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Configurational analysis and relative binding affinities of 16-methyl-5α-androstane derivatives

Pál Tapolcsányi[a], János Wölfling[a], István Tóth[b], Mihály Szécsi[b], Péter Forgó[a], Gyula Schneider[a,*]

[a]*Department of Organic Chemistry, University of Szeged, Dóm tér 8, H-6720 Szeged, Hungary*
[b]*Endocrine Unit and Research Laboratory, University of Szeged, Korányi fasor 8, H-6720 Szeged, Hungary*

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Abstract

The four possible isomers 16β-hydroxymethyl-5α-androstane-3β,17β-diol **1**, 16α-hydroxymethyl-5α-androstane-3β,17β-diol **2**, 16β-hydroxymethyl-5α-androstane-3β,17α-diol **3** and 16α-hydroxymethyl-5α-androstane-3β,17α-diol **4** with proven configuration were converted into the corresponding 16β-methyl-5α-androstane-3β,17β-diol **5**, 16α-methyl-5α-androstane-3β,17β-diol **6**, 16β-methyl-5α-androstane-3β,17α-diol **7**, 16α-methyl-5α-androstane-3β,17α-diol **8**, furthermore into the 16β-methyl-17β-hydroxy-5α-androstane-3-one **13**, 16α-methyl-17β-hydroxy-5α-androstan-3-one **14**, 16β-methyl-17α-hydroxy-5α-androstan-3-one **15** and 16α-methyl-17α-hydroxy-5α-androstan-3-one **16**. The steric structures of the resulting epimers were determined by means of ^{1}H-, and ^{13}C-NMR spectroscopy. In this way, comparison was possible with the C-16 epimers **5, 6** and **13, 14** prepared earlier by a different route, and the series of isomers could be completed with the steric structures of 16β-methyl-17α-hydroxy-5α-androstan-3β-ol **7** and 16α-methyl-17α-hydroxy-5α **8** and with their 3-keto derivatives **15** and **16**. The relative binding affinities of the 16-methyl-5α-androstane-3β,17-diols **5, 6, 7, 8** and 17-hydroxy-16-methyl-5α-androstan-3-ones **13, 14, 15, 16** were studied. The introduction of a 16-methyl substituent into 5α-androstane molecules substantially decreases the binding affinity to the androgen receptor and 16α-methyl derivatives were always bound more weakly than the 16β-methyl isomers.

Keywords: 16-Methyl steroids; Configurational analysis; Androgen receptor binding

1. Introduction

The presence of a C-16 alkyl group in steroids often enhances, sometimes significantly, the biological properties of the parent compound. In the corticoid series, the introduction of a C-16 methyl group enhances the activity of the parent compound [1,2]. In other steroids, such as androstane, estrane and the aldosterone antagonists, alkyl substitution at a similar site causes a reduction in hormone activity [3,4]. This observation assumed importance when the antihormone effects of 16-alkylestrene [5,6,7] and 16,16-dimethyl-4-estren-3-one were recognized [8]. The literature provides a large number of methods for the introduction of a 16-methyl group onto the sterane skeleton. The site of substitution of this group follows unequivocally from the method of synthesis, but the literature reveals uncertainties as concerns the steric situation of the alkyl group.

Ruggieri et al. [9,10] prepared 16α- and 16β-methyl-3β-hydroxypregn-5-en-20-one with confirmed configuration by effecting a Grignard reaction between 3β-hydroxypregna-5,16-dien-20-one and methyl iodide, and 1,3-dipolar cycloaddition with diazomethane with subsequent decomposition. Side-chain isomerization of 16β-methyl-3β-hydroxypregn-5-en-20-one in alkaline medium yielded 16β-methyl-17-*iso*-3β-hydroxypregn-5-en-20-one. Baeyer-Villiger oxidation of pregnane derivatives substituted in different manners yielded three of the four possible isomers of 16-methylandrost-5-ene-3β,17-diol. At the same time, Beckmann rearrangement of 16α- and 16β-methyl-3β-hydroxypregn-5-en-20-one resulted in 16α- and 16β-methyl-3β-hydroxyandrost-5-en-17-one, which were hydrogenated on Pd-$CaCO_3$ to furnish the two 16-methyl-3β-hydroxy-5α-

* Corresponding author. Tel.: +36-62-544-276; fax: +36-62-544-200.
E-mail: schneider@chem.u-szeged.hu (G. Schneider).

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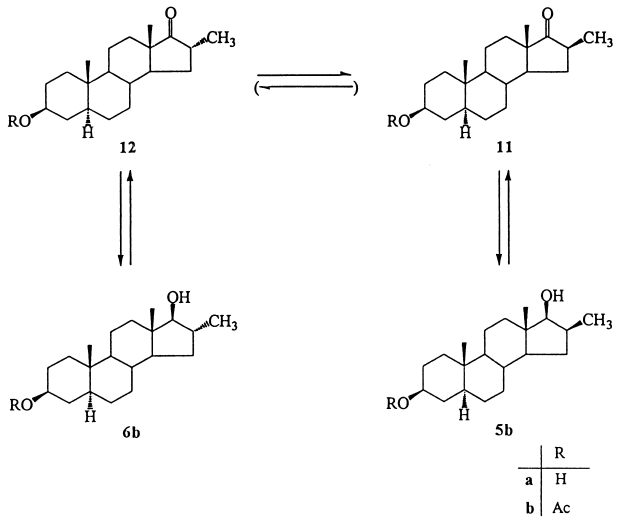


Scheme 1.

androstan-17-ones **11a, 12a**. In the reduction with $NaBH_4$, their acetylated derivatives afforded the 16-methyl-3β-acetoxy-5α-androstan-17-ol isomers **5b, 6b** [9].

The above synthesis of the 16-methyl-3β-acetoxy-17β-hydroxy-5α-androstane epimers **5b, 6b** were achieved by the reduction at C-17 of 16-methyl-17-oxo steroids **11b, 12b**. Neef et al. [11] have pointed out that the 16-methyl-17-ketosteroids undergo interconversion in equilibrium reactions under both acidic and alkaline conditions. The stereochemical homogeneity of 17-hydroxy-16-methyl epimers obtained by the reduction of 16-methyl-17-oxo steroids is therefore strongly in doubt because of the possible equilibrium isomerization of the starting compounds (Scheme 1).

The present work aimed at the preparation of the four possible isomers of 16-methyl-5α-androstane-3β,17-diol **5a, 6a, 7a, 8a** and 16-methyl-17-hydroxy-5α-androstan-3-one **13a, 14a, 15a, 16a** with confirmed configurations, independently from the reaction path described in the literature. This would allow a configurational comparison of the isomers **5a, 6a** and **13a, 14a** prepared earlier by other methods, and also completion of the isomer series with compounds **7a, 8a** and **15a, 16a**. In possession of the different isomers, it seemed of interest to study their binding to the androgen receptor, the specific binding protein in target cells mediating androgen steroid action (Schemes 2 and 3).

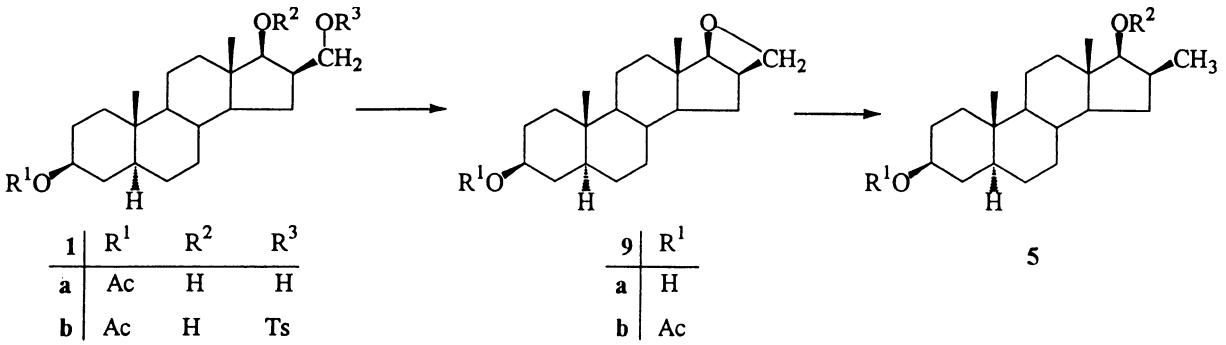
2. Experimental

Melting points (mps) were determined with a Kofler hot-stage apparatus and are uncorrected. Specific rotations were measured with a POLAMAT-A (Zeiss-Jena) polarimeter in chloroform, methanol or acetic acid solutions (*c* 1) and are given in units of 10^{-1} deg cm^2 g^{-1}. Elemental analyses were performed with a Perkin-Elmer CHN analyser model 2400. Thin-layer chromatography: silica gel 60 F_{254}; layer thickness 0.2 mm (Merck); solvent system (ss): (A) chloroform, (B) ethyl acetate/chloroform (5:95 v/v), (C) ethyl acetate/chloroform (10:90 v/v); detection with iodine or UV (365 nm) after spraying with 50% phosphoric acid and heating at 100–120°C for 10 min. Flash chromatography: silica gel 60, 40–63 μm. Column chromatography: Al_2O_3 (standardized according to Brockmann) with activity of III-IV. ^{1}H-NMR spectra were recorded with a Bruker DRX-400 instrument at 400 MHz in $CDCl_3$ solution (if not otherwise given), using Me_4Si as internal standard. ^{13}C-NMR spectra were recorded with the same instrument at 100 MHz under the same conditions.

2.1. 3β,17α-Diacetoxy-16β-hydroxymethyl-5α-androstane (3a) and 3β-acetoxy-16α-hydroxymethyl-5α-androstan-17α-ol (4a)

Alkaline alumina (25 g) was added to a solution of 16β- or 16α-acetoxymethyl-5α-androstane-3β,17α-diacetate (**3c** or **4c**) [12] (448 mg, 1 mmol) dissolved in dichloromethane (5 ml) and the solvent was evaporated off in vacuo. The air-dried material, in a small beaker, was placed inside a microwave oven. After 6 min (in the case of **3c**) or 10 min (in the case of **4c**) of irradiation at 90 W, the product was extracted into chloroform (5 × 20 ml). Following evaporation, the residue was subjected to chromatographic separation on silica gel. During the column chromatography of the **3c** mixture, ethyl acetate/chloroform (10:90) first eluted **3c**, and then **3a** (244 mg, 60%). Mp of **3a** 154–156°C, R_f = 0.30 (ss C); $[\alpha]_D^{20}$ −42 (*c* 1 in chloroform). (Found: C, 70.82; H, 9.50. $C_{24}H_{38}O_5$ requires C, 70.90; H, 9.42%); ^{1}H-NMR δ ppm 0.70(m, 1H, 9-H), 0.76(s, 3H, 18-H), 0.80(s, 3H, 19-H), 2.00(s, 3H) and 2.05(s, 3H): 3- and 17-CH_3CO, 3.60(m, 2H, 16-CH_2), 4.44(d, 1H, J=1.6 Hz, 17-H), 4.68(m, 1H, 3-H). ^{13}C-NMR δ ppm 12.2(C-19), 17.5(C-18), 20.4(C-11), 21.3 and 21.4(2C, CH_3CO), 27.4, 28.4, 29.5, 32.1, 32.2, 34.0, 35.2(C-8), 35.6, 36.8, 44.3(C-13), 44.6(C-5), 50.4 and 50.5(2C, C-14 and C-16), 53.8(C-9), 66.1(C-16), 73.6(C-3), 84.8(C-17), 170.7 and 172.1(2C, CH_3CO).

Chromatography with ethyl acetate/chloroform (10:90) of the **4c,4d** mixture resulted first in **4c**, and then in **4d** (40 mg, 10%). Mp of **4d** 189–192°C, R_f = 0.35 (ss C); $[\alpha]_D^{20}$ −4 (*c* 1 chloroform). (Found: C, 70.97; H, 9.45. $C_{24}H_{38}O_5$ requires C, 70.90; H, 9.42%); ^{1}H-NMR δ ppm 0.68(m, 1H, 9-H), 0.82(s, 3H) and 0.83(s, 3H):18-H and 19-H, 2.02(s, 3H, 3-CH_3CO), 2.12(s, 3H, 17-CH_3CO), 2.60(m, 1H), 3.52(m, 2H, 16-CH_2), 4.68(m, 1H, 3-H), 4.96(d, 1H, J=5.5 Hz, 17-H). ^{13}C-NMR δ ppm 12.9(C-19), 17.7(C-18), 21.1(C-11), 21.7 and 22.1(2C, CH_3CO), 28.1, 28.6, 29.1, 32.2, 32.8, 34.6, 36.2(C-10), 36.3(C-8), 37.4, 43.5(C-16), 45.3(C-5), 46.4(C-13), 49.9(C-14), 54.6(C-9), 63.3(C-16′), 74.3(C-3), 83.3(C-17), 171.4 and 172.3(2C, CH_3CO). Continued elution with ethyl acetate/chloroform (30:70) gave **4a** (183 mg, 51%). Mp 203–205°C, R_f = 0.20 (ss C); $[\alpha]_D^{20}$ +6 (*c* 1 chloroform). (Found: C, 72.55; H 9.82. $C_{22}H_{36}H_4$



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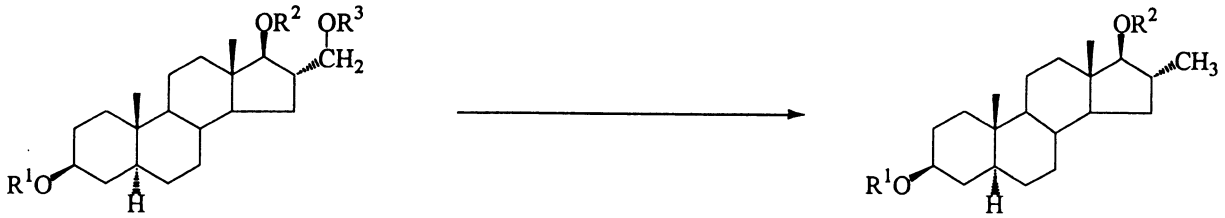
a	Ac	H	H
b	Ac	H	Ts

9	R^1
a	H
b	Ac

5



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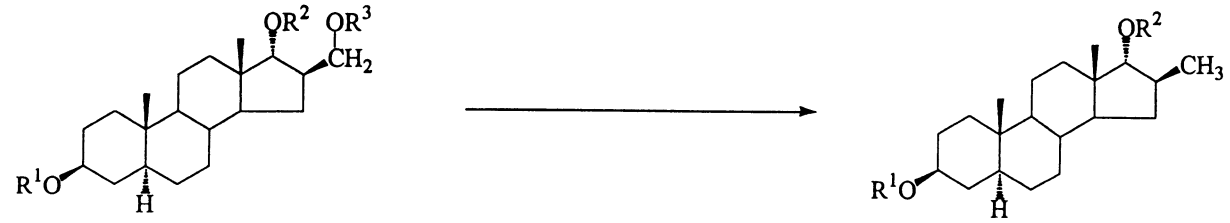
a	Ac	H	H
b	Ac	H	Ts

6



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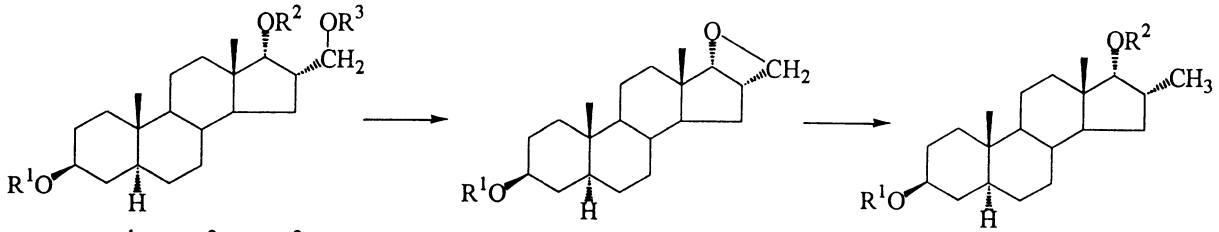
a	Ac	Ac	H
b	Ac	Ac	Ts
c	Ac	Ac	Ac

7

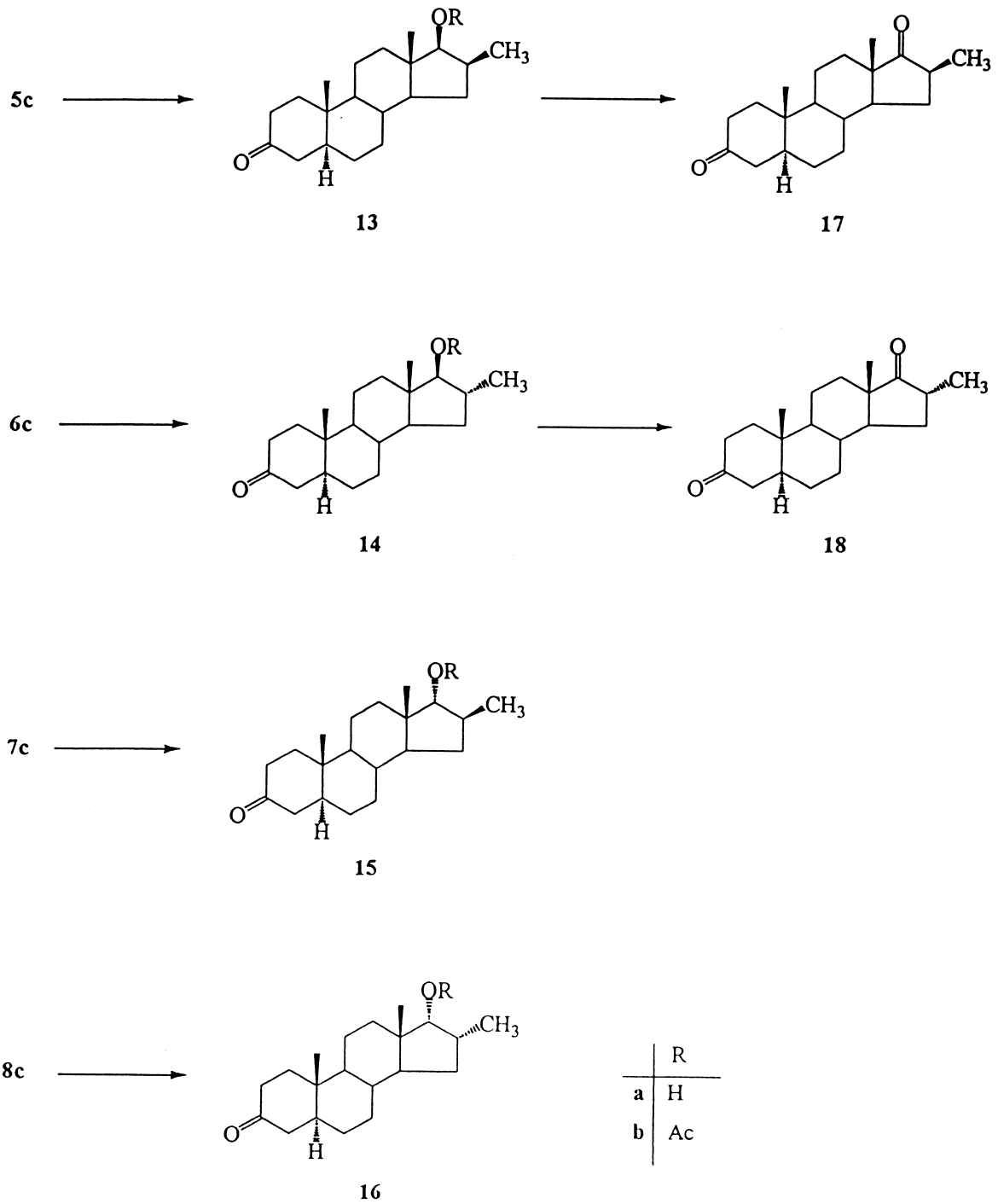


4	R^1	R^2	R^3
a	Ac	H	H
b	Ac	H	Ts
c	Ac	Ac	Ac
d	Ac	Ac	H

10	R^1
a	H
b	Ac

8	R^1	R^2
a	H	H
b	Ac	H
c	H	Ac
d	Ac	Ac

Scheme 2.



Scheme 3.

requires C, 72.49; H, 9.95%); ^{1}H-NMR δ ppm 0.66(m, 1H, 9-H), 0.70(s, 3H, 18-H), 0.81(s, 3H, 19-H), 2.00(s, 3H, CH_3CO), 2.44(m, 1H), 3.66(m, 1H) and 3.80(m, 1H):16-CH_2, 3.83(d, 1H, J=5.7 Hz, 17-H), 4.68(m, 1H, 3-H). ^{13}C-NMR δ ppm 12.2(C-19), 17.3(C-18), 20.5(C-11), 21.5(CH_3CO), 27.4, 27.8, 28.5, 31.2, 32.2, 34.0, 35.5, 35.7(C-8), 36.8, 41.7(C-16), 44.6(C-5), 46.1(C-13), 48.4(C-14), 53.9(C-9), 63.6(C-16′), 73.8(C-3), 81.7(C-17), 170.9(CH_3CO).

2.2. 16β-Toluene-p-sulfonyloxymethyl-5α-androstane-3β,17α-diacetate ***(3b)***

Compound **3a** (2.03 g, 0.005 mol) was dissolved in pyridine (20 ml) and a solution of toluene-*p*-sulfonyl chloride (1.9 g, 0.01 mol) dissolved in pyridine (10 ml) was added dropwise. The reaction mixture was allowed to stand at room temperature for 24 h. It was then poured onto a mixture of sulfuric acid (12 ml) and ice (200 g), and the

precipitate was filtered off, washed and dried. The product was subjected to chromatographic separation on silica gel with chloroform as eluent, and crystallized from methanol. **3b** (2.45 g, 89%) mp 147–149°C, R_f = 0.80 (ss C); $[\alpha]_D^{20}$ +4 (*c* 1 chloroform). (Found: C, 66.32; H, 8.02. $C_{31}H_{44}O_7S$ requires C, 66.40; H, 7.91%); ^{1}H-NMR δ ppm 0.69(m, 1H, 9-H), 0.70(s, 3H, 18-H), 0.81(s, 3H, 19-H), 2.01(s, 3H) and 2.02(s, 3H):3- and 17-CH_3CO), 2.15(m, 1H), 2.45(s, 3H, Ts-CH_3), 4.09(m, 1H) and 4.18(s, 1H):16-CH_2, 4.43(d, 1H, *J*=2.2 Hz, 17-H), 4.68(m, 1H, 3-H), 7.35(d, 2H, *J*=8.1 Hz, 3′- and 5′-H), 7.79(d, 2H, *J*=8.1 Hz, 2′- and 6′-H). ^{13}C-NMR δ ppm 12.9(C-19) and 17.8(C-18), 21.1(C-11), 21.7 and 22.1(2C, *C*H_3CO), 22.4(Ts-CH_3), 28.1, 29.1, 30.2, 32.7(2C), 34.6, 35.8(C-8), 36.2(C-10), 37.4, 45.0(C-13), 45.2(C-5), 46.5(C-16), 51.6(C-14), 54.4(C-9), 73.0(C-16′), 74.2(C-3), 83.7(C-17), 128.6(2C, C-2′ and C-6′), 130.5(2C, C-3′ and C-5′), 134.3(C-1′), 145.3(C-4′), 171.2 and 171.4(2C, CH_3*C*O).

2.3. 3β-Acetoxy-16α-toluene-p-sulfonyloxymethyl-5α-androstan-17α-ol ***(4b)***

Compound **4a** (3.64 g, 0.01 mol) was dissolved in anhydrous pyridine (30 ml) and a solution of toluene-*p*-sulfonyl chloride (2.85 g, 0.015 mol) in anhydrous pyridine (15 ml) was added dropwise during cooling with ice. The reaction mixture was allowed to stand for 24 h and was then poured onto a mixture of ice (500 g) and sulfuric acid (10 ml). The precipitate that separated out was filtered off and recrystallized from a mixture of benzene/light petroleum. **4b** (4.80 g, 92%) mp 142–144°C, R_f = 0.60 (ss C); $[\alpha]_D^{20}$ −6 (*c* 1 chloroform). (Found: C, 67.22; H, 8.20. $C_{29}H_{42}O_6S$ requires C, 67.15; H, 8.16%); ^{1}H-NMR δ ppm 0.63(m, 1H, 9-H), 0.68(s, 3H, 18-H), 0.81(s, 3H, 19-H), 2.01(s, 3H, CH_3CO), 2.45(s, 3H, Ts-CH_3), 2.57(m, 1H), 3.73(s, 1H, 17-H), 3.98(m, 1H) and 4.21(s, 3H): 16-CH_2, 4.68(m, 1H, 3-H), 7.34(d, 2H, *J*=8.0 Hz, 3′- and 5′-H), 7.79(d, 2H, *J*=8.0 Hz, 2′- and 6′-H). ^{13}C-NMR δ ppm 12.6(C-19), 17.4(C-18), 20.8(C-11), 21.8 and 22.0(2C, *C*H_3CO and Ts-CH_3), 27.8, 28.3, 28.8(2C), 31.4, 34.4, 35.8(C-10), 35.9(C-8), 37.2, 40.5(C-16), 45.0(C-5), 46.4(C-13), 48.0(C-14), 54.3(C-9), 71.6(C-16′), 74.0(C-3), 79.5(C-17), 128.3(2C, C-2′ and C-6′), 130.2(2C, C-3′ and C-5′), 133.5(C-1′), 148.5(C-4′), 171.1(CH_3CO).

2.4. 16-Methyl-5α-androstane-3β-17-diols ***(5a, 6a, 7a, 8a)***

2.4.1. General procedure

$LiAlH_4$ (1.0 g) was suspended in anhydrous tetrahydrofuran (50 ml) cooled in a salt-ice bath, and a solution of 16-toluene-*p*-sulfonyloxymethyl-3β-acetoxy-5α-androstan-17-ol (**1b, 2b** [12] or **4b**) (2.60 g, 0.005 mol) or 16β-toluene-*p*-sulfonyloxymethyl-5α-androstane-3β, 17α-diacetate (**3b**) (2.80, 0.005 mol) in anhydrous tetrahydrofuran (50 ml) was added dropwise. The reaction mixture was stirred at 70°C for 6 h. Aqueous ethanol was added to the cooled reaction mixture, which was then acidified with dilute hydrochloric acid and diluted with an equal volume of water, and the organic fraction was evaporated under reduced pressure. The residual precipitate was filtered off, dissolved in chloroform and subjected to chromatographic separation on alumina with chloroform as eluent. The substance obtained was crystallized from a mixture of chloroform-petroleum ether.

5a (1.20 g, 78%) mp 198–199°C, R_f = 0.15 (ss B); $[\alpha]_D^{20}$ +13 (*c* 1 chloroform) ([9] mp 194–196°C, $[\alpha]_D$ +10.9). (Found: C, 78.42; H, 11.25. $C_{20}H_{34}O_2$ requires: C, 78.38; H, 11.18%); ^{1}H-NMR δ ppm 0.66(m, 1H, 9-H), 0.73(s, 3H, 18-H), 0.81(s, 3H, 19-H), 0.99(d, 3H, *J*=7.35 Hz, 16-CH_3), 3.54–3.65(2H, m, 1H, 3-H and d, 1H, *J*=10.4 Hz, 17-H), 3.60(d, 1H, *J*=10.4 Hz, 17-H).

6a (1.05 g, 68%) mp 182–184°C, R_f = 0.10 (ss B); $[\alpha]_D^{20}$ −12 (*c* 1 chloroform) ([9] mp 179–181°C, $[\alpha]_D$ −15). (Found: C, 78.30; H, 11.30. $C_{20}H_{34}O_2$ requires: C, 78.38; H, 11.18%); ^{1}H-NMR δ ppm 0.64(m, 1H, 9-H), 0.75(s, 3H, 18-H), 0.81(s, 3H, 19-H), 1.09(d, 3H, *J*=7.0 Hz, 16-CH_3), 3.10(d, 1H, *J*=7.5 Hz, 17-H), 3.6(m, 1H, 3-H).

7a (0.98 g, 64%) mp 243–245°C, R_f = 0.10 (ss B); $[\alpha]_D^{20}$ +3 (*c* 1 methanol). (Found: C, 78.40; H, 11.02. $C_{20}H_{34}O_2$ requires: C, 78.38; H, 11.18%); ^{1}H-NMR (DMSO-d_6) δ ppm 0.56(m, 1H, 9-H), 0.63(s, 3H, 18-H), 0.74(s, 3H, 19-H), 1.07(d, 3H, *J*=7.3 Hz, 16-CH_3), 4.24(d, 1H, *J*=4.7 Hz, 17-H), 4.39(d, 1H, *J*=4.6 Hz, 3-H).

8a (1.00 g, 65%) mp 265–267°C, R_f = 0.15 (ss B); $[\alpha]_D^{20}$ +5 (*c* 1 in acetic acid). (Found: C, 78.51; H, 11.06. $C_{20}H_{34}O_2$ requires: C, 78.38; H, 11.18%). ^{1}H-NMR (DMSO-d_6) δ ppm 0.56(m, 1H, 9-H), 0.64(s, 3H, 18-H), 0.73(s, 3H, 19-H), 0.87(d, 3H, *J*=7.2 Hz, 16-CH_3), 4.12(d, 1H, *J*=5.2 Hz, 17-H), 4.39(d, *J*=4.7 Hz, 3-H).

*2.5. 16-Methyl-3β-acetoxy-5α-androstan-17-ols (***5b, 6b, 7b, 8b***)*

2.5.1. General procedure

Compound **5a, 6a, 7a**, or **8a** (3.00 g, 0.01 mol) was dissolved in anhydrous pyridine (20 ml), and a solution of acetic anhydride (2 ml, 0.02 mol) in pyridine (10 ml) was then added dropwise while cooling in ice under continuous stirring. The progress of the reaction was monitored by TLC. The reaction mixture was poured onto a mixture of sulfuric acid (10 ml) and ice (100 g) and extracted with chloroform. The chloroform phase was evaporated to dryness and subjected to chromatographic separation on silica gel in ethyl acetate/chloroform (5:95). The product was crystallized from methanol.

5b (3.10 g, 89%) mp 161–163°C, R_f = 0.35 (ss B); $[\alpha]_D^{20}$ +2 (*c* 1 in chloroform) ([9] mp 159–161°C, $[\alpha]_D$ +3). (Found: C, 75.76; H, 10.55. $C_{22}H_{36}O_3$ requires: C, 75.82; H, 10.41%). ^{1}H-NMR δ ppm 0.66(m, 1H, 9-H), 0.73(s, 3H, 18-H), 0.83(s, 3H, 19-H), 0.99(d, 3H, *J*=7.5 Hz, 16-CH_3), 2.02(s, 3H, CH_3CO), 3.61(d, 1H, *J*=9.9 Hz, 17-

H), 4.68 (m, 1H, 3-H). ^{13}C-NMR δ ppm 12.4 and 12.6 (2C, C-18 and C-19), 16.6(C-16′), 20.9(C-11), 21.6(CH_3CO), 27.6, 28.6, 31.9, 34.1, 34.2 and 35.1 (2C, C-8 and C-16), 34.6, 35.7(C-10), 36.9, 37.9, 44.1(C-13), 44.9(C-5), 49.7(C-14), 54.6(C-9), 73.8(C-3), 82.4(C-17), 170.9(CH_3CO).

6b (2.95 g, 84%) mp 168–171°C, R_f = 0.30 (ss B); $[\alpha]_D^{20}$ −20 (*c* 1 in chloroform) ([9] mp 159–161°C, $[\alpha]_D$ −21). (Found: C, 75.68; H, 10.58. $C_{22}H_{36}O_3$, requires: C, 75.82; H, 10.41%). ^{1}H-NMR δ ppm 0.66(m, 1H, 9-H), 0.75(s, 3H, 18-H), 0.83(s, 3H, 19-H), 1.09(d, 3H, *J*=6.9 Hz, 16-CH_3), 2.02(s, 3H, CH_3CO), 3.10(d, 1H, *J*=7.7 Hz, 17-H), 4.68(m, 1H, 3-H). ^{13}C-NMR δ ppm 11.9 and 12.2 (2C, C-18 and C-19), 20.4(C-16′), 20.6(C-11), 21.4(CH_3CO), 27.4, 28.4, 31.5, 32.3, 34.0, 35.2 and 38.2(2C, C-8 and C-16), 35.6(C-10), 36.8(2C), 44.0(C-13), 44.7(C-5), 49.1(C-14), 54.5(C-9), 73.6(C-3), 89.7(C-17), 170.7(CH_3CO).

7b (3.00 g, 86%) mp 178–180°C, R_f = 0.25 (ss B); $[\alpha]_D^{20}$ −8 (*c* 1 in chloroform). (Found: C, 75.74; H, 10.34. $C_{22}H_{36}O_3$ requires: C, 75.82; H, 10.41%). ^{1}H-NMR δ ppm 0.68(m, 1H, 9-H), 0.72(s, 3H, 18-H), 0.83(s, 3H, 19-H), 1.16(d, 3H, 16-CH_3), 2.02(s, 3H, CH_3CO), 3.34(d, 1H, *J*=1.2 Hz, 17-H), 4.68(m, 1H, 3-H). ^{13}C-NMR δ ppm 12.2 (C-19), 17.9(C-18), 20.5(C-11), 21.1(C-16′), 21.4(CH_3CO), 27.5, 28.5, 32.1, 32.3, 34.0, 34.7, 35.4(C-8), 35.6(C-10), 36.8, 43.3(C-16), 44.6(C-5), 45.1(C-13), 50.6(C-14), 53.9(C-9), 72.1(C-3), 87.2(C-17), 170.6(CH_3CO).

8b (3.05 g, 87%) mp 159–161°C, R_f = 0.38 (ss B); $[\alpha]_D^{20}$ −27 (*c* 1 in chloroform). (Found: C, 75.69; C, 10.30. $C_{22}H_{36}O_3$ requires: C, 75.82; H, 10.41%). ^{1}H-NMR δ ppm 0.66(m, 1H, 9-H), 0.72(s, 3H, 18-H), 0.82(s, 3H, 19-H), 0.99(d, 3H, *J*=7.2 Hz, 16-CH_3), 2.02(s, 3H, CH_3CO), 3.53(d, 1H, *J*=4.7 Hz, 17-H), 4.68(m, 1H, 3-H). ^{13}C-NMR δ ppm 12.2(C-19), 15.4(C-16′), 17.4(C-18), 20.6(C-11), 21.4(CH_3CO), 27.5, 28.6, 31.5, 32.2, 33.5, 34.0, 34.4 and 35.7(2C, C-8 and C-16), 35.6(C-10), 36.8, 44.7(C-5), 46.3(C-13), 48.0(C-14), 54.1(C-9), 73.7(C-3), 81.7(C-17), 170.6(CH_3CO).

2.6. 16-Methyl-5α-androstane-3β,17-diacetates ***(5d, 6d, 7d, 8d)***

2.6.1. General procedure

Compound **5a, 6a, 7a**, or **8a** (3.00 g, 0.01 mol) was dissolved in a mixture of pyridine (10 ml) and acetic anhydride (5 ml, 0.05 mol) and allowed to stand for 24 h. It was then poured onto a mixture of sulfuric acid (4 ml) and ice (50 g). The precipitate was filtered off, washed and dried. The product was crystallized from methanol.

5d (3.50 g, 89%) mp 119–120°C, R_f = 0.70 (ss B); $[\alpha]_D^{20}$ +16 (*c* 1 in chloroform) ([9] mp 127–129°C, $[\alpha]_D$ + 12). (Found: C, 73.92; H, 9.75. $C_{24}H_{38}O_4$ requires: C, 73.81; H, 9.81%). ^{1}H-NMR δ ppm 0.68(m, 1H, 9-H), 0.80(s, 3H, 18-H), 0.83(s, 3H, 19-H), 0.87(d, 3H, *J*=7.25 Hz, 16-CH_3), 2.02(s, 3H, 3-CH_3CO), 2.08(s, 3H, 17-CH_3CO), 4.57(d, 1H, *J*=10.0 Hz, 17-H), 4.68(m, 1H, 3-H). ^{13}C-NMR δ ppm 12.2(C-19), 13.4(C-18), 16.8(C-16'), 20.6(C-11), 20.9(17-CH_3CO), 21.4(3-CH_3CO), 27.4, 28.5, 31.7, 32.9 and 34.8(2C, C-8 and C-16), 34.0, 34.3, 35.6, 36.7, 37.7(C-12), 43.2(C-13), 44.6, 49.8(C-14), 54.2, 73.6, 83.7(C-17), 170.6(3-CH_3CO), 171.1(17-CH_3CO).

6d (3.60 g, 92%) mp 140–142°C, R_f = 0.65 (ss B); $[\alpha]_D^{20}$ −49 (*c* 1 in chloroform) ([9] mp 141–143°C, $[\alpha]_D$ −49). (Found: C, 73.75; H, 9.90. $C_{24}H_{38}O_4$ requires: C, 73.81; H, 9.81%). ^{1}H-NMR δ ppm 0.66(m, 1H, 9-H), 0.77(s, 3H, 18-H), 0.82(s, 3H, 19-H), 1.04(d, 3H, *J*=7.0 Hz, 16-CH_3), 2.02(s, 3H, 3-CH_3CO), 2.05(s, 3H, 17-CH_3CO), 4.42(d, 1H, *J*=7.6 Hz, 17-H), 4.68(m, 1H, 3-H). ^{13}C-NMR δ ppm 12.2(C-19), 12.8(C-18), 20.3(C-16′), 20.6(C-11), 21.2(17-CH_3CO), 21.4(3-CH_3CO), 27.4, 28.4, 31.4, 32.4(C-15), 34.0, 35.1 and 35.4(2C, C-8 and C-16), 35.6, 36.7, 37.1, 44.3(C-13), 44.7, 49.2(C-14), 54.2, 73.6, 89.2(C-17), 170.6(3-CH_3CO), 171.2(17-CH_3CO).

7d (3.45 g, 88%) mp 114–117°C, R_f = 0.65 (ss B); $[\alpha]_D^{20}$ +8 (*c* 1 in chloroform). (Found: C, 73.86; H, 9.75. $C_{24}H_{38}O_4$ requires: C, 73.81; H, 9.81%). ^{1}H-NMR δ ppm 0.67(m, 1H, 9-H), 0.80(s, 3H, 18-H), 0.82(s, 3H, 19-H), 1.19(d, 3H, *J*=7.1 Hz, 16-CH_3), 2.02(s, 3H, 3-CH_3CO), 2.04(s, 3H, 17-CH_3CO), 4.42(d, 1H, *J*=1.5 Hz, 17-H), 4.68(m, 1H, 3-H). ^{13}C-NMR δ ppm 12.6(C-19), 17.8(C-18), 20.8(C-11), 21.2, 21.7, 21.9, 27.9, 28.9, 32.6, 32.7, 34.4, 35.0, 35.7(C-8), 35.9, 37.1, 41.1(C-16), 45.0(C-5), 45.0(C-13), 51.9(C-14), 54.2(C-9), 74.1(C-3), 88.9(C-17), 171.1 and 171.2(2C, CH_3CO).

8d (3.70 g, 94%) mp 155–157°C, R_f = 0.68 (ss B); $[\alpha]_D^{20}$ +2 (*c* 1 in chloroform). (Found: C, 73.72; H, 9.90. $C_{24}H_{38}O_4$ requires: C, 73.81; H, 9.81%). ^{1}H-NMR δ ppm 0.67(m, 1H, 9-H), 0.79(s, 3H, 18-H), 0.82(s, 3H, 19-H), 0.87(d, 3H, *J*=7.3 Hz, 16-CH_3), 2.02(s, 3H, 3-CH_3CO), 2.08(s, 3H, 17-CH_3CO), 2.47(m, 1H, 16-H), 4.68(m, 1H, 3-H), 4.85(d, 1H, *J*=5.8 Hz, 17-H). ^{13}C-NMR δ ppm 12.6(C-19), 16.0(C-16′), 17.5(C-18), 20.8(C-11), 21.3 and 21.8(2C, CH_3CO), 27.8, 28.9, 32.2, 32.5, 34.06, 34.11(C-16), 34.4, 35.9, 36.0(C-8), 37.1, 45.1(C-5), 46.3(C-13), 49.4(C-14), 54.4(C-9), 74.0(C-3), 83.7(C-17), 171.1 and 171.2(2C, CH_3CO).

2.7. 16-Methyl-17-acetoxy-5α-androstan-3β-ols ***(5c, 6c, 7c, 8c)***

2.7.1. General procedure

Compound **5d, 6d, 7d**, or **8d** (3.90 g, 0.01 mol) was dissolved in methanol (200 ml), the solution was cooled to 0°C, and a solution of KOH (0.280 g, 0.005 mol) in methanol (100 ml) was added. The progress of the selective hydrolysis was monitored by TLC. After 24 h at 0°C, the solution was poured onto ice and acidified with dilute hydrochloric acid. The precipitate was filtered off and washed with water. It was subjected to column chromatography on silica gel in ethyl acetate/chloroform (5:95) and crystallized from a mixture of methanol and water.

5c (2.90 g, 83%) mp 172–175°C, R_f = 0.25 (ss B);

$[\alpha]_D^{20}$ +37 (*c* 1 in chloroform). (Found: C, 75.74; H, 10.58. $C_{22}H_{36}O_3$ requires: C, 75.82; H, 10.41%). ^{1}H-NMR δ ppm 0.66(m, 1H, 9-H), 0.80 and 0.81(2s, 6H, 18-H and 19-H), 0.87(d, 3H, *J*=7.5 Hz, 16-CH_3), 2.07(s, 3H, 17-CH_3CO), 3.59(m, 1H, 3-H), 4.57(d, 1H, *J*=9.8 Hz, 17-H). ^{13}C-NMR δ ppm 12.2 and 13.4(2C, C-18 and C-19), 16.8(C-16′), 20.6(C-11), 20.9(CH_3CO), 28.6, 31.5, 31.8, 32.9 and 34.8 (2C, C-8 and C-16), 34.3, 35.6(C-10), 37.0, 37.8, 38.2, 43.2(C-13), 44.9(C-5), 49.9(C-14), 54.4(C-9), 71.2(C-3), 83.7(C-17), 171.2(CH_3CO).

6c (3.10 g, 84%) mp 134–136°C, R_f = 0.20 (ss B); $[\alpha]_D^{20}$ −45 (*c* 1 in chloroform). (Found: C, 75.71; H, 10.35. $C_{22}H_{36}O_3$ requires: C, 75.82; H, 10.41%). ^{1}H-NMR δ ppm 0.66(m, 1H, 9-H), 0.77 and 0.80(2s, 6H, 18-H and 19-H), 1.04(d, 3H, *J*=7.1 Hz, 16-CH_3), 2.05(s, 3H, 17-CH_3CO), 3.59(m, 1H, 3-H), 4.42(d, 1H, *J*=7.7 Hz, 17-H). ^{13}C-NMR δ ppm 12.3 and 12.7(2C, C-18 and C-19), 20.3(C-16′), 20.6(C-11), 21.1(*C*H_3CO), 28.5, 31.4, 31.5, 32.4, 35.1 and 35.3(2C, C-8 and C-16), 35.5(C-10), 36.9, 37.1, 38.1, 44.3(C-13), 44.8(C-5), 49.2(C-14), 54.3(C-9), 71.2(C-3), 89.2(C-17), 171.3(CH_3*C*O).

7c (2.85 g, 81%) mp 174–175°C, R_f = 0.22 (ss B); $[\alpha]_D^{20}$ +16 (*c* 1 in chloroform). (Found: C, 75.69; H, 10.48. $C_{22}H_{36}O_3$ requires: C, 75.82; H, 10.41%). ^{1}H-NMR δ ppm 0.65(m, 1H, 9-H), 0.80 and 0.81(2s, 6H, 18-H and 19-H), 1.18(d, 3H, *J*=7.1 Hz, 16-CH_3), 2.03(s, 3H, CH_3CO), 3.59(m, 1H, 3-H), 4.42(d, 1H, *J*=1.5 Hz, 17-H). ^{13}C-NMR δ ppm 12.3(C-19), 17.4(C-18), 20.5(C-11), 20.8(C-16′), 21.2(*C*H_3CO), 28.6, 31.5, 32.3(2C), 34.6, 35.4(C-8), 35.6(C-10), 37.0, 38.2, 40.8(C-16), 44.6(C-13), 44.9(C-5), 51.6(C-14), 54.0(C-9), 71.3(C-3), 88.6(C-17), 170.7(CH_3*C*O).

8c (3.25 g, 93%) mp 172–173°C, R_f = 0.22 (ss B); $[\alpha]_D^{20}$ +3 (*c* 1 in chloroform). (Found: C, 75.73; H, 10.32. $C_{22}H_{36}O_3$ requires: C, 75.82; H, 10.41%). ^{1}H-NMR δ ppm 0.67(m, 1H, 9-H), 0.79 and 0.80(2s, 6H, 18-H and 19-H), 0.88(d, 3H, *J*=7.3 Hz, 16-CH_3), 2.07(s, 3H, CH_3CO), 3.60(m, 1H, 3-H), 4.85(d, 1H, *J*=5.8 Hz, 17-H). ^{13}C-NMR δ ppm 12.3(C-19), 15.6(C-16′), 17.1(C-18), 20.5(C-11), 20.9(*C*H_3CO), 28.7, 31.5, 31.9, 32.3, 33.6, 33.7 and 35.7 (2C, C-8 and C-16), 35.6(C-10), 37.0, 38.2, 44.9(C-5), 45.9(C-13), 49.1(C-14), 54.1(C-9), 71.3(C-3), 83.4(C-17), 170.9(CH_3*C*O).

2.8. 16-Methyl-17-acetoxy-5α-androstan-3-ones ***(13b, 14b, 15b, 16b)***

2.8.1. General procedure

Jones reagent (2 ml) was added dropwise to a solution of compounds **5c, 6c, 7c**, or **8c** (1.75 g, 0.005 mol) in acetone (10 ml) during cooling with ice. The mixture was diluted with ice-water and the precipitate was filtered off, and recrystallized from aqueous methanol.

13b (1.65 g, 95 %) mp 185–187°C, R_f = 0.30 (ss A); $[\alpha]_D^{20}$ +47 (*c* 1 in chloroform) ([9] mp 180–182°C, $[\alpha]_D$ +47). (Found: C, 76.35; H, 9.94. $C_{22}H_{34}O_3$ requires: C, 76.26; H, 9.89%). ^{1}H-NMR δ ppm 0.76(m, 1H, 9-H), 0.83(s, 3H, 18-H), 0.89(d, 3H, *J*=7.5 Hz, 16-CH_3), 1.02(s, 3H, 19-H), 2.08(s, 3H, CH_3CO), 4.58(d, 1H, *J*=10.0 Hz, 17-H). ^{13}C-NMR δ ppm 11.5(C-19), 13.4(C-18), 16.8(C-16′), 20.8(C-11), 20.9(*C*H_3CO), 28.8(C-6), 31.4(C-7), 32.9 and 34.7(2C, C-8 and C-16), 34.3, 35.8(C-10), 37.7, 38.1(C-2), 38.5(C-1), 43.2(C-13), 44.7(C-4), 46.7(C-5), 49.7(C-14), 53.8(C-9), 83.6(C-17), 171.1(CH_3*C*O), 211.8(C-3).

14b (1.60 g, 92%) mp 176–179°C, R_f = 0.28 (ss A); $[\alpha]_D^{20}$ −28 (*c* 1 in chloroform) ([9] 173–174°C, $[\alpha]_D$ +18). (Found: C, 76.14; H, 9.98. $C_{22}H_{34}O_3$ requires: C, 76.26; H, 9.89%). ^{1}H-NMR δ ppm 0.76(m, 1H, 9-H), 0.80(s, 3H, 18-H), 1.01(s, 3H, 19-H), 1.05(d, 3H, *J*=6.95 Hz, 16-CH_3), 2.06(s, 3H, CH_3CO), 4.43(d, 1H, *J*=7.4 Hz, 17-H). ^{13}C-NMR δ ppm 11.5(C-19), 12.5(C-18), 20.3(C-16′), 20.8(C-11), 21.2(*C*H_3CO), 28.8(C-6), 31.2(C-7), 32.4, 35.1 and 35.4 (2C, C-8 and C-16), 35.7(C-10), 37.0, 38.1(C-2), 38.5(C-1), 44.3(C-13), 44.6(C-4), 46.6(C-5), 49.1(C-14), 53.8(C-9), 89.1(C-17), 171.2(CH_3*C*O), 211.8(C-3).

15b (1.48 g, 85%) mp 148–152°C, R_f = 0.32 (ss A); $[\alpha]_D^{20}$ +42 (*c* 1 in chloroform). (Found: C, 76.41; H, 9.77. $C_{22}H_{34}O_3$ requires: C, 76.26; H, 9.89%). ^{1}H-NMR δ ppm 0.76(m, 1H, 9-H), 0.83(s, 3H, 18-H), 1.01(s, 3H, 19-H), 1.21(d, 3H, *J*=7.1 Hz, 16-CH_3), 2.04(s, 3H, CH_3CO), 4.44(d, 1H, *J*=1.5 Hz, 17-H). ^{13}C-NMR δ ppm 11.4(C-19), 17.4(C-18), 20.7(C-11), 20.8(C-16′), 21.2(*C*H_3CO), 28.9(C-6), 32.0, 32.3, 34.6, 35.3(C-8), 35.7(C-10), 38.1(C-2), 38.6(C-1), 40.8(C-16), 44.6(2C, C-4 and C-13), 46.7(C-5), 51.4(C-14), 53.4(C-9), 88.4(C-17), 170.7(CH_3*C*O), 211.8(C-3).

16b (1.40 g, 81%) mp 137–140°C, R_f = 0.32 (ss A); $[\alpha]_D^{20}$ +25 (*c* 1 in chloroform). (Found: C, 76.30; H, 9.97. $C_{22}H_{34}O_3$ requires: C, 76.26; H, 9.89%). ^{1}H-NMR δ ppm 0.76(m, 1H, 9-H), 0.82(s, 3H, 18-H), 0.89(d, 3H, *J*=7.2 Hz, 16-CH_3), 1.01(s, 3H, 19-H), 2.08(s, 3H, CH_3CO), 4.86(d, 1H, *J*=5.6 Hz, 17-H). ^{13}C-NMR δ ppm 11.4(C-19), 15.6(C-16′), 17.0(C-18), 20.7(C-11), 20.8(*C*H_3CO), 28.9(C-6), 31.8, 31.9, 33.6, 33.7 and 35.5(2C, C-8 and C-16), 35.7(C-10), 38.1(C-2), 38.5(C-1), 44.6(C-4), 45.9(C-13), 46.7(C-5), 49.0(C-14), 53.6(C-9), 83.2(C-17), 170.8(CH_3*C*O), 211.8(C-3).

2.9. 16-Methyl-17-hydroxy-5α-androstan-3-ones ***(13a, 14a, 15a, 16a)***

2.9.1. General procedure

Compounds **13b, 14b, 15b** or **16b** (1.73 g, 0.005 mol) was dissolved in methanol (50 ml) and KOH (0.6 g, 0.01 mol) was added. After 24 h of standing at room temperature, the mixture was neutralized with dilute hydrochloric acid and diluted with water. The precipitate was then filtered off, and crystallized from acetone/light petroleum

13a (1.35 g, 90%) mp 186–189°C, R_f = 0.22 (ss B); $[\alpha]_D^{20}$ +35 (*c* 1 in chloroform) ([9] mp 184–186°C, $[\alpha]_D$ +33). (Found: C, 79.02; H, 10.45. $C_{20}H_{32}O_2$ require: C, 78.90; H, 10.59%). ^{1}H-NMR δ ppm 0.76(s, 3H, 18-H),

1.00(d, 3H, J=7.6 Hz, 16-CH_3), 1.02(s, 3H, 19-H), 3.62(d, 1H, J=10.0 Hz, 17-H). ^{13}C-NMR δ ppm 11.5 and 12.5(2C, C-18 and C-19), 16.4(C-16′), 21.0(C-11), 28.8(C-6), 31.5(C-7), 34.1 and 34.9(2C, C-8 and C-16), 34.5, 35.8(C-10), 37.8, 38.1(C-2), 38.6(C-1), 43.9(C-13), 44.7(C-4), 46.7(C-5), 49.4(C-14), 54.1(C-9), 82.1(C-17), 211.8(C-3).

14a (1.25 g, 83%) mp 150–153°C, R_f = 0.20 (ss B); $[\alpha]_D^{20}$ +11 (*c* 1 in chloroform). ([9] mp 156–157°C, $[\alpha]_D$ +9.2). (Found: C, 78.86; H, 10.67. $C_{20}H_{32}O_2$ requires: C, 78.90; H, 10.59%). ^{1}H-NMR δ ppm 0.78(s, 3H, 18-H), 1.01(s, 3H, 19-H), 1.09(d, 3H, J=6.9 Hz, 16-CH_3), 3.11(d, 1H, J=7.6 Hz, 17-H). ^{13}C-NMR δ ppm 11.4 and 11.8 (2C, C-18 and C-19), 20.4(C-16′), 20.9(C-11), 28.8(C-6), 31.2(C-7), 32.3, 35.2 and 38.2(2C, C-8 and C-16), 35.7(C-10), 36.8, 38.1(C-2), 38.5(C-1), 44.0(C-13), 44.6(C-4), 46.8(C-5), 49.0(C-14), 54.0(C-9), 89.6(C-17), 211.9(C-3).

15a (1.40 g, 93%) mp 194–197°C, R_f = 0.18 (ss B); $[\alpha]_D^{20}$ +33 (*c* 1 chloroform). (Found: C, 78.92; H, 10.63. $C_{20}H_{32}O_2$ requires: C, 78.90; H, 10.59%). ^{1}H-NMR δ ppm 0.74(s, 3H, 18-H), 1.02(s, 3H, 19-H), 1.17(d, 3H, J=7.3 Hz, 16-CH_3), 3.36(d, 1H, J=1.6 Hz, 17-H). ^{13}C-NMR δ ppm 11.5(C-19), 17.9(C-18), 20.8(C-11), 21.1(C-16′), 28.9(C-6), 32.0, 32.1, 34.7, 35.3(C-8), 35.7(C-10), 38.1(C-2), 38.6(C-1), 43.4(C-16), 44.7(C-4), 45.1(C-13), 46.6(C-5), 50.5(C-14), 53.5(C-9), 87.1(C-17), 211.9(C-3).

16a (1.38 g, 92%) mp 224–225°C, R_f = 0.18 (ss B); $[\alpha]_D^{20}$ +9 (*c* 1 chloroform). (Found: C, 79.05; H, 10.47. $C_{20}H_{32}O_2$ requires: C, 78.90; H, 10.59%). ^{1}H-NMR δ ppm 0.76(s, 3H, 18-H), 1.00(d, 3H, J=7.6 Hz, 16-CH_3), 1.01(s, 3H, 19-H), 3.55(d, 1H, J=5.2 Hz, 17-H). ^{13}C-NMR δ ppm 11.4(C-19), 15.3(C-16′), 17.4(C-18), 20.8(C-11), 28.9(C-6), 31.5(C-7), 31.9, 33.5, 34.4 and 35.6(2C, C-8 and C-16), 35.7(C-10), 38.1(C-2), 38.5(C-1), 44.6(C-4), 46.3(C-13), 46.7(C-5), 47.9(C-14), 53.6(C-9), 81.6(C-17), 211.9(C-3).

2.10. 16-Methyl-5α-androstane-3,17-diones ***(17, 18)***

2.10.1. General procedure

Compound **5a** or **6a** (1.50 g, 0.005 mol) was dissolved in acetone (10 ml). Jones reagent (2 ml) was added during cooling with ice. The mixture was diluted with ice-water, and the precipitate was filtered off, and crystallized from acetone-light petroleum.

17 (1.25 g, 82%) mp 160–162°C, R_f = 0.30 (ss A); $[\alpha]_D^{20}$ +115 (*c* 1 in chloroform) ([9] mp 163–164°C, $[\alpha]_D$ +115). (Found: C, 79.54; H, 10.08. $C_{20}H_{30}O_2$ requires: C, 79.42; H, 10.00%). ^{1}H-NMR δ ppm 0.81(m, 1H, 9-H), 0.85(s, 3H, 18-H), 1.04(s, 3H, 19-H), 1.20(d, 3H, J=6.7 Hz, 16-CH_3). ^{13}C-NMR δ ppm 11.4(C-19), 14.1(C-18), 16.9(C-16′), 20.6(C-11), 28.7(C-6), 30.7, 30.8, 31.9, 34.6(C-8), 35.8(C-10), 38.1(C-2), 38.4(C-1), 43.7(C-16), 44.6(C-4), 46.6(C-5), 48.0(C-13), 49.9(C-14), 54.1(C-9), 211.4(C-3), 223.0(C-17).

18 (1.30 g, 86%) mp 159–161°C, R_f = 0.28 (ss A); $[\alpha]_D^{20}$ +105 (*c* 1 in chloroform) ([9] mp 156–158°C, $[\alpha]_D$ +90). (Found: C, 79.35, H, 10.12. $C_{20}H_{30}O_2$ requires: C, 79.42; H, 10.10 %). ^{1}H-NMR δ ppm 0.81(m, 1H, 9-H), 0.92(s, 3H, 18-H), 1.04(s, 3H, 19-H), 1.10(d, 3H, J=7.7 Hz, 16-CH_3). ^{13}C-NMR δ ppm 11.5(C-19), 14.4(C-18), 16.7(C-16′), 20.7(C-11), 28.6(C-6), 30.2, 30.5, 31.8, 34.8(C-8), 35.8(C-10), 38.1(C-2), 38.4(C-1), 39.2(C-16), 44.6(C-4), 46.6(C-5), 48.3(C-13), 48.5(C-14), 53.9(C-9), 211.6(C-3), 222.7(C-17).

2.11. 17β,16β-(Epoxymethano)-5α-androstan-3β-ol ***(9a)*** *and 17α,16α-(epoxymethano)-5α-androstan-3β-ol* ***(10a)***

2.11.1. General procedure

$LiA1H_4$ (1.0 g, 0.03 mol) was suspended in anhydrous tetrahydrofuran (50 ml) cooled in a salt-ice bath and a solution of **1b** or **2b** (2.60 g, 0.005 mol) in anhydrous tetrahydrofuran (50 ml) was added dropwise. The reaction mixture was stirred at room temperature for 2 h. Aqueous ethanol (50 ml) and 20% aqueous NH_4Cl solution (50 ml) were added to the cooled reaction mixture. The organic fraction was evaporated under reduced pressure. The residual precipitate was filtered off, and subjected to chromatographic separation on alumina with chloroform/light petroleum (1:1) as eluent. The substance obtained was crystallized from acetone/light petroleum.

9a (1.10 g, 72%) mp 185–187°C, R_f = 0.45 (ss B); $[\alpha]_D^{20}$ +2 (*c* 1 in chloroform). (Found: C, 78.88; H, 10.65. $C_{20}H_{32}O_2$ requires: C, 78.90; H, 10.59%). ^{1}H-NMR δ ppm 0.60(m, 1H, 9-H), 0.82(s, 3H, 19-H), 1.04(s, 3H, 18-H), 3.12(m, 1H, 16-H), 3.56(m, 1H, 3-H), 4.21(dd, 1H, J=6.3 Hz, 6.1 Hz) and 4.73(dd, 1H, J=8.1 Hz, 6.3 Hz): 16′-H, 4.54(d, 1H, J=8.0 Hz, 17-H). ^{13}C-NMR δ ppm 12.3(C-19), 12.8(C-18), 21.5(C-11), 28.6, 31.2, 31.5, 32.3, 35.1 and 37.1(2C, C-8 and C-16), 35.5(C-10), 37.0, 37.5, 38.1, 44.5(C-13), 44.8(C-5), 54.2(C-14), 55.4(C-9), 71.2 (C-3), 76.9(C-16′), 95.7(C-17).

10a (1.0 g, 65%) mp 196–198°C, R_f = 0.50 (ss B); $[\alpha]_D^{20}$ +34 (*c* 1 in chloroform). (Found: C, 79.02; H, 10.47. $C_{20}H_{32}O_2$ requires: C, 78.90; H, 10.59%). ^{1}H-NMR δ ppm 0.50(s, 3H, 18-H), 0.83(s, 3H, 19-H), 3.04(m, 1H, 16-H), 3.61(m, 1H, 3-H), 3.95(dd, 1H, J=5.5 Hz, 4.1 Hz) and 4.80(dd, 1H, J=6.6 Hz, 5.5 Hz): 16′-H, 4.62(d, 1H, J=4.8 Hz, 17-H). ^{13}C-NMR δ ppm 12.2(C-19), 14.6(C-18), 20.6(C-11), 28.7, 30.0, 31.0, 31.5, 32.7, 34.9 and 36.1(2C, C-8 and C-16), 35.6(C-10), 37.1, 38.2, 44.6(C-13), 44.9(C-5), 48.4(C-14), 54.3(C-9), 71.3(C-3), 75.2(C-16′), 93.5(C-17).

2.12. 3β-Acetoxy-17β,16β-(epoxymethano)-5α-androstane ***(9b)*** *and 3β-acetoxy-17α,16α-(epoxymethano)-5α-androstane* ***(10b)***

2.12.1. General procedure

Compound **9a** or **10a** (304 mg, 1 mmol) was dissolved in a mixture of pyridine (3 ml) and acetic anhydride (3 ml) and the solution was allowed to stand at room temperature for 6 h. The mixture was then diluted with water and the

precipitate that separated out was filtered off and crystallized from methanol.

9b (310 mg, 89%), mp 108–111 °C, R_f = 0.50 (ss A); $[\alpha]_D^{20}$ −13 (*c* 1 in chloroform). (Found: C, 76.35, H, 9.78. $C_{22}H_{34}O_3$ requires: C, 76.26; H, 9.89%). ^{1}H-NMR δ ppm 0.60(m, 1H, 9-H), 0.85(s, 3H, 19-H), 1.05(s, 3H, 18-H), 2.01(s, 3H, C*H*$_3$CO), 3.13(m, 1H, 16-H), 4.21(dd, 1H, *J*=6.4 Hz, 6.1 Hz) and 4.73(dd, 1H, *J*=8.0 Hz, 6.4 Hz): 16′-H, 4.55(d, 1H, *J*=8.0 Hz, 17-H), 4.67(m, 1H, 3-H). ^{13}C-NMR δ ppm 12.2(C-19), 12.8(C-18), 21.4(C-11), 21.5(*C*H$_3$CO), 27.5, 28.5, 31.2, 32.2, 33.8, 35.0 and 36.8 (2C, C-8 and C-16), 35.6(C-10), 36.4, 37.2, 44.5(C-13), 44.7(C-5), 54.1(C-14), 55.4(C-9), 73.6(C-3), 76.8(C-16′), 95.7(C-17), 170.6(CH$_3$*C*O).

10b (300 mg, 86%), mp 183–185°C, R_f = 0.55 (ss A); $[\alpha]_D^{20}$ +15 (*c* 1 in chloroform). (Found: C, 76.20; H, 9.94. $C_{22}H_{34}O_3$ requires: C, 76.26; H, 9.89%). ^{1}H-NMR δ ppm 0.50(s, 3H, 18-H), 0.85(s, 3H, 19-H), 2.02(s, 3H, C*H*$_3$CO), 3.04(m, 1H, 16-H), 3.95(dd, 1H, *J*=5.6 Hz, 4.1 Hz) and 4.80(dd, 1H, *J*=6.6 Hz, 5.6 Hz): 16′-H, 4.62(d, 1H, *J*=5.0 Hz, 17-H), 4.70(m, 1H, 3-H). ^{13}C-NMR δ ppm 12.2(C-19), 14.6(C-18), 20.5(C-11), 21.4(*C*H$_3$CO), 27.5, 28.6, 29.9, 31.0, 34.9 and 36.1(2C, C-8 and C-16), 35.6(C-10), 36.8, 44.6(C-13), 44.7(C-5), 48.3(C-14), 54.2(C-9), 73.7(C-3), 75.2(C-16′), 93.4(C-17), 170.6(CH$_3$*C*).

*2.13. 16β-Methyl-3β-acetoxy-5α-androstan-17-one **(11b)** and 16α-methyl-3β-acetoxy-5α-androstan-17-one **(12b)***

2.13.1. General procedure

Compound **5b** or **6b** (350 mg, 1 mmol) dissolved in acetone (3 ml). Jones reagent (1 ml) was added during cooling with ice. The mixture was diluted with ice-water, and the resulting precipitate was filtered off, and crystallized from acetone-light petroleum.

11b (320 mg, 92%) mp 107–110°C, R_f = 0.45 (ss B), $[\alpha]_D^{20}$ +73 (*c* 1 in chloroform) ([10] mp 108–110°C, $[\alpha]_D$ +56). (Found: C, 76.18; H, 9.95. $C_{22}H_{34}O_3$ requires: C, 76.26; H, 9.89%). ^{1}H-NMR δ ppm 0.70(m, 1H, 9-H), 0.82(s, 3H) and 0.85(s, 3H): 18-H and 19-H, 1.19(d, 3H, *J*=7.0 Hz, 16-CH$_3$), 2.02(s, 3H, CH$_3$CO), 2.12(m, 2H), 4.69(m, 3H, 3-H). ^{13}C-NMR δ ppm 12.9(C-19), 14.7(C-18), 17.6(C-16′), 21.1(C-11), 21.3(*C*H$_3$CO), 28.1, 29.0, 31.4, 31.7, 32.6, 34.6, 35.3(C-8), 36.3, 37.3, 44.4(C-16), 45.3(C-5), 48.8(C-13), 50.6(C-14), 55.1(C-9), 74.1(C-3), 171.3(CH$_3$*C*O), 224.1(C-17).

12b (300 mg, 86%) mp 151–153°C, R_f = 0.42 (ss B); $[\alpha]_D^{20}$ +61 (*c* 1 in chloroform) ([10] mp 143–145°C, $[\alpha]_D$ + 46). (Found: C, 76.32; H, 9.70. $C_{22}H_{34}O_3$ requires: C, 76.26; H, 9.89%). ^{1}H-NMR δ ppm 0.70(m, 1H, 9-H), 0.85(s, 3H) and 0.89(s, 3H): 18-H and 19-H, 1.09(d, 3H, *J*=7.7 Hz, 16-CH$_3$), 2.02(s, 3H, CH$_3$CO), 2.50(m, 1H), 4.69(m, 3H, 3-H). ^{13}C-NMR δ ppm 12.9(C-19), 15.1(C-18), 17.3(C-16′), 21.1(C-11), 21.3(*C*H$_3$CO), 28.1, 28.9, 30.8, 31.4, 32.5, 34.6, 35.6(C-8), 36.3, 37.4, 39.8(C-16), 45.3(C-5), 49.0(C-13), 49.2(C-14), 55.0(C-9), 74.3(C-3), 171.3(CH$_3$*C*O), 223.6(C-17).

2.14. In vitro binding of 16-methyl-5α-androstane derivatives to the androgen receptor

The androgen-binding experiments were carried out with cytosol of castrated rat prostate, with [^{3}H]methyltrienolone (R1181; 17β-hydroxy-17α-methylestra-4,9,11-trien-3-one) as radioligand. The competitive receptor assays were performed as previously described [14]. The abilities of the currently synthetized 16-methyl-5α-androstane derivatives (**5a, 6a, 7a, 8a, 13a, 14a, 15a, 16a, 17, 18**) to inhibit the specific binding of the radioligand are characterized quantitatively by their IC_{50} values (the concentration of inhibitor at which 50% of the specific radioligand binding is inhibited). Relative binding affinities (RBAs) are defined by

$$\mathrm{RBA}(\%) = \frac{IC_{50}([^3H]R1881)}{IC_{50}\,(inhibitor)}\,100$$

3. Results and discussion

3.1. Synthetic studies

In an earlier publication we reported on the preparation of the four possible isomers of the 16-hydroxymethyl-5α-androstane-3β,17-diol [12]. 3β-Acetoxy-16β-hydroxymethyl-5α-androstan-17β-ol (**1a**) and 3β-acetoxy-16α-hydroxymethyl-5α-androstan-17β-ol (**2a**) were obtained directly from the reduction of 3β-acetoxy-16-acetoxymethylene-5α-androstan-17-one. 16β-Acetoxymethyl-5α-androstan-3β,17α-diacetate (**3c**) and 16α-acetoxymethyl-5α-androstan-3β,17α-diacetate (**4c**) were prepared by utilizing the neighboring group participation characterized by the general symbol (AcO-6) occurring during the acetolysis of 16β-toluene-*p*-sulfonyloxymethyl-5α-androstane-3β,17β-diacetate and 3β-acetoxy-16α-acetoxymethyl-17β-toluene-*p*-sulfonyloxy-5α-androstane, respectively. Whereas the two isomers prepared by the solvolysis method were obtained in their triacetate forms (**3c, 4c**), their selective deacetylation was carried out at the primary acetoxy group according to an earlier developed method [13]. **3c** and **4c** were subjected to microwave irradiation at 90 W on an alumina surface in household microwave equipment. After irradiation of **3c** at 90 W, the 16β-hydroxymethyl-5α-androstane-3β,17α-diacetate (**3a**) was obtained in 60% yield. Irradiation of **4c** for 10 min gave 51% of 3β-acetoxy-16α-hydroxymethyl-5α-androstan-17α-ol (**4a**) This is explained by the selective deacetylation of the triacetate **4c** at the primary acetoxy group. Subsequent acyl migration was observed for the compounds containing the 16,17 functional groups in the *cis* orientation, and further deacetylation at the primary C-16 position.

The three 3β-acetoxy-16-hydroxymethyl-17-hydroxy

isomers **1a, 2a, 4a** and the 16β-hydroxymethyl-3β,17α-diacetate isomers **3a** were converted into their 16-toluene-*p*-sulfonyloxymethyl derivatives **1b, 2b, 3b, 4b**. The next step was $LiAlH_4$ reduction in tetrahydrofuran, which furnished the desired 16-methyl-5α-androstane-3β,17-diol isomers **5a, 6a, 7a, 8a**. Our earlier results [15] indicated that the reduction of 16,17-*cis* isomers **1a** and **4a** proceeded via oxetanes [16] condensed to ring D in the β and α positions, as in **9a** and **10a**. Whereas the reduction did not involve any chiral center, the configurations of the compounds obtained agreed with those of the starting materials, which had confirmed configurations. The 16-methyl-5α-androstane-3β,17-diacetate isomers **5d, 6d, 7d, 8d** were then selectively deacetylated. Jones oxidation of the resulting 17-acetoxy-16-methyl-5α-androstan-3β-ols **5c, 6c, 7c, 8c** gave the 17-acetoxy-16β-methyl-5α-androstan-3-one isomers **13b, 14b, 15b, 16b**. Since the oxidation did not affect the chiral centers C-16 and C-17, the configurations of the products and the starting compounds were identical.

In agreement with the literature, the coupling constants $J_{16,17}$ display the following sequence [17,18]: $J_{16\alpha H,17\alpha H} > J_{16\beta H,17\alpha H} > J_{16\beta H,17\beta H} > J_{16\alpha H,17\beta H}$ **5d**: 10.00 **6d**: 7.6 **8d**: 5.8 **7d**: 1.5 **13b**: 10.00 **14b**: 7.4 **16b**: 5.6 **15b**: 1.5.

The configurational correlation with compounds published in the literature [9,10] was carried out by comparing their physical data. We found that the stereochemical assignments of substituents on the basis of empirical rules were correct except in the case of 16α-methyl-17β-acetoxy-5α-androstan-3-one **14b**. The specific rotation of this compound, which was prepared by the reduction and hydrolysis of 16α-methyl-3-N-pyrrolidyl-5α-androst-3-en-17-one, was +18 [9]. Compound **14b** which we prepared by another method had a specific rotation of −47 (*c* 1 chloroform). The structure was confirmed via the NMR spectrum, and the negative value confirmed the empirical rule that a 16α substituent in the androstane series is generally more negative than a 16β substituent [19,20]. On the other hand, $NaBH_4$ reduction of 3β-acetoxy-16β-methyl-5α-androstan-17-one (**11b**) by the procedure of Ruggieri et al. [9] led to a mixture. We detected the presence of two isomers by TLC. ^{13}C-NMR spectroscopy allowed determination of their stereochemistry without separation. Besides the 16β,17β derivative **5b**, the only isomer prepared by Ruggieri et al., the 16α,17β-epimer **6b** was also formed in the reaction.

3.2. Receptor-binding studies

The IC_{50} values and the RBAs of the 16-methyl-5α-androstane derivatives (**5a, 6a, 7a, 8a, 13a, 14a, 15a, 16a, 17, 18**) and the reference compounds 17β-hydroxy-5α-androstan-3-one (**19**) and 5α-androstane-3β,17β-diol (**20**) obtained in competitive binding receptor assays are illustrated in Table 1. The natural androgen hormone 17β-hydroxy-5α-androstan-3-one (**19**) is a powerful competitor and binds strongly to the androgen receptor (RBA = 72.5%). The introduction of a 16β-methyl substituent into 5α-androstan-3-on-17β-ol (**13a**) decreases RBA from 72.5% to 6.4%, while introduction of a 16α-methyl group (**14a**) leads to a more significant decreased, to 1.8%. In the other reference compound, **20**, which is a major metabolite of 5α-dihydrotestosterone (**19**) in living organisms, the 3-oxo function is changed to 3β-ol and this compound has a relatively low binding affinity for the androgen receptor (RBA = 1.3%). The 16β-methyl and 16α-methyl derivatives (**5a, 6a**) of this compound exhibit lower binding affinities, with RBA 0.33% and 0.15%, respectively.

Table 1
Relative binding affinities (RBAs) of 16-methyl-5α-androstane derivatives and reference compounds for androgen receptor in rat prostate cytosol

Compounds tested	Range of concentration (nM)	IC_{50} (nM) ± SD	RBA (%)
R1881[a]	0.5–5.0	1.53 ± 0.12	100
19[b]	1.0–10	2.11 ± 0.70	72.5
20[c]	50–500	115 ± 15	1.3
5a	200–2000	460 ± 65	0.33
6a	500–2000	993 ± 50	0.15
7a	500–2000	>1000	<0.15
8a	500–2000	>1000	<0.15
13a	10–200	24 ± 3.0	6.4
14a	10–200	83 ± 16	1.8
15a	500–2000	>1000	<0.15
16a	500–2000	>1000	<0.15
17	500–2000	>1000	<0.15
18	200–2000	>1000	<0.15

[a] 17β-Hydroxy-17α-methylestra-4,9,11-trien-3-one.
[b] 17β-Hydroxy-5α-androstan-3-one.
[c] 5α-Androstane-3β,17β-diol.

All 16-methyl-5α-androstane molecules containing a 17α-hydroxy (**7a, 8a, 15a, 16a**), or 17-oxo group (**17, 18**) do not possess the structural requirement essential androgen receptor binding a (17β-hydroxy group), and they therefore practically lack binding affinity (RBA < 0.5%) (Table 1).

In conclusion, in vitro receptor binding studies and the determination of IC_{50} and RBA data are important in investigations relating to biological activity, but they do not reveal the pharmacological type (agonist or antagonist) of the currently synthetized competitor molecules. The introduction of a 16-methyl substituent into 5α-androstane molecules substantially decreases the binding affinity to the androgen receptor and 16α-methyl derivatives were always bound more weakly than the 16β-methyl isomers.

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