α-yl acetate 9

A solution of steroid **8** (1 g, 2.6 mmol), acetic anhydride (110 mL), and 36% aqueous hydrochloric acid (60 mL) was stirred for 24 h at room temperature. Ice (50 g) was added and the precipitated crude product was isolated by filtration; it was purified by silica gel (70–30 mesh, ASTM) column chromatography (column size, 50 cm long and 0.9 cm wide) using a solvent system of hexane:ethyl acetate (85:15). Yield 900 mg, 2.2 mmol (85.9%) of pure product **9**, mp 210–212°C. UV (nm) 283 (ε = 22,800). IR (KBr) cm^{-1}: 2964, 1730, 1710, 1675, 880. ^{1}H-NMR ($CDCl_3$) δ: 0.73 (3H, s, H-18), 1.2 (3H, s, H-19), 2.3 (3H, s, H-21), 4.8 (1H, s, hydroxyl proton), 5.9 (1H, d, *J* = 2.4 Hz, H-7), 6.3 (1H, s, H-4). ^{13}C-NMR ($CDCl_3$) δ: 15.2 (C-18), 16.5 (C-19), 27.6 (C-21), 123.8 (C-4), 128.3 (C-7), 130.2 (C-6), 198.8 (C-3), 210.7 (C-20). FAB-MS (*m/z*) calcd for $C_{23}H_{27}ClO_3$ 362.3145 (M + H), found 362.3137.

6-Chloro-17α-hydroxypregna-4,6-diene-3,20-dione 10

A solution of steroid **9** (1 g, 2.76 mmol), methanol (50 mL), and 2% aqueous sodium hydroxide solution was allowed to reflux for 2 h. Part of the methanol (25 mL) was eliminated by distillation from the reaction mixture and ice (30 g) was added. The precipitated crude product was isolated by filtration; it was recrystallized from methanol. Yield 510 mg, 1.32 mmol (56.9%) of pure product **10**, mp 195°C. UV (nm) 284.7 (ε = 23,235). IR (KBr) cm^{-1}: 3428, 3029, 1708, 1670, 884.^{1}H-NMR ($CDCl_3$) δ: 0.8 (3H, s, H-18), 1.2 (3H, s, H-19), 2.2 (3H, s, H-21), 4.8 (1H, s, hydroxyl proton), 6.2 (2H, m, H-6 and H-7), 6.3 (1H, s, H-4). ^{13}C-NMR ($CDCl_3$) δ: 15.3 (C-18), 16.5 (C-19), 27.4 (C-21), 129.0 (C-4), 130.5 (C-6), 138.3 (C-7), 198.8 (C-3), 210.7 (C-20). FAB-MS (*m/z*) calcd for $C_{23}H_{27}Cl0_3$ 362.6239 (M + H), found 362.5439.

Biological activity of steroidal compounds

The human prostate of a man aged 74 years, who had died from myocardial infarction, was kindly provided by Dr Avissai Alcántara from the Department of Pathology, the General Hospital (SS) in Mexico City, and stored at –70°C. Frozen human prostate was thawed on ice and minced with scissors. Unless specified, the following procedures were carried out at 4°C.

Animals and tissues

Adult male golden hamsters, 2.5 months old (150–200g), were obtained from the Metropolitan University in Xochimilco, Mexico. Gonadectomies were performed under pentobarbital anesthesia and the castrated hamsters were kept in a room with controlled temperature (22°C) and light–dark periods of 12 h. Food and water were provided *ad libitum*. After 30 days of maintaining these conditions, the hamsters were sacrificed with CO_2. This protocol was approved by the Institutional Care and Use Committee of the Metropolitan University of Mexico (UAM). Experiments with gonadectomized animals were carried out on eight groups of four animals/experiment.

In order to determine the binding of steroids **6–10** to the androgen receptors, adult rats, 8 months old, weighing 500 g, were gonadectomized 48 h prior to the experiment. In this study we used rats because the prostate gland is bigger, and there is no difference in binding activity between rat and hamster cytosol.

Prostates of rats were removed, blotted, weighed, and soaked in cold TEMD (40 mM tris-HCl, 3 mM ethylenediaminetetraacetic acid (EDTA), 20 mM sodium molybdate, dithiothreitol 0.5 mM, 10% glycerol at pH 8) prior to their use. Unless specified, all procedures were carried out in an ice bath.

Tissues were homogenized in one volume of buffer TEMD plus protease inhibitors (2 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg/mL antipain, 5 mM leupeptin[9]) in an ice bath with a tissue homogenizer (Teckmar, Cincinnati, OH). Homogenates were centrifuged at 140,000 × *g* for 60 min[10] in a SW 60 Ti rotor (Beckman Instruments, Palo Alto, CA).

The cytosolic fraction obtained from the supernatant liquid of the rat prostate homogenate described above was

stored at –70°C. Prostatic cytosol proteins (6 mg of protein in 200 μL) were determined by the Bradford method[11].

In vitro experiments

Human prostate was homogenized in two volumes of medium A (20 mM sodium phosphate, pH 6.5 containing 0.32 M sucrose, 0.1 mM dithiothreitol; Sigma-Aldrich) with a tissue homogenizer. Homogenates were centrifuged at 1500 × *g* for 20 min[12,13] in a SW 60 Ti rotor (Beckman Instruments). The pellets were separated, suspended in medium A, and kept at –70°C. The suspension, 5 mg of protein/mL for human prostate, determined by the Bradford method[11] was used as the source of 5α-reductase.

Determination of 5α-reductase activity

The enzyme 5α-reductase was assayed as previously described[12,13]. The reaction mixture contained a final volume of 1 mL: 1 mM dithiothreitol, sodium phosphate buffer 40 mM, at pH 6.5 for human prostate, 2 mM NADPH, 2 nM [1,2,6,7-^{3}H]T[14]. The reaction in duplicate was started when it was added to the enzymatic fraction (500 μg protein in a volume of 80 μL) incubated at 37°C for 60 min[12] and stopped by mixing with 1 mL of dichloromethane; this was considered as the end point. Incubation without tissue was used as a control. The mixture (incubation medium/dichloromethane) was agitated on a vortex for 1 min and the dichloromethane phase was separated and placed in another tube. This procedure was repeated four more times. The dichloromethane extract was evaporated to dryness under a nitrogen stream and suspended in 50 mL of methanol that was spotted on high-performance thin layer chromatography (HPTLC) Keiselgel 60 F_{254} plates. T and DHT were used as carriers, and were applied in different lanes on both lateral sides of the plates (T, T + DHT, and DHT). The plates were developed in chloroform–acetone, 9:1, and were air-dried; the chromatography was repeated twice more. The steroid carriers were detected using phosphomolybdic acid reagent (DHT) and with an ultraviolet (UV) lamp (254 nm) (T). After the plates were segmented into pieces of 1 cm each, these were cut off and the strips soaked in 5 mL of Ultima Gold (Packard). The radioactivity was determined in a scintillation counter (Packard Tri-Carb 2100 TR). The radioactivity content in the segment corresponding to T and DHT carriers was identified. Radioactivity with identical chromatographic behavior to the DHT standard was considered as the DHT transformation. Control incubations, chromatography separations, and identifications were carried out in the same manner as described above, except that the tubes did not contain tissue. The DHT transformation yields were calculated from the strips, accounting for the total radioactivity in the plate.

Determination of 50% inhibitory concentration of steroids 6–10 in human prostatic 5α-reductase

In order to calculate the IC_{50} values (the concentration of steroids **6–10** or finasteride required to inhibit 5α-reductase activity by 50%), six series of tubes containing increasing concentrations of these steroids (10^{-11}–10^{-3} M) were incubated in duplicate, in the presence of: 1 mM dithiothreitol, 40 mM sodium phosphate buffer pH 6.5, 2 mM NADPH, 2 nM [1,2,6,7-^{3}H]T, and 500 μg of protein from the enzymatic fraction in a final volume of 1 mL. The reaction was carried out in duplicate at 37°C for 60 min; 1 mL of dichloromethane was added to stop the reaction. The extraction and chromatographic procedures were carried out as described above. The radioactivity contained in the fraction corresponding to the DHT standard was determined as described above.

Androgen receptor competitive binding assay

For competitive binding studies, tubes containing 1 nM of [^{3}H] MIB plus a range of increasing concentrations (1×10^{-10}–4×10^{-7} M) of cold MIB **5** (Figure 1) and **6–10** in ethanol or chloroform, or in the absence of the competitor, were prepared[14]. Incubates also contained 200 nM triamcinolone, in ethanol (Sigma), to prevent interaction of MIB with glucocorticoid receptors and progesterone receptor. In all experiments the solvent was evaporated.

Aliquots of 200 μL of prostate cytosol were added and incubated in the presence of 300 μL of TEMD buffer containing protease inhibitors (duplicate) for 24 h at 4°C in the tubes as previously described. After incubation, 0.27 mL of saturated ammonium sulfate in TEMD buffer (35%) was added[10]. The mixture was further incubated for 1 h with occasional shaking to facilitate precipitation of the [^{3}H]MIB complex. The precipitate was collected by centrifugation at 10,000 × *g*, 10 min, and the pellet was redissolved in 0.5 mL of TEMD and mixed with 0.5 mL of 0.1% dextran-coated 1% charcoal in TEMD buffer. The mixture was incubated for 40 min at 4°C. To prepare the dextran-coated charcoal mixture, the dextran was agitated for 30 min before adding the charcoal. The tubes were agitated on a vortex and immediately centrifuged at 800 × *g* for 10 min to pellet the charcoal; aliquots (600 μL) were taken and submitted for radioactive counting. The IC_{50} of each compound was calculated according to a plot of concentration versus percentage of binding.

In vivo experiments

For the daily subcutaneous injections, 2 mg/kg of steroids **6–10** were dissolved in 200 μL of sesame oil and administered for 6 days to gonadectomized animals, together with 1 mg/kg of testosterone. Three groups of gonadectomized animals were kept as control; one was injected with 200 μL of sesame oil, the second with 1 mg/kg of testosterone, and the third with T plus 1 mg/kg of finasteride, for 6 days. After the treatment, the animals were sacrificed with CO_2. The prostate gland of each animal was 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 and Dunnett's method to compare means, using JMP IN 5.1 software.

Results

In vitro experiments

The radioactive zone that had identical chromatographic behavior to the standard T (retention factor (Rf) value of

0.56) corresponded to 70% of the total radioactivity in the plate. The radioactivity contained in the zone corresponding to the DHT standard (Rf value of 0.67) of the experimental chromatogram was identified as the transformed DHT and corresponded to 27% of the total radioactivity in the plate. This result was considered to be 100% of the activity of 5α-reductase for the development of inhibition plots. Unmodified [^{3}H]T was identified (Rf value of 0.56) from control incubations which did not contain tissue and had identical chromatographic behavior to the non-labeled standard. The radioactivity contained in the zone corresponding to the DHT standard (R_f value of 0.67) of the control chromatogram was 1% of the total radioactivity determined in the plate and was considered an error; it was subtracted from the experimental chromatograms.

Determination of 50% inhibitory concentration of steroidal compounds in human prostate

The concentrations of finasteride **3** and compounds **6–10** required for inhibiting 5α-reductase activity by 50% (IC_{50}) were determined from inhibition plots using different concentrations of the tested steroids; the results are shown in Table 1. These data show that steroids **6–9** inhibited the activity of human 5α-reductase. However, compound **10** did not exhibit this capacity. It appears that the presence of an acetoxy group in C-17 is a necessary requirement for inhibition of the enzyme 5α-reductase.

Competitive studies

The IC_{50} value of each compound was calculated according to the plots of concentration versus percentage of binding. MIB **5** (Figure 1) showed an IC_{50} of 1 nM, whereas compounds **6–10** did not inhibit MIB binding to the androgen receptor.

In vivo experiments

Weight of the prostate

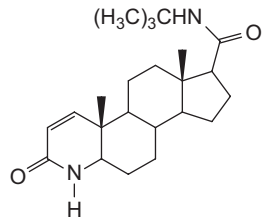
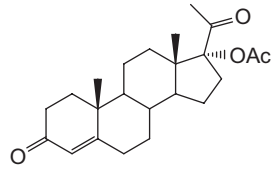
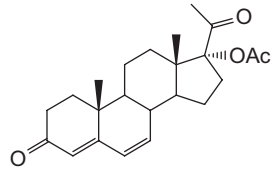
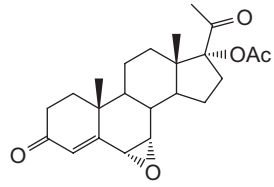
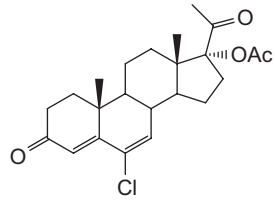
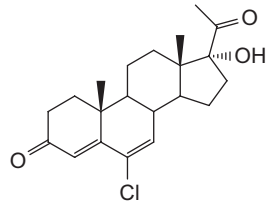
After castration, the weight of the hamster prostate decreased ($p < 0.05$) compared to normal glands. Treatment with vehicle alone did not change this condition, whereas subcutaneous injections of 1 mg/kg of T for 6 days significantly increased ($p < 0.05$) the weight of these glands in castrated male hamsters (Figure 3). When T (1 mg/kg) and finasteride or compounds **6–9** (2 mg/kg) were injected together, the weight of the prostate decreased significantly ($p < 0.05$) as compared to that of T-treated animals (Figure 3). Compound **8** showed the lowest prostate weight, thus indicating the highest effect, whereas **10** did not significantly reduce the weight of this gland in the treated hamsters ($p > 0.05$).

Discussion

In this article we report the synthesis and biological activity of several steroidal compounds (**6–10**). Steroids **6–9**, which contain an acetate ester in C-17, competed with T as a substrate for 5α-reductase enzyme. These compounds were shown to be good inhibitors for human 5α-reductase activity. The free alcohol **10**, which does not contain the ester moiety at C-17, showed a low inhibitory activity for this enzyme. These data indicate that the presence of an acetate ester moiety at 17α is a necessary requirement for the inhibition of 5α-reductase activity. Nevertheless, the double bond in ring B (**7** and **8**) seems to decrease the inhibitory potency as compared to steroid **6**, which does not have this functionality; this compound showed the highest inhibitory potency (lowest IC_{50} value).

Finasteride **3** and steroids **6–9** showed pharmacological activity *in vivo*; these results correlate very well with

Table 1. *In vitro* experiment.

Compound	Steroid structure	IC_{50} (nM)
3		8.5
6		10
7		70
8		22
9		19
10		NA

Note. IC_{50} values were determined for finasteride **3** and synthesized steroids **6–10** with human prostate 5α-reductase enzyme. They represent the concentration of steroid that inhibits 50% of 5α-reductase activity and were determined as described in the "Experimental" section. NA, not active.

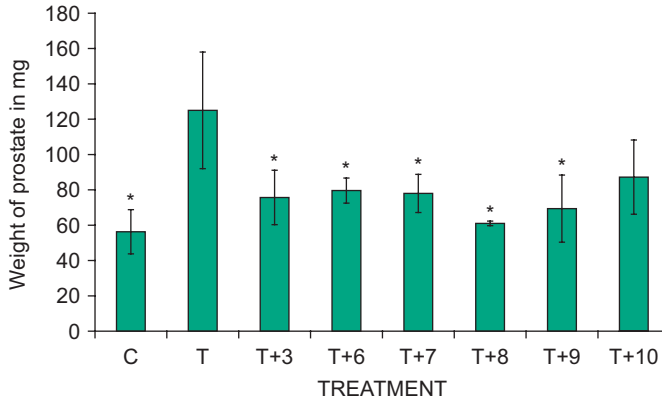


Figure 3. Weight of prostate (± standard deviation) obtained from groups of castrated hamsters (four animals/group) receiving different subcutaneous treatments for 6 days. The control animals (C) were treated with vehicle only. The pharmacological experiment was carried out in duplicate. T, testosterone; **3**, finasteride. *Weight of prostate decreased significantly ($p < 0.05$) as compared to that of T-treated animals.

the analysis of IC_{50} values obtained in the *in vitro* experiments. Based on this, the decrease of prostate weight of the hamsters produced by compounds **6–9** was a consequence of the inhibition of 5α-reductase present in this tissue[3]. Previous reports from our group have provided evidence about the similarity between hamster and human 5α-reductase enzyme behavior in the presence of inhibitors at their corresponding pH and concentration optimum[15].

Another mechanism of action of **6–9** that could explain the pharmacological effect could be the fact that these steroids bind to the androgen receptors. However, the results indicated that compounds **6–9** did not bind to the androgen receptors since they did not inhibit labeled MIB binding to this receptor.

On the other hand, the acetate ester moiety in C-17 and the presence of the chlorine atom in C-6 (**9**) seem to increase the capability of the molecule to decrease the weight of the prostate in treated animals, since this compound showed higher potency in reducing the prostate weight as compared to steroidal derivative **7**. Furthermore, the free alcohol **10** lacking this functional group did not significantly inhibit the weight of this gland in the treated hamsters.

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Declaration of interest: We acknowledge that no conflict of interest exists.

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