Synthesis and Pharmacological Evaluation of 4-Halo Progesterone Derivatives as Antiandrogen

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The pharmacological activity of eight pregnane derivatives 17-α acetoxyprogesterone 9, 17-α acetoxy-4, 5-epoxypregnan-3, 20-dione 10, 17-α acetoxy-4-chloro-4-pregnene-3, 20-dione 11, 17-α acetoxy-4-bromo-4-pregnene-3, 20-dione 12, 17-α hydroxy-4-bromo-4-pregnan-3, 20-dione 13, 4-chloro-17-α hydroxy-4-pregnen-3, 20-dione 14, 17-α benzoyloxy-4-bromo-4-pregnen-3, 20-dione 15 and 17-α benzoyloxy-4-chloro-4-pregnen-3, 20-dione 16 was determined. These compounds were evaluated as antiandrogens on gonadectomized hamster seminal vesicles.

The pharmacological data in this study indicate that compounds 15 and 16 having a C-17 benzoyloxy moiety showed the highest antiandrogenic activity as measured by the reduction of the weight of the seminal vesicles, followed by the steroids 11 and 12 (17-α acetoxy group). The free alcohols 13 and 14 exhibited a lower antiandrogenic activity. Apparently, the ester moiety at C-17 is a necessary requirement for the presence of high antiandrogenic activity. The inhibitory effect on the conversion of testosterone (T) to DHT, of the above described steroids as measured by the amount of produced DHT 2 expressed as pmoles of DHT/g of protein/h.

Steroids 11, 12 and 16 showed a much higher inhibitory activity on the conversion of testosterone (T) to dihydrotestosterone (DHT) than presently used finasteride 3.

Key words seminal vesicles; 5α-reductase; testosterone-conversion; halogen substituents; progesterone derivatives

Enzymatic 5α reduction of testosterone (T) 1 (Fig. 1) which produces the more potent androgen, 5α dihydrotestosterone (DHT) 2, occurs in those organs classically categorized as androgen target organs, chiefly the male accessory glands.1) This tissue enzyme profile results in the accumulation of the potent androgen 2 in the classical androgen target organs.

In several endocrine abnormalities, including benign prostate hyperplasia (BPH), male pattern baldness, acne and hirsutism, 5α reduction plays a principal role. For this reason it is important to develop 5α reductase inhibitors for the treatment of these afflictions.

The most extensively studied class of 5α reductase inhibitors are the 4-azasteroids2,3) which includes the drug finasteride 3 (Fig. 1). Finasteride 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 decreases prostatic DHT 2 levels by 70—90% and reduces prostate size, while T tissue levels remain constant.4) The use of finasteride demonstrated a sustained improvement in the treatment of androgen dependent diseases and reduction in prostate specific antigen (PSA) levels.5) Related analogs 4, 5 and 6 (Fig. 1) have also demonstrated effectiveness in vitro and in vivo.6,7)

Androstane-3-carboxylic acids 7 and 8 (Fig. 1) were recently synthesized and have shown a potent uncompetitive inhibition of type 2 5α reductase.8,9) Epristeride 7 has exhibited the ability to lower serum DHT 2 levels by 50%.10)

In this study we evaluated the following compounds as antiandrogens: 17-α acetoxyprogesterone 9 (Fig. 2), 17-α acetoxy-4, 5-epoxypregnan-3, 20-dione 10, 17-α acetoxy-4-chloro-4-pregnene-3, 20-dione 11, 17-α acetoxy-4-bromo-4-pregnene-3, 20-dione 12, 17-α benzoyloxy-4-bromo-4-pregnen-3, 20-dione 13, 4-chloro-17-α benzoyloxy-4-chloro-4-pregnen-3, 20-dione 14, 17-α benzoyloxy-4-bromo-4-pregnen-3, 20-dione 15 and 17-α benzoyloxy-4-chloro-4-pregnen-3, 20-dione 16 was determined.

![Steroidal Antiandrogens](image-url)
chboro-4-pregnene-3, 20-dione 11, 17-α acetoxy-4-bromo-4-pregnene-3, 20-dione 12, 17-α hydroxy-4-bromo-4-pregnene-3, 20-dione 13, 4-chloro-17-α hydroxy-4-pregnene-3, 20-dione 14, 17-α benzyloxy-4-bromo-4-pregnene-3, 20-dione 15 and 17-α benzyloxy-4-chloro-4-pregnene-3, 20-dione 16.

The biological activity of these steroids was determined on in vitro metabolic change in the T (1) molecule produced by 5-α reductase11,12 and by the in vivo steroid action upon the seminal vesicles,13 where the compound is bound to its cognate receptors.12,13

To test the antiandrogenic effect of the synthesized compounds, we used the seminal vesicles of gonadectomized male hamsters.11,12 Seminal vesicles are male accessory glands that are androgen dependent. These organs are capable of reducing T (1) to DHT (2) in both intact and gonadectomized animals.14

Chemistry As shown in Fig. 2 the active compounds 11, 12, 15 and 16 were synthesized from the commercially available 17-α acetoxyprogesterone 9. Previous reports indicated that compound 9 was synthesized by Mukawa in Japan.15 In the first step, the C-4 double bond of 9 was epoxidized with hydrogen peroxide in basic medium6,17, the desired compound 10 underwent a ring opening reaction with hydrogen chloride or hydrogen bromide to form the corresponding chlorodervative18) 11 and the bromo compound18) 12. Treatment of 11 and 12 with aqueous sodium hydroxide solution19 afforded the corresponding alcohols 13 and 14. The esterification of the C-17 hydroxyl group in 13 and 14 with benzoic acid and trifluoroacetic anhydride19 afforded the desired final compounds 15 (4-bromo derivative) and 16 (4-chloro derivative).

Methods and Results

Seminal Vesicles When 1 (T) and finasteride 3 were injected together, the weight of the seminal vesicles decreased significantly (p<0.005) as compared to T (1) treated animals (Table 1). The starting material 9 and the intermediate epoxy compound 10 failed to show a substantial decrease of the weight of the seminal vesicles (lack of an antiandrogenic effect). Steroids 11—16 decreased the weight of the glands significantly (p<0.005) as compared to the T (1) treated animals. Table 1 shows that compound 16 (17-α benzyloxy-4-chlorodervative) has the highest antiandrogenic effect; the corresponding 4-bromodervative 15 is slightly less active. On the other hand, compound 12 (17-α acetoxy-4-bromodervative) is more active than the corresponding chlorodervative 11. Surprisingly the benzyloxyderivatives 15 and 16 have a much higher antiandrogenic activity than the corresponding acetoxy compounds, 11 and 12. The starting material 9, showed a very weak antiandrogenic effect, whereas the alcohols 13 and 14, the epoxyderivative 10 did not show a comparable pharmacological activity.

Since the weight of the seminal vesicles depends on the 5-α reduced androgens,1,20 it was important to determine the effect of these compounds on the conversion of T (1) to DHT (2) in this tissue.

The hexane extract from castrated male hamster seminal vesicles was subjected to TLC analysis. The zone corresponding to the DHT (2) standard (Rf value 0.33) of each experimental chromatogram was eluted and the radioactivity determined.

The results (Fig. 3) obtained from two separate experiments performed in duplicate demonstrated a difference between the conversion of [3H]T to [3H]DHT in the T plus finasteride 3-treated animals thus indicating that finasteride 3 is a good inhibitor for the conversion of T to DHT at pH 6. The radioactive purity of the formed DHT is shown in Table 1.

### Table 1. Weight and Protein Content of Seminal Vesicles from Animals Receiving Different S.C. Treatments

<table>
<thead>
<tr>
<th>Treatment (mg)</th>
<th>Weight of seminal vesicles (mg)</th>
<th>Protein content mg/mg of tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>99.0±5.0</td>
<td>0.0064±0.0010</td>
</tr>
<tr>
<td>T+3</td>
<td>81.0±2.1</td>
<td>0.0096±0.0005</td>
</tr>
<tr>
<td>T+9</td>
<td>86.1±10.0</td>
<td>0.0130±0.0005</td>
</tr>
<tr>
<td>T+10</td>
<td>95.7±8.0</td>
<td>0.0090±0.0002</td>
</tr>
<tr>
<td>T+11</td>
<td>79.6±3.0</td>
<td>0.0124±0.0006</td>
</tr>
<tr>
<td>T+12</td>
<td>76.8±2.8</td>
<td>0.0137±0.0005</td>
</tr>
<tr>
<td>T+13</td>
<td>86.7±8.0</td>
<td>0.0120±0.0020</td>
</tr>
<tr>
<td>T+14</td>
<td>73.9±11.0</td>
<td>0.0110±0.0020</td>
</tr>
<tr>
<td>T+15</td>
<td>68.4±3.0</td>
<td>0.0137±0.0030</td>
</tr>
<tr>
<td>T+16</td>
<td>56.1±3.0</td>
<td>0.0149±0.0010</td>
</tr>
</tbody>
</table>

Significant differences were observed between the weights of castrated testosterone-treated animals (T) and testosterone+finasteride (3)-treated animals. Compounds 11—16 decreased the weight significantly (p<0.05) compared with T-treated animals.
atom at C-4 whereas and were previously prepared by Japanese workers. All at the same position. Compounds logically inactive. Steroids mediate inhibition of DHT formation and showed a weak activity as T conversion expressed as pmoles of DHT/g of protein/h. The most active compounds of DHT formation expressed as pmoles of DHT/g of protein/h. Finasteride (3) reduces the conversion of T to DHT substantially and therefore can be considered as a 5α-reductase inhibitor at pH 6. Other authors who reported the inhibitory effect of similar compounds in agreement with those obtain these results. Steroids 11, 12, and 16 inhibited significantly (p<0.005) the conversion of T to DHT at pH 6 in seminal vesicles homogenates from castrated male hamsters treated with a dose of 200 μg as compared to T (1).

This antiandrogenic effect of 11—16 is probably due to the presence of a halogen substituent at C-4 on the progestosterone molecule (Fig. 2). Similar compounds with a halogen atom at C-6 have shown a very high antiandrogenic activity. The results in Table 1 clearly indicate that compounds 15 and 16 with the benzoyloxy moiety at C-17 show a much higher antiandrogenic effect as compared to 11 and 12 with an acetoxy group at C-17. In the benzoyloxy series, compound 16 (chlorine at C-4) shows a higher antiandrogenic effect than the analog bromo derivative 15. In the 17α-acetoxy series, this behavior is reversed; the chloro derivative 11 shows a lower antiandrogenic activity than the corresponding bromo compound 12.

The data for protein synthesis (Table 1) indicate that when a significant decrease (p<0.005) in the weight of the seminal vesicles has taken place, protein formation is increased. On the other hand, when the T conversion is inhibited, protein synthesis is increased (lower DHT formation) (Table 1). These proteins could be enzymes, membrane proteins, or cytosol receptors. These data are in agreement with those previously published by our group and by Takayasu and Itami who demonstrated that the synthesis of 5α reductase enzyme is induced by androgen deprivation in hamster sebaceous glands.

Probably in the future, compounds 11, 12, and 16 could have a potential application for the treatment of androgen dependent diseases.

### Experimental

**Chemicals and Radioactive Material** Solvents were laboratory grade or better. Melting points were determined on a Fisher Johns melting point apparatus and are uncorrected. 1H-NMR and 13C-NMR were taken on a Varian Gemini 200 and VRX-300 respectively. Chemical shifts are given in ppm relative to that of Me4Si (δ=0) in CDCl₃. 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 obtained on a Perkin-Elmer 549B and the UV spectra were recorded on a Perkin-Elmer 200 s spectrometers. The elemental analysis were determined at the Christopher Ingold Laboratories, University College, London.

Ci/mmol, was provided by New England Nuclear Co. (Boston, MA) and radioinert T was kindly provided by Syntex of Cuernavaca (Mexico). d(α)-Glucose, which had a melting point of 146°C, was purchased from Merck (Mexico). Sigma Chemical Co. (St. Louis, MO) supplied finasteride, 5α-DHT, and NADPH.

2. Figure 3 shows the effect of steroidal structure on the rate of DHT formation expressed as pmoles of DHT/g of protein/h. The most active compounds 11, 12 and 16 show a lower rate of DHT formation than the control samples, T and T plus finasteride (T+3). The 4-bromo-17α-benzyloxy compound 15 although showed relatively high antiandrogenic activity (Table 1) in this case, it did not reduce the rate of DHT formation and showed a weak activity as T conversion inhibitor.

### Discussion

This paper describes the synthesis of 6 steroidal compounds 11—16 (Fig. 2). The starting material 9 and the intermediate 10 are devoid of a halogen atom at C-4 and are biologically inactive. Steroids 12, 14 and 16 have a chlorine atom at C-4 whereas 11, 13 and 15 contain a bromine atom at the same position. Compounds 11, 12 and 14 are known and were previously prepared by Japanese workers. All of the bromo and chloro derivatives are biologically active as T conversion inhibitors and also show a significant decrease of the weight of seminal vesicles (Table 1) and therefore could be considered as antiandrogens.

Figure 3 shows the conversion of [3H]T to [3H]DHT expressed as pmoles of DHT/g of protein/h. Finasteride (3) reduces the conversion of T to DHT substantially and therefore can be considered as a 5α-reductase inhibitor at pH 6. Other authors who reported the inhibitory effect of similar compounds in agreement with those obtain these results. Steroids 11, 12, and 16 inhibited significantly (p<0.005) the conversion of T to DHT at pH 6 in seminal vesicles homogenates from castrated male hamsters treated with a dose of 200 μg as compared to T (1).

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The data for protein synthesis (Table 1) indicate that when a significant decrease (p<0.005) in the weight of the seminal vesicles has taken place, protein formation is increased. On the other hand, when the T conversion is inhibited, protein synthesis is increased (lower DHT formation) (Table 1). These proteins could be enzymes, membrane proteins, or cytosol receptors. These data are in agreement with those previously published by our group and by Takayasu and Itami who demonstrated that the synthesis of 5α-reductase enzyme is induced by androgen deprivation in hamster sebaceous glands.

Probably in the future, compounds 11, 12, and 16 could have a potential application for the treatment of androgen dependent diseases.
17-α-Acetoxy-4-chloro-4-pregnene-3, 20-dione (11) To a solution of 10 (1.26 mmol) in acetone (50 ml) was added concentrated hydrochloric acid (1 ml). The solution was allowed to reflux for 30 min. The organic solvent was evaporated in vacuum and the desired crude product 11 was precipitated upon addition of 200 g of ice. Yield 0.99 g, 2.4 mmol (92%). Recrystallization of the crude product afforded the pure product 0.78 g (78%), mp 180—182°C. UV: 253 nm (log ε, 4.08). IR (KBr): 1689, 1714, 1722. H-NMR (CDCl3) δ: 0.71 (3H, s), 1.31 (3H, s), 2.08 (3H, s), 2.23 (3H, s). 13C-NMR, δ: 14.3 (C-18), 17.7 (C-19), 21.18 (C-21), 170.0 (CH3-C). MS (m/z): 407 (M+). Calcd for C23H31O4Cl: C, 67.88; H, 7.68; O, 15.73; Cl, 8.71. Found: C, 67.79; H, 7.75; O, 15.81; Cl, 8.75.

17-α-Acetoxy-4-bromo-4-pregnene-3, 20-dione (12) To a solution of 10 (1.26 mmol) in methanol (30 ml) was added concentrated hydrobromic acid (15 ml). The mixture was stirred for 30 min. The resulting solution was stirred under nitrogen for 17 h at room temperature. Ice water was added and the reaction mixture was neutralized with sodium bicarbonate to a pH of 7. The solution was extracted with chloroform; the combined organic extracts were washed with water, dried over sodium sulfate and the solvent evaporated in vacuum. The crude product was purified by column chromatography (hexane : AcOEt = 7 : 3) to give 26 mg, 0.55 mmol (39%) of the pure compound 12. mp 223—226°C. UV: 251 nm (log ε, 4.12). IR (KBr): 1671, 1709, 1714. H-NMR (CDCl3) δ: 0.81 (3H, s), 1.39 (3H, s), 2.23 (3H, s), 8.17 (2H, m), 7.53 (3H, m). 13C-NMR, δ: 14.5 (C-18), 17.6 (C-19), 20.7 (C-21), 170.7 (PhCOO), 203.9 (C-20), 128.3 (C=C-Br), 190.4 (C-3). MS (m/z): 468 (M+). Calcd for C23H31O4Br: C, 71.70; H, 7.09; O, 13.64; Cl, 7.55. Found: C, 71.79; H, 7.15; O, 13.73; Cl, 7.68.

Animals and Tissues Adult male Syrian golden hamsters (150—200 g) were obtained from Metropolitan University-Xochimilco of Mexico. 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.

Gonadectomies were performed under light ether anesthesia 15 d before treatments. Animals were sacrificed by ether anesthesia. The seminal vesicles were immediately removed, blotted, and weighed prior to their use. Tissues used in the metabolic experiments were homogenized with a tissue homogenizer (model 985-370; variable speed 5000—3000 rpm, Biospec Products, Inc.).

Semenal Vesicles Tests The effect of steroids on seminal vesicles from castrated male hamsters was determined in 10 groups of 5 animals/experiment selected at random and gonadectomized 15 d prior to experimentation. 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.

Hormone Treatment of Seminal Vesicles Daily subcutaneous injections of 200 μg of the steroids dissolved in 20 μl of sesame oil were administered to the animals for 3 d. After this treatment, the animals were sacrificed by ether anesthesia, and the seminal vesicles were dissected out and weighed on a balance. The following table gives the composition of each treatment:

<table>
<thead>
<tr>
<th>Group</th>
<th>n = 4, 200 μg of T</th>
<th>n = 4, 200 μg of T + 20 μg of DHT</th>
<th>n = 4, 200 μg of T + 20 μg of E2</th>
<th>n = 4, 200 μg of T + 40 μg of E2</th>
<th>n = 4, 200 μg of T + 80 μg of E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>200 μg of T</td>
<td>200 μg of T + 20 μg of DHT</td>
<td>200 μg of T + 20 μg of E2</td>
<td>200 μg of T + 40 μg of E2</td>
<td>200 μg of T + 80 μg of E2</td>
</tr>
<tr>
<td>Group 2</td>
<td>200 μg of T</td>
<td>200 μg of T + 20 μg of DHT</td>
<td>200 μg of T + 20 μg of E2</td>
<td>200 μg of T + 40 μg of E2</td>
<td>200 μg of T + 80 μg of E2</td>
</tr>
<tr>
<td>Group 3</td>
<td>200 μg of T</td>
<td>200 μg of T + 20 μg of DHT</td>
<td>200 μg of T + 20 μg of E2</td>
<td>200 μg of T + 40 μg of E2</td>
<td>200 μg of T + 80 μg of E2</td>
</tr>
<tr>
<td>Group 4</td>
<td>200 μg of T</td>
<td>200 μg of T + 20 μg of DHT</td>
<td>200 μg of T + 20 μg of E2</td>
<td>200 μg of T + 40 μg of E2</td>
<td>200 μg of T + 80 μg of E2</td>
</tr>
<tr>
<td>Group 5</td>
<td>200 μg of T</td>
<td>200 μg of T + 20 μg of DHT</td>
<td>200 μg of T + 20 μg of E2</td>
<td>200 μg of T + 40 μg of E2</td>
<td>200 μg of T + 80 μg of E2</td>
</tr>
<tr>
<td>Group 6</td>
<td>200 μg of T</td>
<td>200 μg of T + 20 μg of DHT</td>
<td>200 μg of T + 20 μg of E2</td>
<td>200 μg of T + 40 μg of E2</td>
<td>200 μg of T + 80 μg of E2</td>
</tr>
<tr>
<td>Group 7</td>
<td>200 μg of T</td>
<td>200 μg of T + 20 μg of DHT</td>
<td>200 μg of T + 20 μg of E2</td>
<td>200 μg of T + 40 μg of E2</td>
<td>200 μg of T + 80 μg of E2</td>
</tr>
<tr>
<td>Group 8</td>
<td>200 μg of T</td>
<td>200 μg of T + 20 μg of DHT</td>
<td>200 μg of T + 20 μg of E2</td>
<td>200 μg of T + 40 μg of E2</td>
<td>200 μg of T + 80 μg of E2</td>
</tr>
<tr>
<td>Group 9</td>
<td>200 μg of T</td>
<td>200 μg of T + 20 μg of DHT</td>
<td>200 μg of T + 20 μg of E2</td>
<td>200 μg of T + 40 μg of E2</td>
<td>200 μg of T + 80 μg of E2</td>
</tr>
</tbody>
</table>

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.

In Vitro Metabolic Studies with Seminal Vesicles Homogenates from male hamster seminal vesicles (around 3 mg protein) were prepared from intact adult male animals, using Krebs–Ringer–phosphate buffer solution, at pH 6. Tissue preparations were incubated25 in duplicate with 5.1 μmol [3H]T in the presence of 1 μmol NADPH, 0.013 μmol of unlabeled T, finasteride 3, compounds 11—16 in a Dubnoff metabolic incubator at 37°C for 60 min with O2:CO2 (95:5) as the gas phase. The final incubation volume was 1 ml. Incubations without tissues were used as controls. Incubation was terminated by addition of dichloromethane, and the [3H]steroid was extracted (4×) using 3 vol of dichloromethane. The solvent was dried under vacuum and the extract washed with hexane in order to remove the remaining lipids. The protein content of the homogenates was determined by Bradford's dye-binding method4 using bovine serum albumin (BSA) as the standard.

Isolation and purity assessment of radioactive DHT was carried out by the reverse isotope dilution technique. The isolated compound was purified with steroid carriers (T, DHT) in a thin-layer chromatographic system (chloroform–acetone, 9 : 1). The radioactive conversion product was identified on chromatographic plates by autoradiography, while nonradioactive steroid carriers (T, DHT) in a thin-layer chromatographic system (chloroform–acetone, 9 : 1). The radioactive conversion product was identified on chromatographic plates by autoradiography, while nonradioactive steroid carriers (T, DHT) in a thin-layer chromatographic system (chloroform–acetone, 9 : 1).
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References