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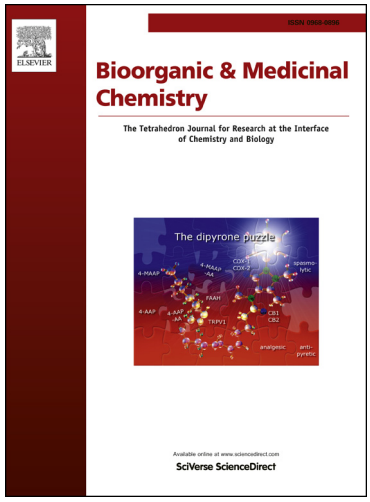

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Synthesis and activity of novel 16-dehydropregnenolone acetate derivatives as inhibitors of type 1 5α-reductase and on cancer cell line SK-LU-1

Aylin Viviana Silva-Ortiz[a], Eugene Bratoeff[†], Teresa Ramírez-Apan[b], Yvonne Heuze[c], Araceli Sánchez[c], Juan Soriano[d], Marisa Cabeza[c,*].

[a] Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Av. Universidad 3000, Copilco Universidad, Coyoacán, 04510 Ciudad de México, Distrito Federal, México D.F.

[b] Intituto de Quimica, Universidad Nacional Autónoma de México, Av. Universidad 3000, Copilco Universidad, Coyoacán, 04510 Ciudad de México, Distrito Federal, México D.F.

[c] Departamento de Sistemas Biológicos y de Producción Agrícola y Animal, Universidad Autónoma Metropolitana-Xochimilco, Calzada del Hueso 1100. Col. Villa Quietud, 04960 Ciudad de México. México, D. F.

[d] Departamento de Patología, Hospital General de México, Dr. Balmis 148, México, D. F.

ASO: aylinsilva@hotmail.es
TRA: mtrapan@unam.mx
ASM: ara_sm89@live.com.mx
EB: eugene@unam.mx
YH: ymheuze@gmail.com
JS: juansorianor@hotmail.com
MC: marisa@correo.xoc.uam.mx

***Address correspondence to:**
Marisa Cabeza Ph.D.
Departamento de Sistemas Biológicos
Universidad Autónoma Metropolitana-Xochimilco
Calzada Del Hueso No. 1100
México, D.F., C.P. 04960, México
Phone: [011-52-55] 5483-72-60
Fax: [011-52-55] 5483-72-60
E-mail: marisa@correo.xoc.uam.mx

Abstract

Testosterone (T) plays a crucial role in prostate growth. In androgen-dependent tissues T is reduced to dihydrotestosterone (DHT) because of the presence of the 5α-reductase enzyme. This androgen is more active than T, since it has a higher affinity for the androgen receptor (AR). When this mechanism is altered, androgen-dependent diseases, including prostate cancer, could result.

The aim of this study was to synthesize several 16-dehydropregnenolone acetate derivatives containing a triazole ring at C-21 and a linear or alicyclic ester moiety at C-3 of the steroidal skeleton. These steroids were designed as potential inhibitors of the activity of both types (1 and 2) of 5α-reductase. The cytotoxic activity of these compounds was also evaluated on a panel of PC-3, MCF7, and SK-LU-1 human cancer cell lines.

The results from this study showed that with the exception of steroids 20-oxo-21-(1*H*-1,2,4-triazole-1-yl)pregna-5,16-dien-3β-yl-propionate and 20-oxo-21-(1*H*-1,2,4-triazole-1-yl)pregna-5,16-dien-3β-yl-pentanoate, the compounds exhibit a lower inhibitory activity for both isoenzymes of 5α-reductase than finasteride. Furthermore the 3β-hydroxy-21-(1*H*-1,2,4-triazole-1-yl)pregna-5,16-dien-20-one and 20-oxo-21-(1*H*-1,2,4-triazole-1-yl)pregna-5,16-dien-3β-yl-acetate derivatives display 80% cytotoxic activity on the SK-LU-1 cell line.

These results also indicated that the triazole derivatives, which have a hydroxyl or acetoxy group at C-3, could have an anticancer effect, whereas the derivatives with a alicyclic ester group at C-3 do not show biological activity.

Keywords

Synthesis of derivatives dehydropregnenolone, 5α-reductase 1 inhibitors, antiproliferative activity on SKLU-1 cells

1. Introduction

Steroids are transcription factors that regulate gene expression; these messengers are able to cross the plasma membrane and bind to specific receptors. The complex comprising the steroid receptor has a direct interaction with DNA, altering gene expression. The result of this process induces growth and differentiation of its target tissues, so any alteration in this mechanism can cause deregulation of the expression, resulting in the modification of several signaling pathways and therefore of functions. [1-2]

It is known that in some metastatic tumors such as lung adenocarcinoma as well as in cancer cell line SK-LU-1, gene CYP24A1 is highly expressed. [3] This gene is a member of the CYP450 superfamily, which codes for 24 hydroxylase enzyme. [3] It is also recognized that the expression of glyoxalase enzyme genes is regulated by testosterone (T) and that altered expression of genes encoding this glyoxalase is present in various types of cancerous tumors of the prostate. [4] Furthermore, androgens also induce the development of benign prostatic hyperplasia (BPH). Both cancer and BPH have also been associated with an increase of dihydrotestosterone (DHT) in this gland. [4] T is converted to DHT by the action of 5α-reductase (5α-R) isoenzymes. There are three 5α-reductase isoenzymes in prostate tissue. Type 1 5α-R (5α-R1) is expressed in the prostate epithelial cells and is related to the development of prostate cancer. [5] Type 2 5α-R (5α-R2) is associated with benign prostatic hyperplasia (BPH) [1], and type 3 5α-R (5α-R3) is found in the brain and pancreas, and is related to hormone-refractory prostate cancer (HRPC) [6, 7] Each is encoded by a different gene; these have been characterized in several species. [8]

Therefore the development of new inhibitors of enzymes involved in the synthetic routes of steroidal hormones and antagonists of steroidal receptors could improve current anticancer therapies. [9, 10]

The cytotoxic effect of dehydroepiandrosterone derivatives with a triazole moiety and an aromatic ester at C-3 was demonstrated by our group. [9] These derivatives displayed high cytotoxic activity in cell lines PC-3, MCF7 and SK-LU-1. We also demonstrated that several progesterone derivatives inhibited the activity of both types (1 and 2) of 5α-R. These steroids also showed a high pharmacological activity inhibiting prostate growth. [10]

Based on these results we decided to study the synthesis and biological activity of a series of 21-(1H-1,2,4-triazole-1-yl)-5,16-pregnadiene-20-one-3β-yl derivatives (**9a-j**). Their biological activity was evaluated by measuring their antiproliferative effect in a panel of human cancer cell lines; PC-3 (prostate), MCF7 (breast) and SK-LU-1 (lung). In addition we also evaluated their effect as inhibitors of the activity of 5α-R1 and 5α-R2 and their binding capacity to the androgen receptor (AR). The pharmacological effect of these pregnane derivatives was evaluated in an animal model.

In the cancer cell lines tested, ketoconazole **10** (Fig. 1) was used as a positive control. This compound is an antimycotic drug and an agent that inhibits cytochrome P450 enzymes, which are required for the synthesis of androgens. [11]

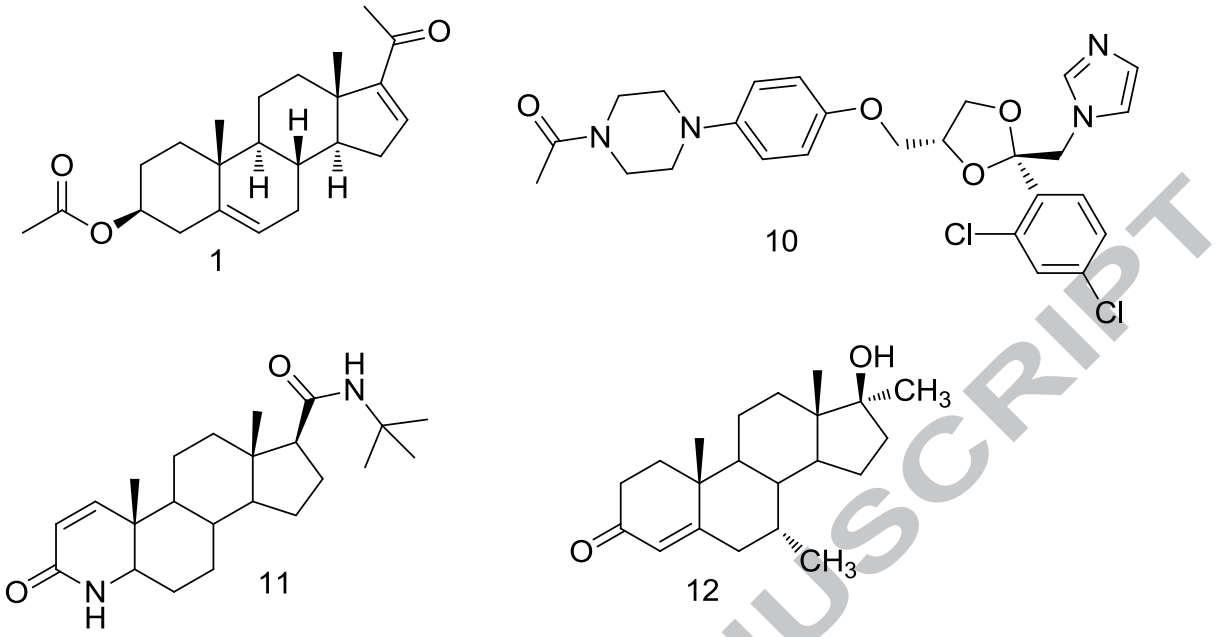


Figure 1. 16-dehydropregnenole acetate (**1**), Ketoconazole (**10**), Finasteride (**11**) and Mibolerone (**12**).

2. Material and methods

2.1. Chemical and radioactive materials

2.1.1. Reagents

Solvents were purchased from commercial sources and used without further purification. Melting points were determined on a Fisher Johns melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 200 spectrometer. The UV lamp (254 nm) was from UVP. ^{1}H and ^{13}C NMR were taken on a Varian VRX-400 spectrometer operating at 400 (^{1}H) and 100 (^{13}C) MHz with TMS as internal standard (δ=0) in $CDCl_3$ (the abbreviations of signal patterns are as follows: s, singlet; d, doublet, t, triplet; m, multiplet). High resolution mass spectra (HRMS) were obtained with a Thermo DFS spectrometer by direct infusion and using FAB^+ ionization mode.

(1,2,6,7-^{3}H) Testosterone [^{3}H] T specific activity: 95 Ci/mmol and Mibolerone (17a-methyl-^{3}H) [^{3}H] MIB 10 specific activity 70–87 Ci/mmol were provided by Perkin Elmer Life and Analytical Sciences (Boston, MA).

Radioinert T, 5α-DHT and MIB were supplied by Steraloids (Wilton, NH, USA). Sigma Chemical Co. (St. Louis, MO) provided NADPH. Finasteride was obtained by extraction from Proscar® (Merck, Sharp & Dohme). The tablets were crushed, extracted with chloroform and the solvent was eliminated in vacuum; the crude product was purified by silica gel 60 (63–200 μm) column chromatography (Sigma–Aldrich) with ethyl acetate as eluant. The melting point of the isolated finasteride (252–254 °C) was identical to that reported in the literature.

The ketoconazole used as a reference compound was purchased from Sigma Life Sciences.

2.1.2. Synthesis of derivates of the 16-dehydropregnenolone acetate (8, 9a−j)

The synthetic pathway for the preparation of compounds **8** and **9a−j** are outlined in Fig. 2. These steroids were prepared from the commercially available 16-dehydropregnenolone acetate (1). The synthesis of these novel compounds is described below.

2.1.2.1. Synthesis of 16α,17α-epoxy-3β-hydroxypregn-5-en-20-one 2. A solution the steroid **1** (1 g, 2.82 mmol), sodium hydroxide 4 N (2 mL, 8 mmol) and H_2O_2 30 % (4 mL, 135 mmol) in hot methanol (66 mL) was stirred at room temperature for 4 h. After this time the methanol was evaporated and the product was washed with water, obtaining a white solid.

Yield 0.665 g (72%), m.p. 197–199 °C, IR (KBr) cm^{-1}: 3457, 1692, 1642 and 1042. ^{1}H RMN (400 MHz, $CDCl_3$) δ: 1.00 (s, H-18, 3H), 1.03 (s, H-19, 3H), 2.01 (s, H-21, 3H), 2.29 (s, H-16, 1H), 3.47 (m, *J* = 3.4 Hz, H-3, 1H), 3.66 (s, OH, 1H), 5.32 (t, *J* = 5.3 Hz, H-6, 1H). ^{13}C RMN (100 MHz, $CDCl_3$) δ: 15.38 (C-18), 19.30 (C-19), 27.51 (C-21), 60.55 (C-16), 71.16 (C-17), 71.75 (C-3), 121.13 (C-6), 141.27 (C-5), 205.07 (C-20). HRMS cal. for $C_{21}H_{30}O_3$ 330.2195, found 330.2187.

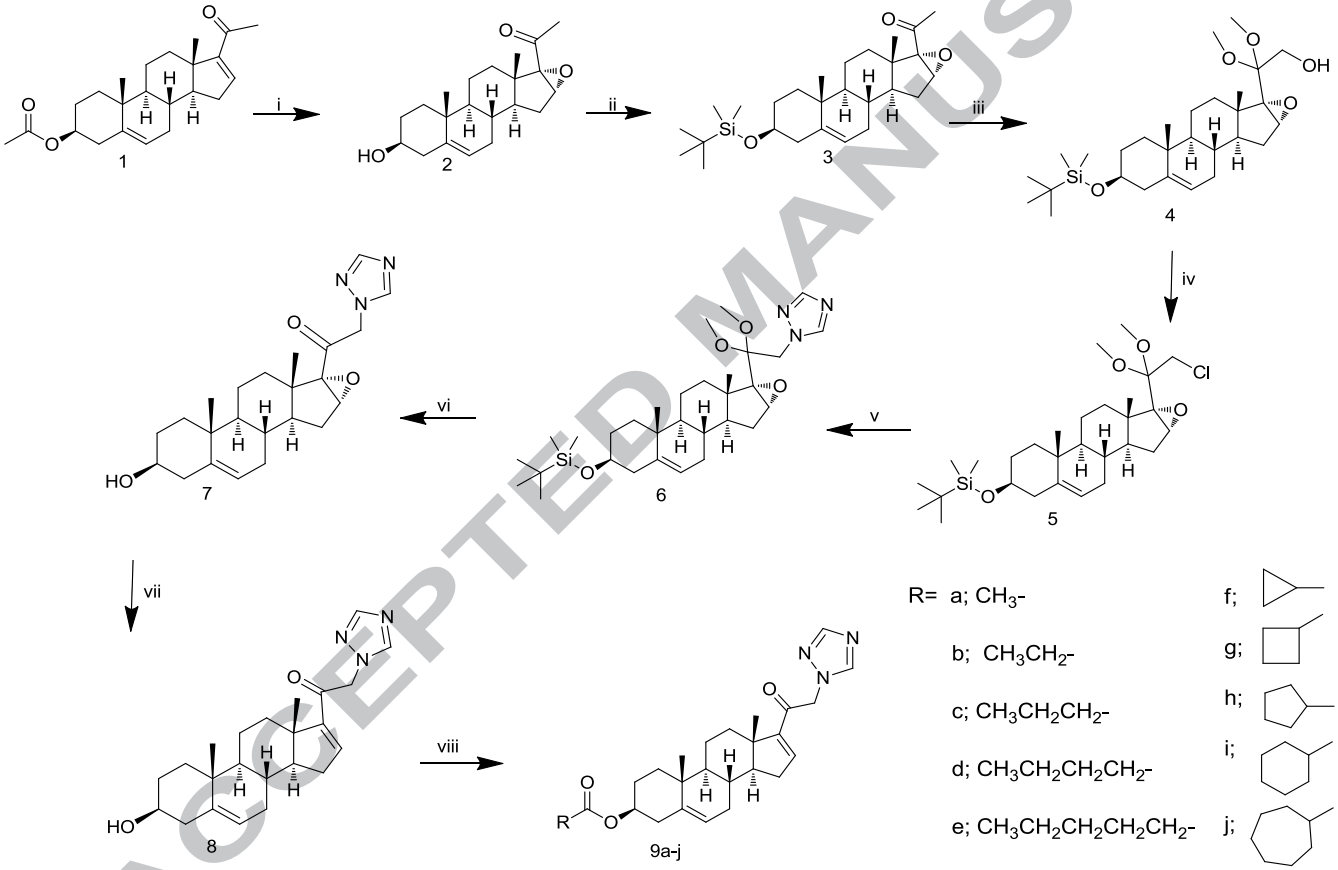


Fig. 2. Reagents and conditions: *i*) H_2O_2, NaOH 4N; *ii*) TBDMS, imidazole, DMF; *iii*) NaOH, $C_6H_5I(OAc)_2$, MeOH; *iv*) $SOCl_2$, Py, CH_2Cl_2; *v*) 1,2,4-triazole, K_2CO_3, 80 °C, 5 h; *vi*) HCl, acetone; *vii*) $CrCl_2$, Ac. Acetic; *viii a*) acetic anhi**y**dride, Py; *viii b-e*) R-COOH, DCC, DMAP, $CHCl_3$.

2.1.2.2. Synthesis of 3β-{[tert-butyl(dimethyl)silyl]oxy}-16α,17α-epoxypregn-5-en-20-one 3. To a solution of steroid **2** (1 g, 3.02 mmol), t-butyl dimethylsilylbchloride (0.75 g, 5.51 mmol) and imidazole (0.488g, 7.17 mmol) in dry DMF (15 mL) were added. The solution was stirred at room temperature for 2 h. When the reaction was finished, the DFM was evaporated at vacuum and the product was washed with water, obtaining a white solid.

Yield 1.17 g (87%), m.p. 118–120 °C, IR (KBr) cm^{-1}: 2935, 1704, 1642, 1698 and 1083. ^{1}H RMN (400 MHz, $CDCl_3$) δ: -0.051 (s, CH_3 of protector group, 6H), 0,048 (s, CH_3 of protector group, 9H), 0.88 (s, H-18, 3H), 1.00 (s, H-19, 3H), 2.02 (s, H-21, 3H), 2.25 (s, H-16, 1H), 3.47 (m, *J* = 3.4 Hz, H-3, 1H), 5.29 (1H, d, *J* = 5.2 Hz, H-6),. ^{13}C RMN (100 MHz, $CDCl_3$) δ: -4.33 (CH_3 of protector group) 15.40 (C-18), 19.32 (C-19), 27.70 (C-21), 26.08 (CH_3 of protector group), 60.57 (C-16), 71.11 (C-17), 72.55 (C-3), 120.43 (C-6), 141.93 (C-5), 205.08 (C-20). HRMS cal. for $C_{27}H_{44}O_3Si$ 444.3060, found 444.3052.

2.1.2.3. Synthesis of 3β-{[tert-butyl(dimethyl)silyl]oxy}-20,20-dimethoxy-16α, 17α-epoxypregn-5-en-21-ol 4. To a solution of steroid **3** (1 g, 2.2 mmol), (diacetoxyiodo) benzene (1.2 g, 3.72 mmol) and sodium hydroxide (1 g, 25 mmol) in methanol (21 mL) and dichloromethane (10 mL) were added, and stirred at room temperature overnight. The solvent was evaporated under reduced pressure. Finally the purification was made with a basic aluminum column chromatography using mixture of hexane and ethyl acetate (95:5).

Yield 0.959 g (84 %), m.p. 215-218 °C, IR (KBr) cm^{-1}: 3597, 2930, 1666, 1070 and 1033. ^{1}H RMN (400 MHz, $CDCl_3$) δ: -0.051 (s, CH_3 of protector group, 6H), 0.048 (s, CH_3 of protector group, 9H), 0.88 (s, H-18, 3H), 1.01 (s, H-19, 3H), 2.25 (s, H-16, 1H), 3.29 (s,

OCH_3, 6H), 3.44 (m, *J* = 3.6, H-3, 1H), 3.69 (s, OH, 1H), 5.30 (d, *J* = 5.3 Hz, H-6, 1H). ^{13}C RMN (100 MHz, $CDCl_3$) δ: -4.33 (CH_3 of protector group), 15.30 (C-18), 19.66 (C-19), 26.08 (CH_3 of protector group), 49.51 (OCH_3), 62.73 (C-21), 69.78 (C-16), 72.50 (C-3), 101.99 (C-20), 120.74 (C-6), 141.86 (C-5). HRMS cal. for $C_{29}H_{50}O_5Si$ 506.3428, found 506.3423.

2.1.2.4. Synthesis of 3β-{[tert-butyl(dimethyl)si1yl]oxy}-21-chloro-20,20-dimethoxy-16α,17α-epoxypregn-5-ene 5. To a cold solution of steroid **4** (1 g, 1.97 mmol) in a mixture of dry dicholoromethane (8 mL) and pyridine (1 mL) thionyl chloride (0.25 mL) was added dropwise under nitrogen atmosphere and stirred at room temperature for 30 min. Chloroform (100 mL) was added and it was washed two times with 10% aqueous hydrochloric acid, aqueous sodium bicarbonate and water. It was dried over sodium sulfate and the solvent was evaporated in vacuum. The crude product was recrystallized from methanol.

Yield 0.830 g (80%), m.p. 150–152 °C, IR (KBr) cm^{-1}: 2930, 1668, 1088 and 1033. ^1H RMN (400 MHz, $CDCl_3$) δ: -0.051 (s, CH_3 of protector group, 6H), 0.048 (s, CH_3 of protector group, 9H), 0.88 (s, H-18, 3H), 1.00 (s, H-19, 3H), 2.24 (s, H-16, 1H), 3.24 (s, OCH_3, 6H), 3.46 (m, *J* = 3.4 Hz, H-3, 1H), 4.01 (s, 2H, H-21), 5.28 (d, *J* = 5.2 Hz, H-6, 1H). ^{13}C RMN (100 MHz, $CDCl_3$) δ: -4.33 (CH_3 of protector group), 15.99 (C-18), 18.83 (C-19), 26.08 (CH_3 of protector group), 49.87 (OCH_3), 59.57 (C-21), 69.50 (C-16), 72.96 (C-3), 101.96 (C-20), 121.18 (C-6), 142.08 (C-5). HRMS cal. for $C_{29}H_{49}ClO_4Si$ 524.3089, found 524.3093.

2.1.2.5. Synthesis of 3β-{[tert-butyl(dimethyl)silyl]oxy)}-20,20-dimethoxy-16α,17α-epoxy-21-(1*H*-1,2,4-triazol-1-yl)pregn-5-ene 6. A mixture of 1,2,4-triazole

(0.069 g, 1.0 mmol), K_2CO_3 (0.263 g, 1.90 mmol) and the corresponding compound **5** (1 g, 1.90 mmol) dissolved in dry DMF (5 mL) was heated at 90 °C under N_2 atmosphere for 4 h. Cold water was added and the resulting solid was filtered. The compound was purified by a column chromatography with florisil.

Yield 0.753 g (71%), m.p. 188–190 °C, IR (KBr) cm^{-1}: 2929, 1616, 1086 and 1058. ^1H RMN (400 MHz, $CDCl_3$) δ: -0.051 (s, CH_3 of protector group, 6H), 0.048 (s, CH_3 of protector group, 9H), 0.88 (s, H-18, 3H), 1.00 (s, H-19, 3H), 2.30 (s, H-16, 1H), 3.24 (s, OCH_3, 6H,), 3.48 (m, *J* = 3.3 Hz, H-3, 1H), 4.01 (s, 2H, H-21), 5.29 (d, *J* = 5.3 Hz, H-6, 1H,), 7.47 (s, H-Het., 1H), 7.84 (s, H-Het., 1H) . ^{13}C RMN (100 MHz, $CDCl_3$) δ: -4.84 (CH_3 of protector group),15.20 (C-18), 18.03 (C-19), 26.08 (CH_3 of protector group), 49.40 (OCH_3), 60.14 (C-21), 68.99 (C-16), 71.99 (C-17), 72.65 (C-3), 101.77 (C-20), 121.08 (C-6), 121.60 (C-Het.), 141.44 (C-Het.), 142.25 (C-5). HRMS cal. for $C_{31}H_{51}N_3O_4Si$ 557.3649, found 557.3645

2.1.2.6. Synthesis of 16α,17α-epoxy-3β-hydroxy-21-(1*H*-1,2,4-triazol-1-yl)pregn-5-en-20-one 7. This compound was prepared from compound **6** (0.5 g, 0.89 mmol). The hydrolysis was carried out in acetone (1.5 mL) with 36% chlorhydric acid (1.3 mL), at room temperature 1h. The solvent was evaporated under reduced pressure and the crude product was recrystallized from methanol.

Yield 0.275 g (77%), m.p. 155–157 °C, IR (KBr) cm^{-1}: 3346, 2928, 1724, 1378, 1274 and 1051. ^1H RMN (400 MHz, $CDCl_3$) δ: 0.90 (s, H-18, 3H), 1.20 (s, H-19, 3H), 3.24 (m, *J* = 3.5, H-3, 1H), 3.51 (s, OH, 1H), 3.68 (s, H-16, 1H), 4.18 (s, 2H, H-21), 5.33 (d, *J* = 5.3 Hz, H-6, 1H), 7.51 (s, H-Het, 1H), 7.70 (s, H-Het, 1H). ^{13}C RMN (100 MHz, $CDCl_3$) δ: 15.32

(C-18), 19.56 (C-19), 62.26 (C-21), 71.74 (C-3), 121.04 (C-6), 121.2 (C-Het.), 131.2 (C-Het.), 141.24 (C-5), 206,08 (C-20). HRMS cal. for $C_{23}H_{31}N_3O_3$ 397.2365, found 397.2369.

2.1.2.7. Synthesis of 3β-hydroxy-21-(1*H*-1,2,4-triazol-1-yl)pregna-5,16-dien-20-one 8. A solution of steroid **7** (0.5 g, 1.25 mmol) and chromous chloride (0.4 g, 3.25 mmol) in acetic acid (20 mL) was stirred at room temperature for 30 min. The mixture was diluted with cold water (150 mL) and the precipitate was filtered and dried. The product was purified by silica gel column chromatography using a mixture of hexane and ethyl acetate (9:1). From this compound, compounds (**9a–j**) were derived.

Yield 0.370 g (77%), m.p. 120–122, IR (KBr) cm^{-1}: 3355, 2927, 1709, 1670, 1276 and 1051. ^{1}H RMN ($CDCl_3$) δ: 0.90 (s, H-18, 3H), 1.20 (s, H-19, 3H), 3.25 (m, *J* = 3.5 Hz, H-3, 1H), 3.52 (s, OH, 1H), 4.18 (s, 2H, H-21), 5.34 (d, *J* = 5.3 Hz, H-6, 1H), 6.74 (s, H-16, 1H),7.50 (s, H-Het., 1H), 7.70 (s, H-Het., 1H). ^{13}C RMN (100 MHz, $CDCl_3$) δ: 15.32 (C-18), 20.01 (C-19), 62.15 (C-21), 71.78 (C-3), 121.04 (C-6), 141.23 (C-5), 141.38 (C-16), 141.47 (C-Het.), 144.83 (C-Het.), 196.66 (C-20). HRMS cal. For $C_{23}H_{31}N_3O_3$ 381.2416, found 381.2420.

2.1.2.8. Preparation of 20-oxo-21-(1*H*-1,2,4-triazol-1-yl)pregna-5,16-dien-3β-yl acetate 9a. The synthesis of this compound was conducted from a solution of steroid **8** (0.1 g, 2.62 mmol), pyridine (0.4 mL) and acetic anhydride (0.4 mL). The resulting solution was stirred at room temperature for 1 h. A solution of water and salt was added and the precipitated was filtered. The compound was washed with water 3 times.

Yield 0.10 g (90%), m.p. 119–121 °C, IR (KBr) cm^{-1}: 2927, 1731, 1270 and 1087. ^{1}H RMN (40 MHz, $CDCl_3$) δ: 1.15 (s, H-18, 3H), 1.30 (s, H-19, 3H), 2.03 (t, ester-H-2′, 3H), 3.25

(m, *J* = 3.2 Hz, H-3, 1H), 4.19 (s, 2H, H-21), 5.34 (d, *J* = 5.3 Hz, H-6, 1H), 6.74 (s, *J* = 6.7, C-16, 1H), 8.01 (s, H-Het., 1H), 8.30 (s, H-Het., 1H). ^{13}C RMN (100 MHz, $CDCl_3$) δ: 15.30 (C-18), 19.40 (C-19), 21.3 (C-2′ of ester), 65.50 (C-21), 73.61 (C-3), 121.97 (C-6), 129.62 (C-16), 140.02 (C-Het.), 141.24 (C-5), 148.32 (C-Het.), 156.57 (C-17), 173.46 (ester carbonyl), 201.02 (C-20). HRMS cal. for $C_{25}H_{33}N_3O_3$ 423.2522, found 423.2525.

2.1.2.9. General procedure for obtention of 20-oxo-21-(1*H*-1,2,4-triazol-1-yl)pregna-5,16-dien-3β-ylpropionate (9b) to 20-oxo-21-(1*H*-1,2,4-triazol-1-yl)pregna-5,16-dien-3β-ylcycloheptanecarboxylate (9j).

To a solution of steroid **8** (0,1 g, 0.262 mmol), DCC (0.110 g, 0.524 mmol) and DMAP (0.064 g, 0.524 mmol) in chloroform (3 mL) the corresponding acid (0.6 mmol) was stirred at room temperature for 2 h. Ethyl acetate (8 mL) was added and the precipitated dicyclohexyl urea was filtered. The organic phase was washed three times with 10% aqueous hydrochloric acid, 5% aqueous sodium bicarbonate, water and was dried over anhydrous sodium sulfate. The crude ester was recrystallized from chloroform-methanol.

2.1.2.9.1. 20-oxo-21-(1*H*-1,2,4-triazol-1-yl)pregna-5,16-dien-3β-yl-propionate 9b. Yield 0.097 g (85%), m.p. 119–121 °C, IR (KBr) cm^{-1}: 2927, 1728, 1571, 1270 and 1087. ^{1}H RMN (400 MHz, $CDCl_3$) δ: 0.90 (s, H-18, 3H), 1.03 (s, H-19, 3H), 1.3 (t, H-3′ of ester, 3H), 2.1 (q, H-2′ of ester, 2H), 3.25 (m, *J* = 3.2, H-3, 1H), 4.19 (s, 2H, H-21), 5.34 (d, *J* = 5.3 Hz, H-6, 1H), 6.74 (s, *J* = 6.7 Hz, C-16, 1H), 8.02 (s, H-Het., 1H), 8.30 (s, H-Het., 1H). ^{13}C RMN (100 MHz, $CDCl_3$) δ: 8.30 (C-3′ of ester), 10.3 (C-18), 12.03 (C-19), 73.61 (C-3), 121.97 (C-6), 129.62 (C-16), 140.02 (C-Het.), 141.24 (C-5), 148.32 (C-Het.), 156.57 (C-17), 173.48 (ester carbonyl), 201.02 (C-20). HRMS cal. for $C_{26}H_{35}N_3O_3$ 437.2678, found 437.2673.

2.1.2.9.2. 20-oxo-21-(1*H*-1,2,4-triazol-1-yl)pregna-5,16-dien-3β-yl-butirate 9c. Yield 0.095 g (80%), m.p. 202–205 °C, IR (KBr) cm^{-1}: 2927, 1732, 1575, 1270 and 1087. ^{1}H RMN (400 MHz, $CDCl_3$) δ: 0.90 (t, ester-H-4′, 3H), 1.03 (s, H-18, 3H), 1.20 (s, H-19, 3H), 2.20 (q, ester-H-3′, 2H), 3.25 (m, *J* = 3.2 Hz, H-3, 1H), 4.20 (s, 2H, H-21), 5.34 (d, *J* = 5.3 Hz, H-6, 1H), 6.74 (s, *J* = 6.7 Hz, C-16, 1H), 7.51 (s, H-Het., 1H), 7.84 (s, H-Het., 1H). ^{13}C RMN (100 MHz, $CDCl_3$) δ: 10.1 (C-3′ of ester), 12.32 (C-18), 15.20 (C-19), 18.6 (C-4′ of ester), 67.01 (C-21), 73.61 (C-3), 121.97 (C-6), 129.62 (C-16), 140.02 (C-Het.), 142. 2 (C-5), 148.32 (C-Het.), 156.57 (C-17), 173.48 (ester carbonyl), 201.02 (C-20). HRMS cal. for $C_{27}H_{37}N_3O_3$ 451.2835, found 451.2830.

2.1.2.9.3. 20-oxo-21-(1*H*-1,2,4-triazol-1-yl)pregna-5,16-dien-3β-yl-pentanoate 9d. Yield 0.095 g (78%), m.p. 195–197 °C, IR (KBr) cm^{-1}: 2927, 1731, 1570, 1275 and 1087. ^{1}H RMN (400 MHz, $CDCl_3$) δ: 0.98 (t, ester-H-5′, 3H), 1.03 (s, H-18, 3H), 1.20 (s, H-19, 3H), 2.4 (q, ester-H-4′, 2H), 3.25 (m, *J* = 3.2 Hz, H-3, 1H), 4.20 (s, 2H, H-21), 5.34 (d, *J* = 5.3 Hz, H-6, 1H), 6.74 (s, *J* = 6.7 Hz, C-16, 1H), 7.51 (s, H-Het., 1H), 7.82 (s, H-Het., 1H). ^{13}C RMN (100 MHz, $CDCl_3$) δ: 9.82 (C-5′ of ester), 10.1 (C-4′ of ester), 10.31 (C-18), 15.4 (C-19), 67.31 (C-21), 73.59 (C-3), 121.97 (C-6), 129.62 (C-16), 140.02 (C-Het.), 142.25 (C-5), 148.32 (C-Het.), 156.57 (C-17), 173.46 (ester carbonyl), 200.02 (C-20). HRMS cal. for $C_{28}H_{39}N_3O_3$ 465.2991, found 465.2993.

2.1.2.9.4. 20-oxo-21-(1*H*-1,2,4-triazol-1-yl)pregna-5,16-dien-3β-yl-hexanoate 9e. Yield 0.098 g, (73%), m.p. 197–199 °C, IR (KBr) cm^{-1}: 2927, 1725, 1571, 1270 and 1087. ^{1}H RMN (400 MHz, $CDCl_3$) δ: 1.0 (t, ester-H-6′, 3H), 1.23 (s, H-18, 3H), 1.30 (s, H-19, 3H), 2.32 (q, ester-H-5′, 2H), 3.25 (m, *J* = 3.2 Hz, H-3, 1H), 4.20 (s, 2H, H-21), 5.33 (d, *J* = 5.3 Hz, H-6, 1H), 6.74 (s, *J* = 6.7 Hz, C-16, 1H), 7.51 (s, H-Het., 1H), 7.80 (s, H-Het., 1H).

^{13}C RMN (100 MHz, $CDCl_3$) δ: 9.80 (C-6′), 12.30 (C-18), 15.42 (C-19), 67.30 (C-21), 73.62 (C-3), 121.97 (C-6), 129.62 (C-16), 140.02 (C-Het.), 142.31(C-5), 148.32 (C-Het.), 156.57 (C-17), 173.46 (ester carbonyl), 200.02 (C-20). HRMS cal. for $C_{29}H_{41}N_3O_3$ 479.3148 for, found 479.3141.

2.1.2.9.5. 20-oxo-21-(1*H*-1,2,4-triazol-1-yl)pregna-5,16-dien-3β-yl-cyclopropanecarboxylate 9f. Yield 0.059 g, (50%), m.p. 131–134 °C, IR (KBr) cm^{-1}: 2928, 1722, 1625, 1575, 1270 and 1172. ^{1}H RMN (400 MHz, $CDCl_3$) δ: 0.90 (s, H-18, 3H), 1.31 (s, H-19, 3H), 1.83 (m, ester-H-1′, 1H), 3.45 (m, *J* = 3.5 Hz, H-3, 1H), 4.90 (s, 2H, H-21), 5.35 (d, *J* = 5.3 Hz, H-6, 1H), 6.74 (s, *J* = 6.7 Hz, C-16, 1H), 7.70 (s, H-Het., 1H), 8.01 (s, H-Het., 1H). ^{13}C RMN (100 MHz, $CDCl_3$) δ: 13.6 (C-1′ of ester), 14.53 (C-18), 15.23 (C-19), 63.2 (C-21), 73.55 (C-3), 121.03 (C-6), 121.1 (C-16), 141.52 (C-5), 144.04 (C-Het.), 152.15 (C-Het.), 157.13 (C-17), 174.44 (ester carbonyl), 190.89 (C-20). HRMS cal. for $C_{27}H_{35}N_3O_3$ 449.2678, found 449.2672.

2.1.2.9.6. 20-oxo-21-(1*H*-1,2,4-triazol-1-yl)pregna-5,16-dien-3β-yl-cyclobutanecarboxylate 9g. Yield 0.068 g, (56%), m.p. 136–138 °C, IR (KBr) cm^{-1}: 2930, 1723, 1625, 1575, 1270 and 1167. ^{1}H RMN (400 MHz, $CDCl_3$) δ: 0.98 (s, H-18, 3H), 1.01 (s, H-19, 3H), 2.30 (m, ester-H-1′, 1H), 3.45 (m, *J* = 3.4 Hz, H-3, 1H), 4.90 (s, 2H, H-21), 5.36 (d, *J* = 5.3 Hz, H-6, 1H), 6.74 (m, *J* = 6.7 Hz, C-16, 1H), 7.80 (s, H-Het, 1H), 8.2 (s, H-Het., 1H). ^{13}C RMN (100 MHz, $CDCl_3$) δ: 12.18 (C-18), 15.86 (C-19), 30.10 (C1′ of ester), 65.51 (C-21), 73.55 (C-3), 121.93 (C-6), 121.96 (C-16), 141.53 (C-5), 143.98 (C-Het.), 152.15 (C-Het.), 157.21 (C-17.), 174.96 (ester carbonyl), 190.92 (C-20). HRMS cal. for $C_{28}H_{37}N_3O_3$ 463.2835, found 463.2837.

2.1.2.9.7. 20-oxo-21-(1*H*-1,2,4-triazol-1-yl)pregna-5,16-dien-3β-yl-cyclopentanecarboxylate 9h. Yield 0.073 g, (58%), m.p. 118–120 °C, IR (KBr) cm^{-1}: 2928, 1724, 1625, 1574, 1270 and 1168. ^{1}H RMN (400 MHz, $CDCl_3$) δ: 0.96 (s, H-18, 3H), 1.20 (s, H-19, 3H), 2.31 (m, ester-H-1′, 1H), 3.48 (m, *J* = 3.4 Hz, H-3, 1H), 4.90 (s, 2H, H-21), 5.36 (d, *J* = 5.3 Hz, H-6, 1H), 6.74 (m, *J* = 6.7 Hz, C-16, 1H), 7.70 (s, H-Het., 1H), 8.01 (s, H-Het., 1H). ^{13}C RMN (100 MHz, $CDCl_3$) δ: 15.87 (C-18), 19.25 (C-19), 30.10 (C1′ of ester), 65.10 (C-21), 74.45 (C-3), 121.92 (C-6), 140.01 (C-16), 141.23 (C-5), 144.0 (C-Het.), 152.17 (C-Het.), 157.29 (C-17), 176.37 (ester carbonyl), 190.96 (C-20). HRMS cal. for $C_{29}H_{39}N_3O_3$ 477.2991, found 477.2997.

2.1.2.9.8. 20-oxo-21-(1*H*-1,2,4-triazol-1-yl)pregna-5,16-dien-3β-yl-cyclohexanecarboxylate 9i. Yield 0.071 g, (55%), m.p. 115–118 °C, IR (KBr) cm^{-1}: 2930, 1724, 1627, 1585, 1275 and 1166. ^{1}H RMN (400 MHz, $CDCl_3$) δ: 0.96 (s, H-18, 3H), 1.31 (s, H-19, 3H), 2.30 (m, ester-H-1′, 1H), 3.78 (m, *J* = 3.7 Hz, H-3, 1H), 4.90 (s, 2H, H-21), 5.36 (d, *J* = 5.3 Hz, H-6, 1H), 6.74 (m, *J* = 6.7 Hz, C-16, 1H), 7.70 (s, H-aromatic, 1H), 8.01 (s, H-aromatic, 1H). ^{13}C RMN (100 MHz, $CDCl_3$) δ: 15.80 (C-18), 19.25 (C-19), 30.10 (C1′ of ester), 65.50 (C-21), 73.52 (C-3), 121.92 (C-6), 140.10 (C-16), 141.50 (C-5), 143.92 (C-Het.), 152.20 (C-Het.), 175.57 (ester carbonyl), 199.37 (C-20). HRMS cal. for $C_{30}H_{41}N_3O_3$ 491.3148 for, found 491.3144.

2.1.2.9.9. 20-oxo-21-(1*H*-1,2,4-triazol-1-yl)pregna-5,16-dien-3β-yl-cycloheptanecarboxylate 9j. Yield 0.068 g, (51%), m.p. 197–199 °C, IR (KBr) cm^{-1}: 2932, 1726, 1629, 1583, 1270 and 1160. ^{1}H RMN (400 MHz, $CDCl_3$) δ: 0.90 (s, H-18, 3H), 1.33 (s, H-19, 3H), 2.30 (m, ester-H-1′, 1H), 3.48 (m, *J* = 3.4 Hz, H-3, 1H), 4.90 (s, 2H, H-21), 5.35 (d, *J* = 5.3 Hz, H-6, 1H), 6.73 (m, *J* = 6.7 Hz, C-16, 1H), 7.70 (s, H-aromatic, 1H),

7.98 (s, H-aromatic, 1H). ^{13}C RMN (100 MHz, $CDCl_3$) δ: 15.87 (C-18), 19.31 (C-19), 30.71 (C1′ of ester), 65.50 (C-21), 72.05 (C-3), 121.07 (C-6), 140.30 (C-16), 141.50 (C-5), 143.94 (C-Het.), 152.20 (C-Het.), 176.58 (ester carbonyl), 191.05 (C-20). HRMS cal. for $C_{31}H_{43}N_3O_3$ 505.3304, found 505.3310.

2.2. Animal and human tissues

2.2.1 Type 1 5α-R isoenzyme isolated from rat liver

Two adult (8-month old) rats were obtained by the Metropolitan University in Xochimilco from Animal Care Facility, Mexico. All procedures with animals were approved by the Institutional Care and Use Committee of UAM.

The adult rats weighing 500 g had been fasted overnight to decrease glycogen levels before their livers were extirpated for use as a source of 5α-R1 [12]. To prepare microsomes, the livers (30 g) were minced in one volume of buffer A (20 mM sodium phosphate, pH 6.5, containing 0.32 M sucrose, 0.1 mM DTT (Sigma-Aldrich, Mexico City, Mexico) with an IKA−A11 basic tissue mill (IKA Laboratory Equipment, Mexico City, Mexico). Unless otherwise specified, the following procedures were carried out at 4 °C.

The tissue was homogenized and the suspension was centrifuged (13,200 rpm; 20 min; 0 °C) (Beckman L70 K ultracentrifuge). The pellet was discarded. The supernatant was filtered through a nylon mesh filter (pore size 11 μm, distributed by OEM-Membrane Solution, Dallas TX) and centrifuged again (10,000 rpm; 60 min, 4 °C); the microsomal pellet was resuspended in five volumes of buffer A with a homogenizer. The protein amount was measured by the Bradford method. [13]

The suspension was re-centrifuged (13,200 rpm; 20 min, 4 °C) and the pellet was resuspended in buffer A to give a final concentration of 20 mg protein/mL. The microsomal suspension was stored at −70 °C prior to the preparation of the sample steroid.

2.2.2. Type 2 5αR isoenzyme isolated from human prostate

Four hours after a 53-year-old patient had died of a heart attack, his normal prostate was extirpated in the Pathology Department of the General Hospital in Mexico City. The Ethical Committee of the General Hospital in Mexico City approved this protocol.

The tissue was rinsed and immediately chilled in ice-cold 150 mM NaCl and stored at −20 ºC. The frozen human prostate was thawed on ice, rinsed, and minced in buffer A (20 mM sodium phosphate, pH 6.5, containing 0.32 M sucrose, 0.1 mM DTT; Sigma-Aldrich, Mexico City, Mexico) with an IKA® A11 basic tissue mill (IKA Laboratory Equipment, Mexico City, Mexico). Unless otherwise specified, the following procedures were carried out at 4 ºC.

Human prostate was used in this experiment because this tissue is an abundant source of 5α-R2, but not of 5α-R1; the AR study in this model is very complex.

The human prostate tissue was homogenized in two volumes of buffer A with a tissue homogenizer Ultra-Turrax IKA, T18 basic (Wilmington, NC). The homogenates were centrifuged (1500×g; 60 min) [10] in a SW 60 Ti rotor (Beckman Instruments, Palo Alto, CA). The pellets were resuspended in buffer A, and stored at −70 °C. This suspension

had a final concentration of 5 mg protein/mL, as determined by the Bradford method, and was used as source of 5α-R2 isozyme.

2.2.3. Rat prostate cytosol as source of AR

In order to evaluate the binding of steroids **8** and **9a−j** to the androgen receptor, the prostates of 50 adult rats (8 months old; 500 g) were removed, blotted, weighed and soaked in cold TEMD (40 mM tris–HCl, 3 mM EDTA and 20 mM sodium molybdate, dithiothreitol 0.5 mM, 10% glycerol at pH 8) before use. All procedures were carried out in an ice bath at 0 °C.

The tissues were homogenized with a tissue homogenizer (Teckmar, Cincinnati, OH) in one volume of buffer TEMD plus protease inhibitors (2 mM PMSF, 10 µg/mL antipain, 5 mM leupeptin) [14] in an ice bath with a tissue homogenizer; subsequently the homogenates were centrifuged (14,000 rpm, 60 min) [15] 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. The protein (6 mg of protein in 200 µL) was determined by the Bradford Method.

In this study we used rats because the prostate gland is bigger than that of the hamster and there is no difference in the binding activity of the AR between rat and hamster cytosol. [15]

2.2.4 Characteristics of cell lines

The following panel of cells was used in this study: PC-3 (human prostate cancer cell line), MCF7 (human breast cancer cell line), and SK-LU-1 (human lung cancer cell line), supplied by the National Cancer Institute (USA).

2.2.5 *In vivo* experiments

For the experiments *in vivo*, 80 adult male golden hamsters (2.5 months old; 150–200 g) were used. After gonadectomies had been performed on the hamsters under isoflurane anesthesia, the castrated hamsters were allowed to recover for 30 days prior to experimentation. The castrated hamsters and the remaining eight intact hamsters were housed in a room with controlled temperature (22 °C) and light-dark periods of 12 h; the hamsters were fed with food and water *ad libitum*. Thirty days post gonadectomy, the hamsters were separated into nine groups consisting of four animals per group. The hamsters were treated for six days, and thereafter sacrificed with CO_2 [10]. This experiment was carried out twice under the same conditions.

2.3 Biological activity of the new compounds

2.3.1 *In vitro* experiments

The effect of steroids **8** and **9a–j**, (Fig. 2) on the activity of 5α-R1 and 5α-R2 was evaluated under the same *in vitro* conditions that we described previously [10]

We give a brief description of some of the details of the technique to evaluate the 50% inhibitory concentration (IC_{50}) of **8** and **9a–j** required to inhibit the activity of 5α-R isozymes. Different concentrations (1×10^{-10} to 10^{-4} M) of each of the steroid derivatives were added to tubes containing the culture medium [10]. The transformation of T to DHT in the presence of **8** and **9a–j** was calculated from radioactive compounds present in each of

the lanes of the chromatographic plate, taking into account the entire radioactivity present in the rest of the plate. These data were plotted using SigmaPlot software 12 inhibition curves. [10].

2.3.2 Competitive studies in AR

The binding capacity of steroids **8** and **9a–j** to the AR was determined using the same technique that was described previously by our group [9] and the IC_{50} of each compound was calculated according to the plots of concentration versus percentage of binding, using SigmaPlot 12.

2.3.3 *In vivo* experiments

2.3.3.1 Weight of the prostate, seminal vesicles and diameter of the pigmented spot

For six consecutive days, each of the steroid derivatives **8**, **9a**–**e** (2 mg/kg body weight (BW)) dissolved in 200 µL of sesame oil, together with 1 mg/kg (BW) of testosterone, was administered by subcutaneous (SC) injection to a group of gonadectomized hamsters (four animals per derivative). Three groups of gonadectomized animals were kept as control; the first group was injected SC with 200 µL sesame oil, the second group with 1 mg /kg (BW) of testosterone, and the third group with 1 mg T plus 1 mg/kg (BW) of finasteride also prepared in sesame oil. Additionally, one group of four intact hamsters was used as the intact control. After treatment, the animals were sacrificed with CO_2. The diameters of the flank organs were measured using a vernier caliper; the prostate and seminal vesicles of each hamster were dissected and weighed. Two separate experiments were performed for each group of steroid treated hamsters. The results were

analyzed by using one-way analysis of variance and Dunnett's method to compare means, using JMP IN 5.1 software (JMP, Statistical Discovery, Cary, NC, USA).

2.3.4 Cytotoxicity assay

Cytotoxic activity of steroids **8** and **9a–j** on three different cell lines; PC-3, MCF7, and SK-LU-1, was evaluated as reported previously. [16, 17]

The percentage of cell growth inhibition was calculated according to the following expression:

$$\textit{Cell growth inhibition (\%)} = \frac{100 - (\textit{simple absorption})}{(\textit{vehicle absorption})} X\ 100$$

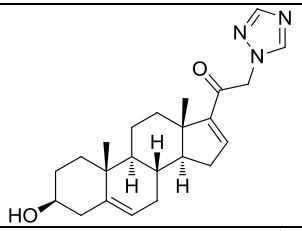
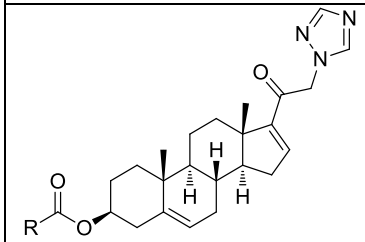
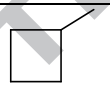
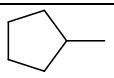
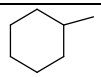
3. Results

3.1. Chemistry

In this synthesis the strategy for the preparation of **8** and **9a−j** (Fig. 2) was the following: we used the commercially available 16-dehydropregnenolone acetate (**1**) treated with hydrogen peroxide and sodium hydroxide 4N to form the 16β, 17β-epoxy compound **2**. The protection in C-3 with tert-butyldimethylsilyl chloride and imidazole in DMF afforded the compound **3.** The oxidation of **3** with (diacetoxyiodo) benzene, sodium hydroxide and methanol generated the compound with the alcohol in C-21 **4**. This compound treated with thionyl chloride yielded the chlorinated compound **5**. **6** was prepared using potassium carbonate and 1,2,4-triazole in DMF and warmed at 80 °C for 5 h. Compound **6** upon treatment with hydrochloric acid in acetone afforded the compound **7**. The 16β,17β-epoxy elimination of **7** was performed with chromium chloride (II) in acetic acid giving

compound **8**. Finally compounds **9b−j** were obtained from **8** by using the Steglish esterification method. [18] Their esters were obtained with a high and regular yields (51-90%). The synthesis of **9a** was done with acetic anhydride and pyridine (77% yield). All compounds were isolated and the melting points showed a maximum of two degrees difference. The desired compounds and intermediates were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry.

Table 1. Biological activity of novel derived compounds on human cancer cell line growth inhibition at 50 µM, IC_{50} value for 5α-R isoenzymes and AR binding assay.

Structure	Compound	R	PC-3	MCF7	SK-LU-1	5α-R1 IC_{50} nM	5α-R2 IC_{50} µM	RBA AR %
Ketoconazole	**10**	-	88.48±3.9	95.16±4.8	73.39±1.6	ND	ND	ND
Finasteride	**11**	-	NA	NA	NA	630	0.0085	NA
	**8**	-	61.3±0.3	62.1±0.8	88.9±5.5	NA	NA	NA
	**9a**	$-CH_3$	56.2±1.3	59.4±2.3	74.8±1.5	NA	100	NA
	9b	$-C_2H_5$	NA	5.9±2.2	8.8±0.7	88	NA	NA
	9c	$-C_3H_7$	9.2±1.6	25.6±2.7	15.6±1.8	NA	NA	NA
	9d	$-C_4H_9$	38.2±2.2	38.5±0.9	20.2±2.8	320	NA	NA
	9e	$-C_5H_{11}$	16.9±1.8	14.4±0.2	6.4±1.7	$100x10^3$	NA	NA
	9f		NA	NA	NA	NA	118	NA
	9g		NA	NA	NA	NA	NA	NA
	9h		NA	NA	NA	NA	NA	NA
	9i		NA	NA	NA	NA	NA	NA
	9j		NA	NA	NA	NA	$98x10^3$	NA

3.2. Effect of steroids 8 and 9a−j as inhibitors of the activity of 5α-R isozymes

The *in vitro* effect of steroids **8, 9a−j** and finasteride (**11 in Fig. 1**) required for inhibiting 5α-R1 and 5α-R2 activity for 50% (IC_{50} value) are shown in Table 1.

These results indicate that **9b** and **9d** with IC_{50} values for 5α-R1 of 88 nM and 320 nM respectively (Table 1) have higher inhibitory activity (lower IC_{50} values) than finasteride (IC_{50}=630). However, the rest of these compounds do not show this inhibitory activity by 5α-R1.

The data in Table 1 also show that **9a** and **9f** inhibited the activity of 5α-R2 with IC_{50} values of 100 and 118 μM respectively. These IC_{50} values are higher than that of finasteride (0.0085 μM), which is the steroid we used as a reference control. Thus **9a** and **9f** are less potent than finasteride for inhibiting the activity of 5αR-2.

3.3. Competitive studies

These studies show that **8** and **9a-j** did not displace the tritium-labeled MIB (**12** in Fig. 1) of AR in the trial. However, the unlabeled MIB used as reference control shifted the labeled MIB from AR, giving an IC_{50} value of binding to the AR of 1 nM. MIB is a very potent agonist of AR; this is the reason we used this steroid as a reference control.

3.4. Cytotoxic activity on cancer cell lines

The percentage of antiproliferative effect produced by steroids **8**, **9a−j** on the growth of human cancer cell lines PC-3 (prostate), MCF7 (breast) and SK-LU-1 (lung) is shown in Table 1. Ketoconazole was used in this study as a reference standard because it is known that this compound has an antiproliferative effect in cancer cell lines.

The data in Table 1 also show that compounds **8** and **9a** have lower antiproliferative activity on PC-3 and MCF7 (61.3%, 56.2%, 62.1% and 59.4% respectively) than in SK-

LU-1 cells. Furthermore these compounds have a high inhibitory activity on this last cell line (88.9% and 74.8% respectively). However steroids **9b-9j** displayed a lower cytotoxic activity than ketoconazole; this low cytotoxic activity could be due to its low solubility in DMSO, which could prevent availability in the cell culture medium.

3.5. Pharmacological results

After castration, the weight of the hamster prostate and seminal vesicles decreased ($p < 0.05$) compared to the normal glands, as did the diameter of the pigmented spot. Treatment with vehicle alone did not change these conditions. However, injections of 1 mg/kg of T for 6 days significantly increased ($p \leq 0.05$) the diameter of the pigmented spot as well as the weight of the prostate and seminal vesicles (Table 2). When T (1 mg/kg) and finasteride were injected together, the diameter of the flank organs and the weight of the prostate and seminal vesicles decreased significantly ($p \leq 0.05$) compared to that of T-treated animals (Table 2). This reduction of the weight of these glands and diameter of the pigmented spot was comparable to that produced by finasteride **11** (Table 2).

The results of this experiment (Table 2) show the effect of steroids **8**, **9a−j** on the size of the pigmented spot of the flank organs as well as the weight of prostate and seminal vesicles. The Dunnett test, which compares the mean obtained in the pharmacological experiments, indicated that no significant differences exist between the response obtained with T-treatment and T plus **8**, **9a−j**.

Table 2. Diameter of the pigmented spot of the flank organs; weight of the prostate and seminal vesicles glands ± standard deviation from castrated hamsters receiving different treatments for 6 days.

Treatment	DIAMETER OF THE PIGMENTED SPOT (mm)±standard error	WEIGHT OF PROSTATE (mg)±standard error	WEIGHT OF SEMINAL VESICLES (mg)±standard error
Control	2.03±.63	50.4±9.9	96.11±12.5
T	**5.65± 1.1**	**98 ±32.6**	**200.6 ±55**
T+F	3.57±0.9	69.6± 21.2	161.4± 44.5
T + 8	3.05 1.5	89.0 ±16	163.33± 41.4
T + 9a	2.9±0.9	87.65±21.8	216.075±67.2
T + 9b	2.79±1.4	89.55±21.1	157.08±32.6
T + 9c	2.71±0.7	81.3±57	138.9±45
T + 9d	3.71±1.0	82.30±21.9	180.03±39.5
T + 9e	2.8±0.4	86.9±19.5	200±56.7
T + 9f	2.88±0.8	107.48±22.8	180.3±39.5
T + 9g	2.14±2.5	100±40	130±58
T + 9h	2.8±.1.0	97±30	120±25
T + 9i	3±0.3	88±10	150±15
T + 9j	4.45±0.4	78.2±18.5	203.98±47.8

The control animals were treated with vehicle only

4. Discussion

In this study we demonstrated that derivatives **8** and **9a-j** were less potent for inhibition of the activity of 5α-R2 than other 16-pregnenolone acetate and 16-pregnandiene acetate derivatives previously studied. [19] This low activity *in vitro* (**8** and **9a-j)** fully explains the lack of pharmacological effect of these steroids observed in these experiments.

However **9b** and **9d** were more potent for inhibition of 5αR1 than the previously reported derivatives. [19] Compounds **9b** and **9d** have ester groups with an even number of carbon atoms in their structure, which may favor union with 5α-R1. This is important because some types of prostate tumor cells, such as LnCaP, proliferate due to the presence of 5α-R1 specifically, so these compounds may have therapeutic potential for this type of condition. [5]

The structure of the two isozymes of 5α-R has not been elucidated because they are membrane proteins, which makes them very difficult to purify in their functional form. [1] For this reason its crystal structure is unknown and docking and SAR studies remain in the theoretical stage. [9, 10, 19]

Data obtained in this study demonstrated that steroid **8** with a trizole moiety at C-21 showed high cytotoxic activity on the cell line SK-LU-1 (lung cancer). In a previous study it has been reported that DHEA derivatives to which triazole groups were added at C-17 were able to inhibit the growth of the same three cell lines used in this study. [9] But these derivatives of DHEA [9] also had antiproliferative action on cell lines PC-3 and MCF7. The triazole group added to the synthesized pregnane derivatives increased their solubility in the culture medium of cells studied. [20] In addition, the ester group attached at C-3 of

these derivatives enhances the value of log P and therefore its permeability through the cell membrane. When the antiproloferative effect produced by steroid derivatives with imidazole or a pyrazole ring is compared with triazole it is observed that the antiproliferative effect increases triazole ring. [21] Other 21-triazolyl derivatives of pregnenolone have displayed a potent antitumor activity. For example the compound 21-{4-[(4-methoxyphenoxy)methyl]-1H-1,2,3-triazol-1-yl}-3-hydroxypregn-5-en-20-one synthesized by the group of Banday, which was very active to inhibit the growth of DU-145 and PC-3 cell lines [22].

These data indicate that the hydroxylated derivative of pregnenolone at C-3 and with a triazole group at C-21 synthesized and studied in this work displays antiproliferative specificity on the SK-LU-1 cells, which could be considered an advantage for its therapeutic use. This activity could be attributed to the presence of a free OH group at C-3 (**8**), which forms hydrogen bonds with different molecules in the cells.

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6. Conflict of Interest

The authors declare that that are no real or perceived conflicts of interest arising from intellectual, personal, or financial circumstances of the research. Additionally, all authors are aware, and approve, of the contents and order of authorship of the manuscript.

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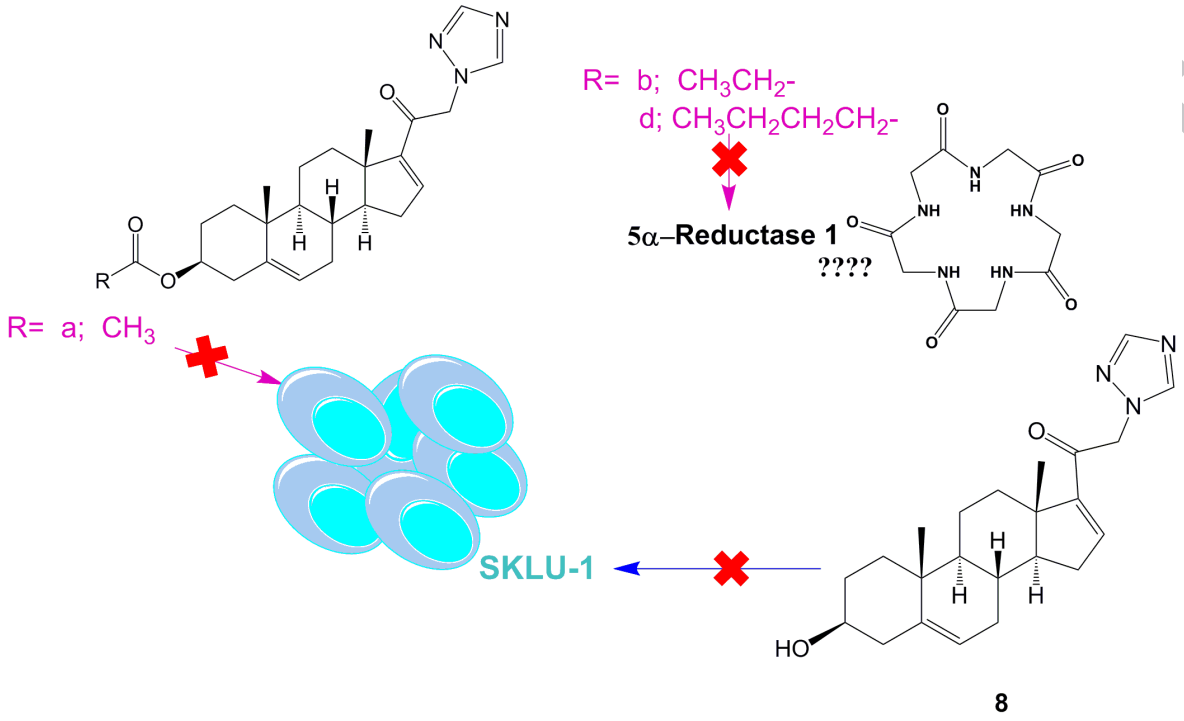
8. **Figure legends**

Fig. 1. 16-dehydropregnenole acetate (**1**), Ketoconazole (**10**), Finasteride (**11**) and Mibolerone (**12**).

Fig. 2. Reagents and conditions: *i*) H_2O_2, NaOH 4N; *ii*) TBDMS, imidazole, DMF; *iii*) NaOH, $C_6H_5I(OAc)_2$, MeOH; *iv*) $SOCl_2$, Py, CH_2Cl_2; *v*) 1,2,4-triazole, K_2CO_3, 80 °C, 5 h; *vi*) HCl, acetone; *vii*) $CrCl_2$, Ac. Acetic; *viii a*) acetic anhydride, Py; *viii b-e*) R-COOH, DCC, DMAP, $CHCl_3$.

Graphical abstract



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