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Synthesis of regioisomeric 17β-*N*-phenylpyrazolyl steroid derivatives and their inhibitory effect on 17α-hydroxylase/$C_{17,20}$-lyase

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ABSTRACT

The reaction of 3β-hydroxy-21-hydroxymethylidenepregn-5-en-3β-ol-20-one (**1**) with phenylhydrazine (**2a**) affords two regioisomers, 17β-(1-phenyl-3-pyrazolyl)androst-3-en-3β-ol (**5a**) and 17β-(1-phenyl-5-pyrazolyl)androst-5-en-3β-ol (**6a**). The direction of the ring-closure reactions of **1** with *p*-substituted phenylhydrazines (**2b**–**e**) depends strongly on the electronic features of the substituents. Oppenauer oxidation of 3β-hydroxy-17β-*exo*-heterocyclic steroids **5a**–**e** and **6a**–**e** yielded the corresponding Δ^4-3-ketosteroids **9a**–**e** and **10a**–**e**. The inhibitory effects (IC_{50}) of these compounds on rat testicular $C_{17,20}$-lyase were investigated by means of an *in vitro* radioligand incubation technique.

1. Introduction

Steroids bearing heterocycles fused to the D-ring of the steroid nucleus have been of pharmaceutical interest [1]. The syntheses of abirateron [17-(3-pyridyl)androst-5,16-dien-3β-ol] and its 4-en-3-one analog were reported recently; these display high inhibitory activities against 17α-hydroxylase/$C_{17,20}$-lyase ($P450_{17\alpha}$). It has been suggested that such activity is related to the presence of the heterocyclic moiety in ring D, with the nitrogen lone pair coordinating to the heme iron atom at the active site of the enzyme [2]. Brodie and coworkers recently described a number of inhibitors of $P450_{17\alpha}$, of which 17-imidazolyl, pyrazolyl, isoxazolyl, oxazolyl and thiazolyl steroids are very potent [3–5].

We recently reported a series of 17β-tetrahydrooxazinolyl, dihydrooxazinyl, oxazolidonyl and oxazolinyl steroids. The inhibitory effects of these compounds on rat testicular $C_{17,20}$-lyase were investigated by means of an *in vitro* radioincubation technique [6–9].

To continue our program, we set out to synthesize a novel series of phenylpyrazolyl steroid regioisomers in which there is a heterocycle containing two N atoms at position 17β of androst-5-en-3β-ol, to obtain answers to the following questions: (1) How is the cyclization process influenced by the substituents on the phenyl ring? (2) How do the decreases in enzyme activity of the regioisomers in the 3β-hydroxy- and Δ^4-3-ketosteroid series **5a**–**e**, **6a**–**e** and **9a**–**e**, **10a**–**e** differ?

2. Experimental

2.1. General

Melting points (mp) were determined on a Kofler block and are uncorrected. Specific rotations were measured in $CHCl_3$ (*c* 1) at 20 °C with a POLAMAT-A (Zeiss-Jena) polarimeter and are given in units of 10^{-1} ° cm^2 g^{-1}. Elementary analysis data were determined with a PerkinElmer CHN analyzer model 2400. The reactions were monitored by TLC on Kieselgel-G (Merck Si 254 F) layers (0.25 mm thick); solvent systems (ss) (A) acetone/toluene/hexane (35:30:30, v/v), (B) diisopropyl ether. The spots were detected by spraying with 5% phosphomolybdic acid in 50% aqueous phosphoric acid. The R_f values were determined for the spots observed by illumination at 254 and 365 nm. Flash chromatography: silica gel 60, 40–63 μm. All solvents were distilled prior to use. NMR spectra were recorded on a Bruker DRX 500 instrument at 500 (^{1}H NMR) or 125 MHz (^{13}C NMR). Chemical shifts are reported in ppm (δ scale), and coupling constants (*J*) in Hertz. For the determination of multiplicities, the *J*-MOD pulse sequence was used.

2.2. General procedure for preparation of 17β-(1-phenyl-, and *p*-substituted-1-phenyl-3-pyrazolyl)androst-5-en-3β-ol (*5a*–*e*) and 17β-(1-phenyl-, and *p*-substituted-1-phenyl-5-pyrazolyl)androst-5-en-3β-ol (*6a*–*e*)

(Method A) Compound **1** [7] (2.07 g, 6 mmol) and potassium acetate (1.2 g, 12 mmol) were dissolved in glacial acetic acid (75 ml) and phenylhydrazine hydrochloride (**2a**) or one of its *p*-substituted derivatives (**2b**–**e**) (1.1 equivalent) was added. The reaction

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mixture was stirred at room temperature for 6 h, and was then poured into ice-cold water (500 ml). The precipitate that formed was filtered off and washed with water. The residue obtained was dissolved in CH_2Cl_2 and chromatographed on silica gel, starting with CH_2Cl_2/hexane (1:1, v/v) as eluent, followed by CH_2Cl_2/hexane (2:1, v/v) and CH_2Cl_2 to afford **5a**–**e** and **6a**–**e**.

(Method B) Compound **1** [7] (2.07 g, 6 mmol) was suspended in CH_2Cl_2 (50 ml) and phenylhydrazine hydrochloride (**2a**) or one of its *p*-substituted derivatives (**2b**–**e**) (1.1 eqivalent) was added, followed by the dropwise addition of $BF_3{\cdot}OEt_2$ (50%) (2 mmol, 0.25 ml). The reaction mixture was stirred for 6 h. After the disappearance of the starting material (TLC monitoring), saturated $NaHCO_3$ solution (100 ml) was added and the mixture was stirred until bubbling ceased. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel starting with CH_2Cl_2/hexane (1:1, v/v) as eluent, followed by CH_2Cl_2/hexane (2:1, v/v) and CH_2Cl_2 to afford **5a**–**e** and **6a**–**e**.

*2.2.1. 17β-(1-Phenyl-3-pyrazolyl)androst-5-en-3β-ol (**5a**) and 17β-(1-phenyl-5-pyrazolyl)androst-5-en-3β-ol (**6a**)*

The resulting crude product was chromatographed on silica gel with CH_2Cl_2/hexane (1:1) to yield pure **5a** (with Method A: 620 mg, 24%; with Method B: 1.95 g, 78%), mp 153–155 °C, R_f = 0.55 (ss A); $[\alpha]_D^{20}$ −60 (*c* 1 in $CHCl_3$) (found C, 80.55; H, 8.82. $C_{28}H_{36}N_2O$ requires C, 80.73; H, 8.71%). ^{1}H NMR (*δ*, ppm, $CDCl_3$): 0.59 (s, 3H, 18-H_3), 1.02 (s, 3H, 19-H_3), 2.80 (t, 1H, *J* = 8.3 Hz, 17-H), 3.54 (m, 1H, 3-H), 5.38 (d, 1H, *J* = 2.3 Hz, 6-H), 6.27 (d, 1H, *J* = 2.0 Hz, 4′-H), 7.23 (t, 1H, *J* = 6.5 Hz, 4″-H), 7.42 (t, 2H, *J* = 6.5 Hz, 3″- and 5″-H), 7.68 (d, 2H, *J* = 6.5 Hz, 2″- and 6″-H), 7.83 (d, 1H, *J* = 2.0 Hz, 5′-H). ^{13}C NMR (125 MHz, $CDCl_3$, *δ* (ppm)): 13.1 (C-18), 19.4 (C-19), 20.9, 24.7, 26.3, 31.7, 32.0, 32.3, 37.3, 37.9, 42.3, 43.7, 50.3, 50.4, 56.2, 71.8 (C-3), 106.8 (C-4′), 118.8 (2C, C-2″ and C-6″), 121.6, 125.7, 126.6, 129.3 (2C, C-3″ and C-5″), 134.7, 140.9, 153.2, 155.2. Continued elution with CH_2Cl_2/hexane (2:1) resulted in **6a** (with Method A: 1.60 g, 64%; with Method B): (400 mg, 16%), mp 223–226 °C (Ref. [10]: mp 222–224 °C), R_f = 0.48 (ss A); $[\alpha]_D^{20}$ −133 (*c* in $CHCl_3$) (found C, 80.61; H, 8.55. $C_{28}H_{36}N_2O$ requires C, 80.73; H, 8.71%). ^{1}H NMR (*δ*, ppm, $CDCl_3$): 0.67 (s, 3H, 18-H_3), 0.96 (s, 3H, 19-H_3), 2.84 (t, 1H, *J* = 8.3 Hz, 17-H), 3.47 (m, 1H, 3-H), 5.31 (d, 1H, *J* = 5.0 Hz, 6-H), 6.27 (d, 1H, *J* = 1.5 Hz, 4′-H), 7.36 (d, 2H, *J* = 6.3 Hz, 2″- and 6″-H), 7.40 (t, 1H, *J* = 6.3 Hz, 4″-H), 7.45 (t, 2H, *J* = 6.3 Hz, 3″- and 5″-H), 7.60 (d, 1H, *J* = 1.5 Hz, 3′-H). ^{13}C NMR (125 MHz, $CDCl_3$, *δ* (ppm)): 13.3 (C-18), 19.3 (C-19), 20.7, 24.4, 29.5, 31.6, 31.7, 32.3, 36.5, 37.2 (2C), 42.2, 44.3, 46.9, 49.9, 56.0, 71.6 (C-3), 105.5 (C-4′), 121.3 (C-6), 126.9 and 128.9 (4C, C-2″, C-3″, C-5″ and C-6″), 128.1, 139.3, 140.4, 140.8, 144.4.

*2.2.2. 17β-(1-p-Chlorophenyl-3-pyrazolyl)androst-5-en-3β-ol (**5b**) and 17β-(1-p-chlorophenyl-3-pyrazolyl)androst-5-en-3β-ol (**6b**)*

The resulting crude product was chromatographed on silica gel with CH_2Cl_2/hexane (1:1) to yield **5b** (with Method A: 300 mg, 11%; with Method B: 1.90 g, 70%), mp 177–179 °C, R_f = 0.57 (ss A); $[\alpha]_D^{20}$ −54 (*c* 1 in $CHCl_3$) (found C, 74.34; H, 7.67. $C_{28}H_{35}ClN_2O$ requires C, 74.56; H, 7.82%).

Continued elution with CH_2Cl_2/hexane (2:1) resulted in **6b** (with Method A: 2.10 g, 77%; with Method B: 530 mg, 19%), mp 133–137 °C, R_f = 0.50 (ss A); $[\alpha]_D^{20}$ −128 (*c* 1 in $CHCl_3$) (found C, 74.42; H, 7.65. $C_{28}H_{35}ClN_2O$ requires C, 74.56; H, 7.82%). ^{1}H NMR (*δ*, ppm, $CDCl_3$): 0.66 (s, 3H, 18-H_3), 0.96 (s, 3H, 19-H_3), 2.78 (t, 1H, *J* = 9.3 Hz, 17-H), 3.47 (m, 1H, 3-H), 5.31 (s, 1H, 6-H), 6.30 (s, 1H, 4′-H), 7.31 (d, 2H, *J* = 8.3 Hz, 2″- and 6″-H), 7.44 (d, 2H, *J* = 8.3 Hz, 3″- and 5″-H), 7.63 (s, 1H, 3′-H). ^{13}C NMR (125 MHz, $CDCl_3$, *δ* (ppm)): 13.3 (C-18), 19.3 (C-19), 20.6, 24.4, 29.4, 31.6, 31.7, 32.3, 36.5, 37.2, 37.4, 42.2, 44.6, 46.9, 49.9, 56.1, 71.6 (C-3), 106.0 (C-4′), 121.2 (C-6), 128.2, and 129.3 (4C, C-2″, C-3″, C-5″, and C-6″), 134.6, 137.8, 138.9 (C-5), 140.9, 145.4.

*2.2.3. 17β-(1-p-Cyanophenyl-3-pyrazolyl)androst-5-en-3β-ol (**5c**) and 17β-(1-p-cyanophenyl-5-pyrazolyl)androst-5-en-3β-ol (**6c**)*

The resulting crude product was chromatographed on silica gel with CH_2Cl_2/hexane (2:1) to yield **5c** (with Method A: 250 mg, 9%; with Method B: 1.9 g, 71%), mp 273–275 °C, R_f = 0.50 (ss A); $[\alpha]_D^{20}$ −47 (*c* 1 in $CHCl_3$) (found C, 78.65; H, 8.06. $C_{29}H_{35}N_3O$ requires C, 78.87; H, 7.99%).

Continued elution with CH_2Cl_2 resulted in **6c** (with Method A: 2.30 g, 85%; with Method B: 250 mg, 9%), mp 183–186 °C, R_f = 0.45 (ss A); $[\alpha]_D^{20}$ −129 (*c* 1 in $CHCl_3$) (found C, 78.69; H, 8.25. $C_{29}H_{35}N_3O$ requires C, 78.87; H, 7.99%). ^{1}H NMR (*δ*, ppm, $CDCl_3$): 0.63 (s, 3H, 18-H_3), 0.95 (s, 3H, 19-H_3), 2.86 (t, 1H, *J* = 9.8 Hz, 17-H), 3.47 (m, 1H, 3-H), 5.31 (s, 1H, 6-H), 6.35 (d, 1H, *J* = 2.0 Hz, 4′-H), 7.53 (d, 2H, *J* = 8.3 Hz, 2″- and 6″-H), 7.67 (d, 1H, *J* = 2.0 Hz, 3′-H), 7.77 (d, 2H, *J* = 8.3 Hz, 3″- and 5″-H). ^{13}C NMR (125 MHz, $CDCl_3$, *δ* (ppm)): 13.3 (C-18), 19.3 (C-19), 20.7, 24.3, 29.2, 31.6, 31.7, 32.3, 36.5, 37.2, 37.4, 42.2, 44.8, 47.0, 49.9, 56.1, 71.6 (C-3), 106.9 (C-4′), 112.2, 118.0 (CN), 121.1, 127.3, and 133.1: (4C, C-2″, C-3″, C-5″ and C-6″), 139.9 (C-3′), 140.9 (C-5), 143.2, 145.3.

*2.2.4. 17β-(1-p-Tolylphenyl-3-pyrazolyl)androst-5-en-3β-ol (**5d**) and 17β-(1-p-toly- 5-pyrazolyl)androst-5-en-3β-ol (**6d**)*

The resulting crude product was chromatographed on silica gel with CH_2Cl_2/hexane (1:1) to yield **5d** (with Method A: 500 mg, 19%; with Method B: 2.45 g, 94%), mp 167–170 °C, R_f = 0.58 (ss A); $[\alpha]_D^{20}$ −63 (*c* 1 in $CHCl_3$) (found C, 80.76; H, 8.75. $C_{29}H_{38}N_2O$ requires C 80.89; H, 8.89%). ^{1}H NMR (*δ*, ppm, $CDCl_3$): 0.59 (s, 3H, 18-H_3), 1.01 (s, 3H, 19-H_3), 2.36 (s, 3H, 4″-CH_3), 2.84 (t, 1H, *J* = 9.8 Hz, 17-H), 3.53 (m, 1H, 3-H), 5.37 (d, 1H, *J* = 2.5 Hz, 6-H), 6.26 (d, 1H, *J* = 2.3 Hz, 4′-H), 7.22 and 7.54 (d, 4H, *J* = 8.0 Hz, 2″-, 3″-, 5″- and 6″-H), 7.78 (d, 1H, *J* = 2.3 Hz, 5′-H). ^{13}C NMR (125 MHz, $CDCl_3$, *δ* (ppm)): 13.1 (C-18), 19.4 (C-19), 20.8, 20.9 (4″-CH_3), 24.7, 26.5, 31.6, 31.9, 32.3, 36.6, 37.3, 37.7, 42.3, 43.8, 49.9, 50.3, 56.2, 71.7 (C-3), 106.5 (C-4′), 119.2 (2C, C-2″ and C-6″), 121.5, 127.1, 129.8 (2C, C-3″ and C-5″), 135.9, 137.7, 140.9 (C-5), 154.7. Continued elution with CH_2Cl_2/hexane (2:1) resulted in **6d** (1.60 g, 46%), mp 166–168 °C, R_f = 0.50 (ss A); $[\alpha]_D^{20}$ −128 (*c* 1 in $CHCl_3$) (found C, 80.68; H, 8.94. $C_{29}H_{38}N_2O$ requires C, 80.89; H, 8.89%). ^{1}H NMR (*δ*, ppm, $CDCl_3$): 0.68 (s, 3H, 18-H_3), 0.95 (s, 3H, 19-H_3), 2.41 (s, 3H, 4″-CH_3), 2.78 (t, 1H, *J* = 9.8 Hz, 17-H), 3.45 (m, 1H, 3-H), 5.29 (s, 1H, 6-H), 6.29 (d, 1H, *J* = 1.5 Hz, 4′-H), 7.24 (overlapping multiplets, 4H, 2″-, 3″-, 5″-, 6″-H), 7.65 (d, 1H, *J* = 1.5 Hz, 3′-H). ^{13}C NMR (125 MHz, $CDCl_3$, *δ* (ppm)): 13.4 (C-18), 19.3 (C-19), 20.7, 21.2 (4″-CH_3), 24.4, 29.6, 31.6, 31.7, 32.3, 36.5, 37.2 37.3, 42.2, 44.5, 46.9, 49.9, 56.0, 71.6 (C-3), 105.6 (C-4′), 121.2 (C-6), 126.8 and 129.7 (4C, C-2″, C-3″, C-5″ and C-6″), 136.2, 137.8 (C-5′), 139.0, 140.9 (C-5), 145.7.

*2.2.5. 17β-(1-p-Methoxyphenyl-3-pyrazolyl)androst-5-en-3β-ol (**5e**) and 17β-(1-p-methoxyphenyl-5-pyrazolyl)androst-5-en-3β-ol (**6e**)*

The resulting crude product was chromatographed on silica gel with CH_2Cl_2/hexane (2:1) to yield **5e** (with Method A: 1.65 g, 61%; with Method B: 280 mg, 10%), mp 150–152 °C, R_f = 0.50 (ss A); $[\alpha]_D^{20}$ −60 (*c* 1 in $CHCl_3$) (found C, 78.05; H, 8.50. $C_{29}H_{38}N_2O_2$ requires C 77.99; H, 8.58%). ^{1}H NMR (*δ*, ppm, $CDCl_3$): 0.58 (s, 3H, 18-H_3), 1.02 (s, 3H, 19-H_3), 2.80 (t, 1H, *J* = 8.3 Hz, 17-H), 3.54 (m, 1H, 3-H), 3.83 (s, 3H, O-CH_3), 5.38 (d, 1H, *J* = 2.0 Hz, 6-H), 6.24 (d, 1H, *J* = 2.0 Hz, 4′-H), 6.94 (d, 2H, *J* = 7.3 Hz, 3″- and 5″-H), 7.56 (d, 2H, *J* = 7.3 Hz, 2″- and 6″-H), 7.72 (d, 1H, *J* = 2.0 Hz, 5′-H). ^{13}C NMR (125 MHz, $CDCl_3$, *δ* (ppm)): 13.1 (C-18), 19.4 (C-19), 20.8, 24.7, 26.4, 31.7, 32.0, 32.3, 36.6, 37.3, 37.8, 42.3, 43.7, 50.3, 50.4, 55.6 (O-CH_3), 56.2, 71.8 (C-3), 106.3 (C-4′), 114.4 (2C, C-3″ and C-5″), 120.6 (2C,

C-2″ and C-6″), 121.6 (C-6), 126.7, 134.3, 140.9, 154.7, 157.8. Continued elution with CH_2Cl_2 resulted in **6e** (with Method A: 590 mg, 22%; with Method B: 2.10 g, 78%), mp 186–188 °C, R_f = 0.38 (ss A); $[\alpha]_D^{20}$ −134 (*c* 1 in $CHCl_3$) (found C, 77.84; H, 8.65. $C_{29}H_{38}N_2O_2$ requires C, 77.99; H, 8.58%). ^{1}H NMR (*δ*, ppm, $CDCl_3$): 0.68 (s, 3H, 18-H_3), 0.97 (s, 3H, 19-H_3), 2.77 (t, 1H, *J* = 8.3 Hz, 17-H), 3.49 (m, 1H, 3-H), 3.86 (s, 3H, O-CH_3), 5.32 (d, 1H, *J* = 2.0 Hz, 6-H), 6.24 (d, 1H, *J* = 1.5 Hz, 4′-H), 6.95 (d, 2H, *J* = 7.0 Hz, 3″- and 5″-H), 7.26 (d, 2H, *J* = 7.0 Hz, 2″- and 6″-H), 7.57 (d, 1H, *J* = 1.5 Hz, 3′-H). ^{13}C NMR (125 MHz, $CDCl_3$, *δ* (ppm)): 13.3 (C-18), 19.4 (C-19), 20.7, 24.4, 29.6, 31.6, 31.8, 32.3, 36.5, 37.2, 37.3, 42.2, 44.1, 47.0, 49.9, 55.5 (O-CH_3), 56.0, 71.7 (C-3), 105.1 (C-4′), 114.0 (2C, C-3″ and C-5″), 121.3 (C-6), 128.2 (2C, C-2″ and C-6″), 133.4 (C-1″), 139.0 (C-3′), 140.8 (C-5), 144.6 (C-5′), 159.3 (C-4″).

2.3. General procedure for preparation of 3β-acetoxy-17β-(1-phenyl- and p-substituted-1-phenyl-3-pyrazolyl)androst-5-ene (**7a–e**) and 3β-acetoxy-17β-(1-phenyl- and p-substituted-1-phenyl-5-pyrazolyl)androst-5-ene(**8a–e**)

The individual compounds **5a–e** and **6a–e** (1 mmol) were dissolved in a mixture of pyridine (5 ml) and acetic anhydride (5 ml) and the solution was allowed to stand at room temperature for 12 h. The mixture was then diluted with water and the precipitate that separated out was filtered off and crystallized from acetone/hexane.

2.3.1. 3β-Acetoxy-17β-(1-phenyl-3-pyrazolyl)androst-5-ene (**7a**)

7a (420 mg, 91%), mp 140–143 °C, R_f = 0.77 (ss B); $[\alpha]_D^{20}$ −49 (*c* 1 in $CHCl_3$) (found C, 78.42; H, 8.44. $C_{30}H_{38}N_2O_2$ requires C, 78.56; H, 8.35%). ^{1}H NMR (*δ*, ppm, $CDCl_3$): 0.58 (s, 3H, 18-H_3), 1.03 (s, 3H, 19-H_3), 2.04 (s, 3H, Ac-CH_3), 2.80 (t, 1H, *J* = 8.0 Hz, 17-H), 4.62 (m, 1H, 3-H), 5.41 (t, 1H, *J* = 2.3 Hz, 6-H), 6.27 (d, 1H, *J* = 2.0 Hz, 4′-H), 7.23 (t, 1H, *J* = 6.3 Hz, 4″-H), 7.42 (t, 2H, *J* = 6.3 Hz, 3″- and 5″-H), 7.68 (d, 2H, *J* = 6.3 Hz, 2″- and 6″-H), 7.83 (d, 1H, *J* = 2.0 Hz, 5′-H). ^{13}C NMR (125 MHz, $CDCl_3$, *δ* (ppm)): 13.1 (C-18), 19.4 (C-19), 20.8, 21.4 (Ac-CH_3), 24.7, 26.3, 27.8, 31.9, 32.3, 36.7, 37.0, 37.8, 38.1, 43.7, 50.2, 50.3, 56.1, 74.0 (C-3), 106.8 (C-4′), 118.8 (2C, C-2″ and C-6″), 122.5 (C-6), 125.7, 126.6, 129.3 (2C, C-3″ and C-5″), 139.8, 155.1, 156.1, 170.5 (Ac-CO).

2.3.2. 3β-Acetoxy-17β-(1-phenyl-5-pyrazolyl)androst-5-ene (**8a**)

8a (435 mg, 94%), mp 205–208 °C (Ref. [10]: mp 205–206 °C), R_f = 0.45 (ss B); $[\alpha]_D^{20}$ −128 (*c* 1 in $CHCl_3$) (Ref. [10]: $[\alpha]_D^{25}$ −110 (*c* 1 in $CHCl_3$)) (found C, 78.33; H, 8.52. $C_{30}H_{38}N_2O_2$ requires C, 78.56; H, 8.35%). ^{1}H NMR (*δ*, ppm, $CDCl_3$): 0.67 (s, 3H, 18-H_3), 0.97 (s, 3H, 19-H_3), 2.02 (s, 3H, Ac-CH_3), 2.85 (t, 1H, *J* = 8.3 Hz), 4.56 (m, 1H, 3-H), 5.34 (d, 1H, *J* = 4.0 Hz, 6-H), 6.27 (d, 1H, *J* = 2.0 Hz, 4′-H), 7.35 (d, 2H, *J* = 6.3 Hz, 2″- and 6″-H), 7.41 (t, 1H, *J* = 6.3 Hz, 4″-H), 7.46 (t, 2H, *J* = 6.3 Hz, 3″- and 5″-H), 7.60 (d, 1H, *J* = 2.0 Hz, 3′-H). ^{13}C NMR (125 MHz, $CDCl_3$, *δ* (ppm)): 13.3 (C-18), 19.3 (C-19), 20.6, 21.4 (Ac-CH_3), 24.4, 27.7, 29.5, 31.7, 32.3, 36.9 (2C), 37.2, 38.1, 44.3, 46.9, 49.8, 55.9, 73.8 (C-3), 105.5 (C-4′), 122.3, 126.9 and 128.9 (4C, C-2″, C-3″, C-5″ and C-6″), 128.2, 139.3, 139.7 (C-5), 140.4, 144.4, 170.5 (Ac-CO).

2.3.3. 3β-Acetoxy-17β-(1-p-chlorophenyl-3-pyrazolyl)androst-5-ene (**7b**)

7b (475 mg, 96%), mp 173–175 °C, R_f = 0.77 (ss B); $[\alpha]_D^{20}$ −42 (*c* 1 in $CHCl_3$) (found C, 72.95; H, 7.43. $C_{30}H_{37}N_2ClO_2$ requires C, 73.08; H, 7.56%). ^{1}H NMR (*δ*, ppm, $CDCl_3$): 0.57 (s, 3H, 18-H_3), 1.03 (s, 3H, 19-H_3), 2.03 (s, 3H, Ac-CH_3), 2.78 (t, 1H, *J* = 12.3 Hz, 17-H), 4.62 (m, 1H, 3-H), 5.40 (d, 1H, *J* = 5.5 Hz, 6-H), 6.27 (d, 1H, *J* = 3.0 Hz, 4′-H), 7.37 (d, 2H, *J* = 11.0 Hz, 2″- and 6″-H), 7.61 (d, 2H, *J* = 11.0 Hz, 3″- and 5″-H), 7.78 (d, 1H, *J* = 3.0 Hz, 5′-H). ^{13}C NMR (125 MHz, $CDCl_3$, *δ* (ppm)): 13.1 (C-18), 19.3 (C-19), 20.8, 21.4 (Ac-CH_3), 24.6, 26.2, 27.8, 31.9, 32.3, 36.7, 37.0, 37.8, 38.1, 43.7, 50.3 (2C), 56.1, 73.9 (C-3), 107.3 (C-4′), 119.8 (2C, C-2″ and C-6″), 122.5 (C-6), 126.5 (C-5′), 129.3 (2C, C-3″ and C-5″), 131.0 (C-4″), 139.0 (C-1″), 139.7 (C-5), 155.5 (C-3′), 170.4 (Ac-CO).

2.3.4. 3β-Acetoxy-17β-(1-p-chlorophenyl-5-pyrazolyl)androst-5-ene (**8b**)

8b (462 mg, 93%), mp 174–177 °C, R_f = 0.60 (ss B); $[\alpha]_D^{20}$ −151 (*c* 1 in $CHCl_3$) (found C, 73.15, H, 7.68. $C_{30}H_{37}N_2ClO_2$ requires C, 73.08; H, 7.56%). ^{1}H NMR (*δ*, ppm, $CDCl_3$): 0.66 (s, 3H, 18-H_3), 0.97 (s, 3H, 19-H_3), 2.02 (s, 3H, Ac-CH_3), 2.79 (t, 1H, *J* = 9.8 Hz, 17-H), 4.57 (m, 1H, 3-H), 5.34 (d, 1H, *J* = 4.5 Hz, 6-H), 6.29 (s, 1H, 4′-H), 7.31 (d, 2H, *J* = 8.5 Hz, 2″- and 6″-H), 7.44 (d, 2H, *J* = 8.5 Hz, 3″- and 5″-H), 7.63 (s, 1H, 3′-H). ^{13}C NMR (125 MHz, $CDCl_3$, *δ* (ppm)): 13.3 (C-18), 19.3 (C-19), 20.6, 21.4 (Ac-CH_3), 24.5, 27.7, 29.4, 31.7, 32.2, 36.6, 36.9, 37.3, 38.0, 44.5, 46.9, 49.8, 56.0, 73.8 (C-3), 105.9 (C-4′), 122.2 (C-6), 128.2 (2C, C-2″ and C-6″), 129.3 (2C, C3″ and C-5″), 134.4 (C-4″), 138.1 (C-1″), 139.1 (C-3′), 139.7 (C-5), 145.1 (C-5′), 170.5 (Ac-CO).

2.3.5. 3β-Acetoxy-17β-(1-p-cyanophenyl-3-pyrazolyl)androst-5-ene (**7c**)

7c (452 mg, 93%), mp 242–244 °C, R_f = 0.58 (ss B); $[\alpha]_D^{20}$ −36 (*c* 1 in $CHCl_3$) (found C, 76.84; H, 7.65. $C_{31}H_{37}N_3O_2$ requires C, 76.99; H, 7.71%). ^{1}H NMR (*δ*, ppm, $CDCl_3$): 0.56 (s, 3H, 18-H_3), 1.02 (s, 3H, 19-H_3), 2.03 (s, 3H, Ac-CH_3), 2.77 (t, 1H, *J* = 9.5 Hz, 17-H), 4.61 (m, 1H, 3-H), 5.40 (s, 1H, 6-H), 6.33 (d, 1H, *J* = 2.0 Hz, 4′-H), 7.70 (d, 2H, *J* = 8.5 Hz, 2″- and 6″-H), 7.80 (d, 2H, *J* = 8.5 Hz, 3″- and 5″-H), 7.89 (d, 1H, *J* = 2.0 Hz, 5′-H). ^{13}C NMR (125 MHz, $CDCl_3$, *δ* (ppm)): 13.1 (C-18), 19.3 (C-19), 20.7, 21.4 (Ac-CH_3) 24.6, 26.0, 27.7, 31.9, 32.2, 36.7, 37.0, 37.8, 38.1, 43.8, 50.1, 50.2, 56.1, 73.9 (C-3), 108.6 (C-4′), 118.3 (2C, C-2″ and C-6″) 118.6 (CN), 122.4 (C-6), 126.7 (C-5′), 133.5 (2C, C3″ and C-5″), 139.7, 143.1 (C-1″), 156.7 (C-3′), 170.5 (Ac-CO).

2.3.6. 3β-Acetoxy-17β-(1-p-cyanophenyl-5-pyrazolyl)androst-5-ene (**8c**)

8c (432 mg, 89%), mp 233–236 °C, R_f = 0.36 (ss B); $[\alpha]_D^{20}$ −122 (*c* 1 in $CHCl_3$) (found C, 77.06; H, 7.65. $C_{31}H_{37}N_3O_2$ requires C, 76.99; H, 7.71%). ^{1}H NMR (*δ*, ppm, $CDCl_3$): 0.63 (s, 3H, 18-H_3), 0.97 (s, 3H, 19-H_3), 2.01 (s, 3H, Ac-CH_3) 2.88 (t, 1H, *J* = 9.8 Hz, 17-H), 4.57 (m, 1H, 3-H), 5.35 (d, 1H, *J* = 2.5 Hz, 6-H), 6.34 (s, 1H, 4′-H), 7.53 (d, 2H, *J* = 8.3 Hz, 2″- and 6″-H), 7.65 (s, 1H, 3′-H), 7.77 (d, 2H, *J* = 8.3 Hz, 3″- and 5″-H). ^{13}C NMR (125 MHz, $CDCl_3$, *δ* (ppm)): 13.3 (C-18), 19.2 (C-19), 20.6, 21.3 (Ac-CH_3), 24.3, 27.7, 29.3, 31.7, 32.3, 36.6, 37.0, 37.4, 38.1, 44.7, 47.0, 49.8, 56.1, 73.7 (C-3), 106.8 (C-4′), 111.9 (C-4″), 118.0 (CN), 122.1 (C-6), 127.2 (2C, C-2″ and C-6″), 133.0 (2C, C3″ and C-5″), 139.7 (C-5), 140.4 (C-3′), 143.9 and 144.7 (C-1″ and C-5′), 170.4 (Ac-CO).

2.3.7. 3β-Acetoxy-17β-(1-p-toly-3-pyrazolyl)androst-5-ene (**7d**)

7d (442 mg, 93%), mp 163–165 °C, R_f = 0.76 (ss B); $[\alpha]_D^{20}$ −47 (*c* 1 in $CHCl_3$) (found C, 78.54; H, 8.77. $C_{31}H_{40}N_2O_2$ requires C, 78.77; H, 8.53%). ^{1}H NMR (*δ*, ppm, $CDCl_3$): 0.59 (s, 3H, 18-H_3), 1.03 (s, 3H, 19-H_3), 2.03 (s, 3H, Ac-CH_3), 2.37 (s, 3H, 4″-CH_3), 2.85 (t, 1H, *J* = 9.5 Hz, 17-H), 4.62 (m, 1H, 3-H), 5.41 (s, 1H, 6-H), 6.27 (s, 1H, 4′-H), 7.22 and 7.55 (d, 4H, *J* = 8.0 Hz, 2″-, 3″-, 5″- and 6″-H), 7.78 (s, 1H, 5′-H). ^{13}C NMR (125 MHz, $CDCl_3$, *δ* (ppm)): 13.1 (C-18), 19.3 (C-19), 20.8, 20.9 (4″-CH_3), 21.4 (Ac-CH_3), 24.7, 26.5, 27.8, 31.9, 32.3, 36.7, 37.1, 37.8, 38.1, 43.8, 50.1, 50.2, 56.1, 74.0 (C-3), 106.6 (C-4′), 119.1 (2C, C-2″ and C-6″), 122.5 (C-6), 126.9 (C-5′), 129.8 (2C, C-3″ and C-5″), 135.8 and 137.8 (C-1″ and C-4″) 139.8 (C-5), 154.7 (C-3′), 170.4 (Ac-CO).

*2.3.8. 3β-Acetoxy-17β-(1-p-tolyl-5-pyrazolyl)androst-5-ene (**8d**)*

8d (426 mg, 90%), mp 202–204 °C, R_f = 0.62 (ss B); $[\alpha]_D^{20}$ −120 (*c* 1 in $CHCl_3$) (found 78.88; H, 8.35. $C_{31}H_{40}N_2O_3$ requires C, 78.77; H, 8.53%).

^{1}H NMR (δ, ppm, $CDCl_3$): 0.68 (s, 3H, 18-H_3), 0.98 (s, 3H, 19-H_3), 2.02 (s, 3H, Ac-CH_3) 2.42 (s, 3H, 4″-CH_3), 2.82 (t, 1H, *J* = 9.8 Hz), 4.57 (t, 1H, *J* = 4.8 Hz, 3-H), 5.35 (s, 1H, 6-H), 6.25 (s, 1H, 4′-H), 7.24 (overlapping multiplets, 4H, 2″-, 3″-, 5″-, 6″-H), 7.58 (s, 1H, 3′-H). ^{13}C NMR (125 MHz, $CDCl_3$, δ (ppm)): 13.3 (C-18), 19.3 (C-19), 20.6, 21.2 (4″-CH_3), 21.4 (Ac-CH_3) 24.4, 27.7, 29.6, 31.7, 32.3, 36.6, 37.0 37.2, 38.0, 44.2, 46.9, 49.8, 55.9, 73.8 (C-3), 105.2 (C-4′), 122.3 (C-6), 126.7 and 129.5 (4C, C-2″, C-3″, C-5″ and C-6″), 137.8, 138.1, 139.1 (C-3′), 139.7 (C-5), 144.3, 170.5 (Ac-CO).

*2.3.9. 3β-Acetoxy-17β-(1-p-methoxyphenyl-3-pyrazolyl)androst-5-ene (**7e**)*

7e (420 mg, 86%), mp 175–179 °C, R_f = 0.65 (ss B); $[\alpha]_D^{20}$ −45 (*c* 1 in $CHCl_3$) (found C, 75.98; H, 8.37; $C_{31}H_{40}N_2O_3$ requires C, 76.19; H, 8.25%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.59 (s, 3H, 18-H_3), 1.02 (s, 3H, 19-H_3), 2.03 (s, 3H, Ac-CH_3), 2.86 (t, 1H, *J* = 9.5 Hz, 17-H), 3.83 (s, 3H, O-CH_3), 4.62 (t, 1H, *J* = 5.3 Hz, 3-H), 5.40 (d, 1H, *J* = 4.5 Hz, 6-H), 6.26 (d, 1H, *J* = 2.0 Hz, 4′-H), 6.95 (d, 2H, *J* = 9.0 Hz, 3″- and 5″-H), 7.58 (d, 2H, *J* = 9.0 Hz, 2″- and 6″-H), 7.73 (d, 1H, *J* = 2.0 Hz, 5′-H). ^{13}C NMR (125 MHz, $CDCl_3$, δ (ppm)): 13.1 (C-18), 19.3 (C-19), 20.8, 21.4 (Ac-CH_3), 24.6, 26.5, 27.8, 31.9, 32.3, 36.7, 37.0, 37.7, 38.1, 43.8, 49.9, 50.2, 55.6 (O-CH_3), 56.1, 73.9 (C-3), 106.4 (C-4′), 114.5 (2C, C-3″ and C-5″), 120.9 (2C, C-2″ and C-6″), 122.5 (C-6), 127.2 (C-5′), 133.6 (C-1″), 139.8 (C-5), 154.5 (C-3′), 158.1 (C-4″), 170.5 (Ac-CO).

*2.3.10. 3β-Acetoxy-17β-(1-p-methoxyphenyl-5-pyrazolyl)androst-5-ene (**8e**)*

8e (438 mg, 89%), mp 153–156 °C, R_f = 0.30 (ss B); $[\alpha]_D^{20}$ −124 (*c* 1 in $CHCl_3$) (found C, 76.05; H, 8.42. $C_{31}H_{40}N_2O_3$ requires C, 76.19; H, 8.25%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.67 (s, 3H, 18-H_3), 0.98 (s, 3H, 19-H_3), 2.02 (s, 3H, Ac-CH_3), 2.77 (t, 1H, *J* = 9.5 Hz, 17-H), 3.86 (s, 3H, O-CH_3), 4.57 (m, 1H, 3-H), 5.35 (s, 1H, 6-H), 6.24 (s, 1H, 4′-H), 6.95 (d, 2H, *J* = 8.8 Hz, 3″- and 5″-H), 7.25 (d, 2H, *J* = 8.8 Hz, 2″- and 6″-H), 7.57 (s, 1H, 3′-H). ^{13}C NMR (125 MHz, $CDCl_3$, δ (ppm)): 13.3 (C-18), 19.2 (C-19), 20.6, 21.4 (Ac-CH_3) 24.4, 27.7, 29.6, 31.7, 32.2, 36.6, 36.9, 37.2, 38.0, 44.1, 46.9, 49.8, 55.5 (O-CH_3), 55.9, 73.8 (C-3), 105.1 (C-4′), 114.0 (2C, C-3″ and C-5″), 122.3 (C-6), 128.2 (2C, C-2″ and C-6″), 133.3 (C-1″), 139.0 (C-3′), 139.7 (C-5), 144.5 (C-5′), 159.3 (C-4″), 170.5 (Ac-CO).

*2.4. General procedure for the preparation of 17β-(1-phenyl-, and p-substituted-1-phenyl-3-pyrazolyl)androst-4-en-3-one (**9a**–**e**) and 17β-(1-phenyl-, and p-substituted-1-phenyl-5-pyrazolyl)androst-4-en-3-one (**10a**–**e**)*

The individual compounds **5a**–**e** and **6a**–**e** (2 mmol) were dissolved in toluene (50 ml), Al(O*i*Pr)$_3$ (1.2 g, 6 mmol) and cyclohexanone (25 ml) were added, and the mixture was stirred at 100 °C. The decrease in the starting material was followed by means of TLC. When the reaction was complete, the mixture was poured into water (200 ml) in which K/Na-tartrate (5 g) was dissolved. Most of the organic solvent was removed *in vacuo* and the residual emulsion was extracted with CH_2Cl_2. The organic phase was evaporated to dryness and the residual product was chromatographed on silica gel with ethyl acetate/CH_2Cl_2 (5:95).

*2.4.1. 17β-(1-Phenyl-3-pyrazolyl)androst-4-en-3-one (**9a**)*

9a (310 mg, 37%), mp 196–198 °C (Ref. [11]: mp 97–99 °C), R_f = 0.67 (ss A); $[\alpha]_D^{20}$ +147 (*c* 1 in $CHCl_3$) (found C, 81.29; H, 8.02. $C_{28}H_{34}N_2O$ requires C, 81.12; H, 8.27%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.62 (s, 3H, 18-H_3), 1.18 (s, 3H, 19-H_3), 2.86 (t, 1H, *J* = 9.8 Hz, 17-H), 5.74 (s, 1H, 4-H), 6.28 (d, 1H, *J* = 2.0 Hz, 4′-H), 7.25 (t, 1H, *J* = 7.5 Hz, 4″-H), 7.43 (t, 2H, *J* = 7.5 Hz, 3″- and 5″-H), 7.68 (d, 2H, *J* = 7.5 Hz, 2″- and 6″-H), 7.84 (d, 1H, *J* = 2.0 Hz, 5′-H). ^{13}C NMR (125 MHz, $CDCl_3$, δ (ppm)): 13.2 (C-18), 17.4 (C-19), 20.8, 24.5, 26.3, 32.0, 32.9, 34.0, 35.7, 36.1, 37.6, 38.7, 43.8, 50.0, 54.0, 55.3, 106.9 (C-4′), 119.0 (2C, C-2″ and C-6″), 123.8 (C-4), 126.1, 127.0 (C-5′), 129.3 (2C, C-3″ and C-5″), 139.9 (C-1″), 154.8 (C-3′), 171.3 (C-5), 199.4 (C-3).

*2.4.2. 17β-(1-Phenyl-5-pyrazolyl)androst-4-en-3-one (**10a**)*

10a (365 mg, 44%), mp 197–200 °C (Ref. [11]: mp 196–197 °C), R_f = 0.55 (ss A); $[\alpha]_D^{20}$ +17 (*c* 1 in $CHCl_3$) (Ref. [10]: $[\alpha]_D^{25}$ +10 (*c* 1 in $CHCl_3$)) (found C, 80.97; H, 8.35. $C_{28}H_{34}N_2O$ requires C, 81.12; H, 8.27%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.70 (s, 3H, 18-H_3), 1.13 (s, 3H, 19-H_3), 2.85 (t, 1H, *J* = 10.0 Hz, 17-H), 5.69 (s, 1H, 4-H), 6.31 (d, 1H, *J* = 1.8 Hz, 4′-H), 7.35 (d, 2H, *J* = 7.5 Hz, 2″- and 6″-H), 7.54 (overlapping multiplets, 3H, 3″-, 4″-, 5″-H), 7.66 (d, 1H, *J* = 1.8 Hz, 3′-H). ^{13}C NMR (125 MHz, $CDCl_3$, δ (ppm)): 13.4 (C-18), 17.3 (C-19), 20.6, 24.3, 29.3, 31.8, 32.7, 33.9, 35.6, 36.0, 37.0, 38.5, 44.4, 46.8, 53.5, 55.1, 105.7 (C-4′), 123.8, 126.9 and 129.1 (4C, C-2″, C-3″, C-5″ and C-6″), 128.8, 138.5 (C-3′), 139.2 (C-1″), 144.9 (C-5′), 170.8 (C-5), 199.3 (C-3).

*2.4.3. 17β-(1-p-Chlorophenyl-3-pyrazolyl)androst-4-en-3-one (**9b**)*

9b (352 mg, 39%), mp 164–167 °C, R_f = 0.60 (ss A); $[\alpha]_D^{20}$ +132 (*c* 1 in $CHCl_3$) (found C, 75.02; H, 7.41. $C_{28}H_{33}ClN_2O$ requires C, 74.90; H, 7.41%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.61 (s, 3H, 18-H_3), 1.19 (s, 3H, 19-H_3), 2.83 (d, 1H, *J* = 9.5 Hz), 5.74 (s, 1H, 4-H), 6.30 (d, 1H, *J* = 2.0 Hz, 4′-H), 7.39 (d, 2H, *J* = 8.3 Hz, 2″- and 6″-H), 7.63 (d, 2H, *J* = 8.3 Hz, 3″- and 5″-H), 7.81 (d, 1H, *J* = 2.0 Hz, 5′-H). ^{13}C NMR (125 MHz, $CDCl_3$, δ (ppm)): 13.2 (C-18), 17.4 (C-19), 20.8, 24.5, 26.3, 32.0, 32.9, 34.0, 35.7, 36.0, 37.6, 38.7, 43.9, 49.9, 54.0, 55.3, 107.3 (C-4′), 120.1 (2C, C-2″ and C-6″), 123.8 (C-4), 127.0 (C-5′), 129.3 (2C, C-3″ and C-5″), 131.5 (C-4″), 138.5 (C-1″), 155.2 (C-3′), 171.3 (C-5), 199.5 (C-3).

*2.4.4. 17β-(1-p-Chlorophenyl-5-pyrazolyl)androst-4-en-3-one (**10b**)*

10b (280 mg, 44%), mp 186–189 °C, R_f = 0.56 (ss A); $[\alpha]_D^{20}$ −6 (*c* 1 in $CHCl_3$) (found C, 74.81; H, 7.63. $C_{28}H_{33}ClN_2O$ requires C, 74.90; H, 7.41%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.68 (s, 3H, 18-H_3), 1.13 (s, 3H, 19-H_3), 2.80 (t, 1H, *J* = 9.8 Hz, 17-H), 5.69 (s, 1H, 4-H), 6.29 (d, 1H, *J* = 1.3 Hz, 4′-H), 7.29 (d, 2H, *J* = 8.5 Hz, 2″- and 6″-H), 7.43 (d, 2H, *J* = 8.5 Hz, 3″- and 5″-H), 7.62 (d, 1H, *J* = 1.3 Hz, 3′-H). ^{13}C NMR (125 MHz, $CDCl_3$, δ (ppm)): 13.4 (C-18), 17.3 (C-19), 20.6, 24.2, 29.3, 31.8, 32.7, 33.9, 35.6, 36.0, 37.2, 38.5, 44.5, 46.8, 53.5, 55.2, 106.0 (C-4′), 123.9 (C-4), 128.1 (2C, C-2″ and C-6″), 129.3 (2C, C-3″ and C-5″), 134.5 (C-4″), 138.3 (C-1″), 139.2 (C-3′), 145.0 (C-5′), 170.7 (C-5), 199.2 (C-3).

*2.4.5. 17β-(1-p-Cyanophenyl-3-pyrazolyl)androst-4-en-3-one (**9c**)*

9c (186 mg, 21%), mp 240–242 °C, R_f = 0.55 (ss A); $[\alpha]_D^{20}$ +149 (*c* 1 in $CHCl_3$) (found C, 79.04; H, 7.72. $C_{29}H_{33}N_3O$ requires C, 79.24; H, 7.57%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.59 (s, 3H, 18-H_3), 1.18 (s, 3H, 19-H_3), 2.78 (t, 1H, *J* = 9.5 Hz, 17-H), 5.74 (s, 1H, 4-H), 6.33 (s, 1H, 4′-H), 7.70 (d, 2H, *J* = 8.5 Hz, 2″- and 6″-H), 7.80 (d, 2H, *J* = 8.5 Hz, 3″- and 5″-H), 7.90 (s, 1H, 5′-H). ^{13}C NMR (125 MHz, $CDCl_3$, δ (ppm)): 13.1 (C-18), 17.3 (C-19), 20.7, 24.4, 25.9, 31.9, 32.8, 33.9, 35.7, 35.9, 37.6, 38.6, 43.8, 50.1 53.9, 55.3, 108.5 (C-4′), 118.3 (2C, C-2″ and C-6″) 118.6 (CN), 123.8 (C-4), 126.7 (C-5′), 133.8 (2C, C3″ and C-5″), 143.0 (C-1″), 156.4 (C-3′), 171.4 (C-5), 199.5 (C-3).

*2.4.6. 17β-(1-p-Cyanophenyl-5-pyrazolyl)androst-4-en-3-one (**10c**)*

10c (230 mg, 26%), mp 217–219 °C, R_f = 0.47 (ss A); $[\alpha]_D^{20}$ +8 (*c* 1 in $CHCl_3$) (found C, 79.38;, 7.63. $C_{29}H_{33}N_3O$ requires C, 79.24; H, 7.57%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.65 (s, 3H, 18-H_3), 1.12 (s, 3H, 19-H_3), 2.88 (t, 1H, *J* = 9.8 Hz, 17-H), 5.69 (s, 1H, 4-H), 6.34 (d, 1H, *J* = 1.8 Hz, 4′-H), 7.51 (d, 2H, *J* = 8.5 Hz, 2″- and 6″-H), 7.65 (d, 1H, *J* = 1.8 Hz, 3′-H), 7.60 (d, 2H, *J* = 8.5 Hz, 3″- and 5″-H). ^{13}C NMR (125 MHz, $CDCl_3$, δ (ppm)): 13.4 (C-18), 17.3 (C-19), 20.6, 24.2, 29.1, 31.8, 32.7, 33.8, 35.6, 36.0, 37.2, 38.5, 44.7, 46.9, 53.5, 55.3, 106.9 (C-4′), 112.0 (C-4″), 118.0 (CN), 123.9 (C-4), 127.2 (2C, C-2″ and C-6″), 133.1 (2C, C3″ and C-5″), 140.3 (C-3′), 143.8 and 144.5 (C-1″ and C-5′), 170.5 (C-5), 199.1 (C-3).

*2.4.7. 17β-(1-p-Tolyl-3-pyrazolyl)androst-4-en-3-one (**9d**)*

9d (295 mg, 34%), mp 214–216 °C, R_f = 0.65 (ss A); $[\alpha]_D^{20}$ +131 (*c* 1 in $CHCl_3$) (found C, 81.06; H, 8.55. $C_{29}H_{36}N_2O_2$ requires C, 81.27; H, 8.47%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.61 (s, 3H, 18-H_3), 1.18 (s, 3H, 19-H_3), 2.36 (s, 3H, 4″-CH_3), 2.82 (t, 1H, *J* = 9.8 Hz, 17-H), 5.74 (s, 1H, 4-H,), 6.24 (d, 1H, *J* = 2.5 Hz, 4′-H), 7.21 and 7.54: (d, 4H, *J* = 8.0 Hz, 2″-, 3″-, 5″-, 6″-H), 7.78 (d, 1H, *J* = 2.5 Hz, 5′-H). ^{13}C NMR (125 MHz, $CDCl_3$, δ (ppm)): 13.2 (C-18), 17.4 (C-19), 20.8, 20.9 (4″-CH_3), 24.5, 26.3, 32.1, 32.9, 34.0, 35.7, 36.1, 37.7, 38.7, 43.7, 50.1, 54.0, 55.3, 106.5 (C-4′), 118.9 (2C, C-2″ and C-6″), 123.8 (C-4), 126.7 (C-5′), 129.8 (2C, C-3″ and C-5″), 135.7 and 138.0: (C-1″ and C-4″), 154.5 (C-3′), 171.3 (C-5), 199.4 (C-3).

*2.4.8. 17β-(1-p-Tolyl-5-pyrazolyl)androst-4-en-3-one (**10d**)*

10d (342 mg, 39%), mp 208–210 °C, R_f = 0.52 (ss A); $[\alpha]_D^{20}$ −9 (*c* 1 in $CHCl_3$) (found C, 81.36; H, 8.22. $C_{29}H_{36}N_2O_2$ requires C, 81.27; H, 8.47%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.70 (s, 3H, 18-H_3), 1.13 (s, 3H, 19-H_3), 2.41 (s, 3H, 4″-CH_3), 2.82 (t, 1H, *J* = 10.0 Hz, 17-H), 5.69 (s, 1H, 4-H,), 6.27 (d, 1H, *J* = 1.8 Hz, 4′-H), 7.23 (overlapping multiplets, 4H, 2″-, 3″-, 5″-, 6″-H), 7.60 (d, 1H, *J* = 1.8 Hz, 3′-H). ^{13}C NMR (125 MHz, $CDCl_3$, δ (ppm)): 13.4 (C-18), 17.3 (C-19), 20.6, 21.2 (4″-CH_3), 24.3, 29.4, 31.9, 32.7, 33.9, 35.7, 36.0, 37.1, 38.6, 44.4, 46.8, 53.5, 55.1, 105.5 (C-4′), 123.8 (C-4), 126.7 (2C, C-2″ and C-6″), 129.6 (2C, C-3″ and C-5″), 138.3, 138.7, 138.8, 144.8, 170.8 (C-5), 199.3 (C-3).

*2.4.9. 17β-(1-p-Methoxyphenyl-3-pyrazolyl)androst-4-en-3-one (**9e**)*

9e (258 mg, 29%), mp 192–194 °C, R_f = 0.58 (ss A); $[\alpha]_D^{20}$ +126 (*c* 1 in $CHCl_3$) (found C, 77.92; H, 8.45. $C_{28}H_{36}N_2O_2$ requires C, 77.74; H, 8.39%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.61 (s, 3H, 18-H_3), 1.19 (s, 3H, 19-H_3), 2.80 (t, 1H, *J* = 9.3 Hz, 17-H), 3.83 (s, 3H, O-CH_3), 5.74 (s, 1H, 4-H), 6.23 (s, 1H, 4′-H), 6.94 (d, 2H, *J* = 7.8 Hz, 3″- and 5″-H), 7.56 (d, 2H, *J* = 7.8 Hz, 2″- and 6″-H), 7.73 (s, 1H, 5′-H). ^{13}C NMR (125 MHz, $CDCl_3$, δ (ppm)): 13.1 (C-18), 17.4 (C-19), 20.7, 24.5, 26.3, 32.0, 32.9, 33.9, 35.7, 36.0, 37.6, 38.7, 43.7, 50.1, 54.0, 55.3, 55.5 (O-CH_3), 106.3 (C-4′), 114.4 (2C, C-3″ and C-5″), 120.5 (2C, C-2″ and C-6″), 123.8 (C-4), 126.7 (C-5′), 134.2 (C-1″), 154.3 (C-3′), 157.8 (C-4″), 171.5 (C-5), 199.6 (C-3).

*2.4.10. 17β-(1-p-Methoxyphenyl-5-pyrazolyl)androst-4-en-3-one (**10e**)*

10e (315 mg, 36%), mp 211–214 °C, R_f = 0.44 (ss A); $[\alpha]_D^{20}$ −13 (*c* 1 in $CHCl_3$) (found C, 77.54; H, 8.36. $C_{28}H_{36}N_2O_2$ requires C, 77.74; H, 8.39%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.69 (s, 3H, 18-H_3), 1.13 (s, 3H, 19-H_3), 2.78 (t, 1H, *J* = 9.8 Hz, 17-H), 3.85 (s, 3H, O-CH_3), 5.69 (s, 1H, 4-H), 6.24 (s, 1H, 4′-H), 6.94 (d, 2H, *J* = 8.5 Hz, 3″- and 5″-H), 7.23 (d, 2H, *J* = 8.5 Hz, 2″- and 6″-H), 7.57 (s, 1H, 3′-H). ^{13}C NMR (125 MHz, $CDCl_3$, δ (ppm)): 13.4 (C-18), 17.3 (C-19), 20.6, 24.2, 29.4, 31.8, 32.7, 33.9, 35.6, 35.9, 37.1, 38.5, 44.1, 46.8, 53.5, 55.1, 55.5 (O-CH_3), 105.2 (C-4′), 114.0 (2C, C-3″ and C-5″), 123.8 (C-4), 128.1 (2C, C-2″ and C-6″), 133.0 (C-1″), 138.9 (C-3′), 144.3 (C-5′), 159.3 (C-4″), 171.0 (C-5), 199.4 (C-3).

2.5. Determination of $C_{17,20}$-lyase activity and its inhibition in the rat testis

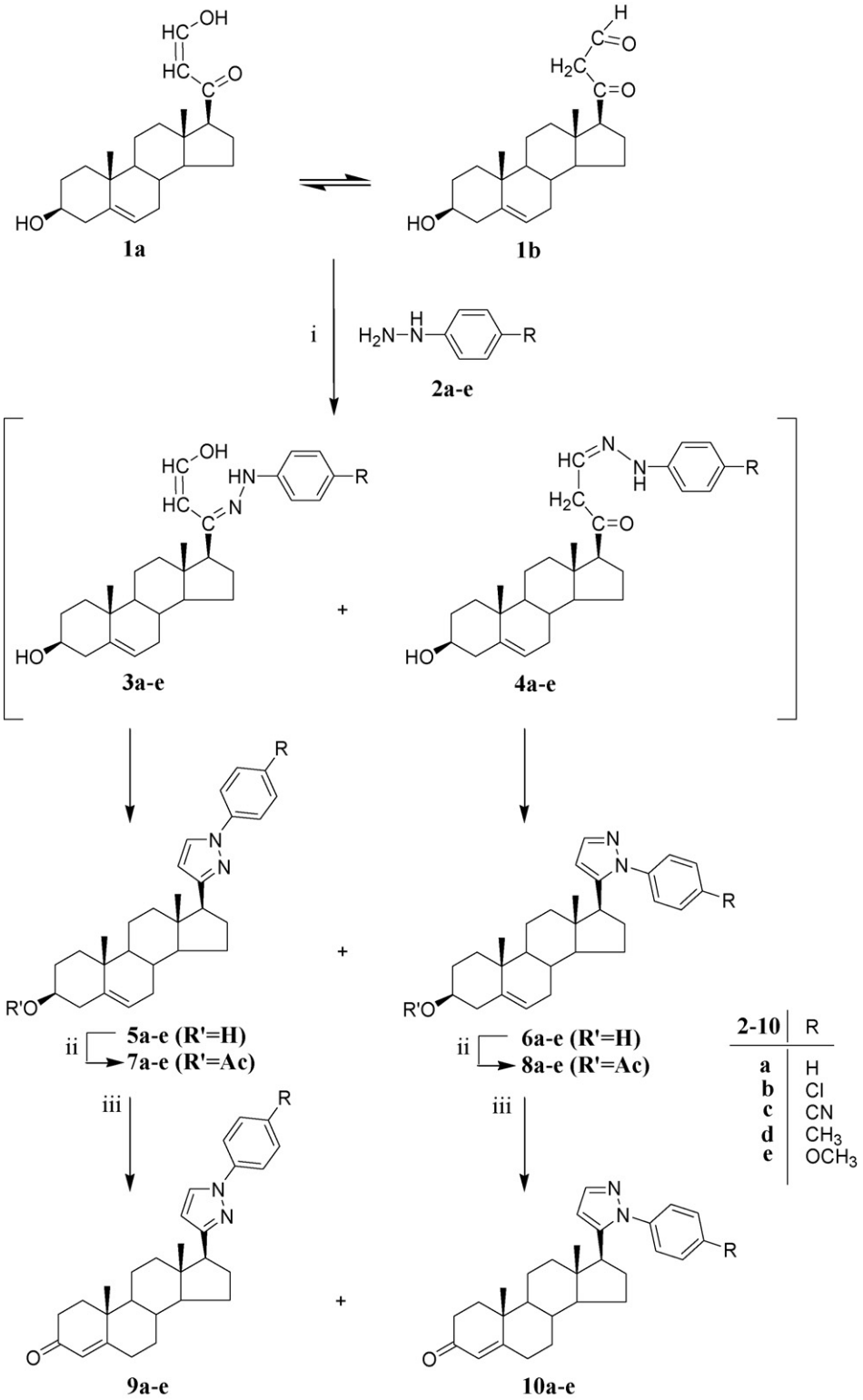
Inhibitory effects exerted on the $C_{17,20}$-lyase activity by the newly synthesized 17β-(1-phenyl- and *p*-substituted-1-phenylpyrazolyl) steroids (**5a**–**e**, **6a**–**e** and **9a**–**e**, **10a**–**e**) were determined by an *in vitro* radiosubstrate incubation method described in earlier publications [7–9].

3. Results and discussion

3.1. Synthetic studies

The reaction between a monosubstituted hydrazine and a nonsymmetrical β-ketoaldehyde leads to the formation of a mixture of pyrazole isomers, even when one of them is present in a very small amount and the process can be considered regioselective [12]. In this manner, the reaction of 3β-hydroxy-21-hydroxymethylidenepregn-5-en-20-one (**1**) with methylhydrazine affords two regioisomers, 17β-(1-methyl-3-pyrazolyl)androst-5-en-3β-ol and 17β-(1-methyl-5-pyrazoly)androst-5-en-3β-ol, in a ratio of 2:1. Despite this observation with methylhydrazine, Doorenbos and Milewich found that the reaction of **1** with phenylhydrazine in acetic acid or ethanol afforded *N*-phenylpyrazolyl derivative **6a** in good yield, as the sole product [10]. It is known that the β-nitrogen atom of phenylhydrazine is the most nucleophilic and that the aldehyde C atom in a β-ketoaldehyde is the most susceptible to nucleophilic attack. The isolated compound was therefore expected to be 17β-(1-phenyl-5-pyrazolyl)androst-5-en-3β-ol (**6a**). The assignment of this structure was confirmed when β-ketoaldehyde **1** was treated with 2-phenylsemicarbazide in an acetate buffered medium to give the 3β-hydroxy-21-formylpregn-5-en-20-one 22-(2-phenyl-semicarbazone) derivative, pyrolysis of which yielded an *N*-phenylpyrazole as the sole product, which proved to be identical with **6a**.

We found that, in contrast with the earlier literature observation, the reaction of **1** with phenylhydrazine hydrochloride in the presence of potassium acetate in acetic acid solution at room temperature for 6 h afforded two compounds in a ratio of 2:3. After chromatographic separation, the minor compound proved to be 17β-(1-phenyl-3-pyrazolyl)androst-5-en-3β-ol (**5a**), while the major compound was 17β-(1-phenyl-5-pyrazolyl)androst-5-en-3β-ol (**6a**). The direction of the ring-closure reactions of **1** with *p*-substituted phenylhydrazines (**2b**–**e**) depended strongly on the electronic features of the substituents on the aromatic moiety. Substituents with an electron-withdrawing character (Cl and CN) afforded predominantly 5-pyrazolyl derivatives **6b**–**c**. Electron-donating substituents (CH_3 and OCH_3) gave mainly 3-pyrazolyl compounds **5d**–**e**. On theoretical grounds, **1** could exist in either of the tautomeric forms **1a** or **1b**, or as some equilibrium mixture of them. In acetic acid medium, **1b** is in dynamic equilibrium with **1a** as indicated by the NMR spectrum in acetic acid-d_4. The spectrum in deuteriochloroform demonstrated that **1a** was the main form present in this solvent [10]. An electron-withdrawing substituent in the *p* position in phenylhydrazine decreases the electron density and the nucleophilicity of the N atom. The favored step in this case is the reaction of the phenylhydrazine with the more reactive free C-21 aldehyde group of **1b** in the equilibrium mixture. The second step is the ring-closure process, resulting in 5-pyrazolyl derivatives. Substituents with an electron-donating character increase the electron density and nucleophilicity of the substituted phenylhydrazines, which react with both the C-20 keto and C-21 formyl groups in **1a** and **1b**, resulting predominantly in 3-pyrazolyl derivatives. We found moreover that in CH_2Cl_2 solution, in the presence of $BF_3{\cdot}OEt_2$, the formation of 3-pyrazolyl derivatives was increased.



Scheme 1. Reagents and conditions: (i) acetic acid, phenylhydrazine or substituted phenylhydrazine, KOAc, rt, 6 h; (ii) pyridine, acetic anhydride, rt, 12 h and (iii) Al(O*i*Pr)$_3$, cyclohexanone, toluene, reflux.

We presumed that **1a** is the main form in this solvent system, and is favorable for the first step of the reaction of phenylhydrazine with the C-20 ketone, resulting mainly in 3-pyrazolyl compounds. Oppenauer oxidation of the 3β-hydroxy compounds **5a**–**e** and **6a**–**e** lead to the corresponding Δ^4-3-ketosteroids without any degradation of the *exo*-heterocyclic system (Scheme 1).

The optical rotation of the compounds, measured in $CHCl_3$, shows a characteristic picture. The $[\alpha]_D^{20}$ values of **5a**–**e** are between −47 and −63, while those of **6a**–**e** are between −128 and −133. For the Δ^4-3-ketosteroids, the corresponding values are less negative: for **9a**–**e** between −13 and +17, or positive for **10a**–**e** between +125 and +149.

Table 1
Inhibition of $C_{17,20}$-lyase activity.

Compounds	Relative conversion, mean ± S.D. (%)	IC_{50} ± S.D. (μM)
5a	65 ± 6	
5b	NI	
5c	NI	
5d	93 ± 4	
5e	95 ± 4	
6a	92 ± 5	
6b	74 ± 3	
6c	NI	
6d	89 ± 5	
6e	NI	
9a	86 ± 2	
9b	91 ± 5	
9c	34 ± 1	22 ± 1
9d	92 ± 2	
9e	54 ± 2	59 ± 2
10a	87 ± 6	
10b	78 ± 1	
10c	69 ± 1	
10d	86 ± 4	
10e	91 ± 6	
Ketoconazole (reference)		0.35 ± 0.05

NI: no inhibition.
S.D.: standard deviation.
Relative conversions (the control incubation with no inhibition is taken as 100%) measured in the presence of 50 μM of the compounds tested, and IC_{50} results for more potent inhibitors.

The structures of the newly synthesized steroidal pyrazoles **5a**–**e**, **6a**–**e**, **7a**–**e**, **8a**–**e**, **9a**–**e** and **10a**–**e** were determined by NMR spectroscopy. In the ^{1}H NMR spectra, the signals of the phenyl (or *p*-substituted phenyl) group appear in the aromatic region. In the case of the 3-pyrazolyl steroids (**5a**–**e**, **7a**–**e** and **9a**–**e**), 4′-H on the heteroaromatic ring resonates at δ = 6.23–6.34 ppm, while the signal of 5′-H can be found at lower fields: δ = 7.72–7.90 ppm. 4′-H of the 5-pyrazolyl derivatives (**6a**–**e**, **8a**–**e** and **10a**–**e**) can be observed at 6.24–6.35 ppm, and 3′-H at 7.57–7.67 ppm. The ^{13}C NMR spectra of the synthesized steroids also contain the signals of the heteroaromatic ring, one for C-4′ at ∼106 ppm, the C-5′ signal at ∼127 ppm (**5a**–**e**, **7a**–**e** and **9a**–**e**), and the C-3′ signal at ∼139 ppm (**6a**–**e**, **8a**–**e** and **10a**–**e**). In **5a**–**e** and **6a**–**e**, the multiplet at ∼3.50 ppm can be assigned to 3-H. Acetylation resulted in a shift of the 3-H signal toward lower fields ($\delta \sim 4.60$ ppm), while the Ac-CH_3 group resonates at $\delta \sim 2.02$ ppm in the ^{1}H NMR spectra, and at δ = 21.4 in the ^{13}C NMR spectra (**7a**–**e** and **8a**–**e**). In the Oppenauer products (**9a**–**e** and **10a**–**e**), 4-(sp^2-)H is to be found at δ = 5.69–5.74 ppm (singlet) in the ^{1}H NMR spectra, and the 3-H signal disappears, while the C-3s resonate at ∼199.5 ppm (^{13}C).

Apart from 17β-(1-phenyl-5-pyrazolyl)androst-5-en-3β-ol (**6a**) and 17β-(1-phenyl-5-pyrazolyl)androst-4-en-3-one (**10a**) in the Δ^4-3-keto series, 17β-(1-phenyl-3-pyrazolyl)androst-5-en-3β-ol (**5a**) and the substituted derivatives **5b**–**e** and **6b**–**e** appear to be unknown in the literature. The Δ^4-3-keto compounds **9b**–**e** and **10b**–**e** have not been synthesized earlier either. Mohareb and Hana recently reacted 21-*N*,*N*-dimethylaminomethylidene-pregn-4-en-3-one with phenylhydrazine and obtained a compound presumed to be 17β-(1-phenyl-3-pyrazolyl)androst-4-en-3-one (**9a**) [11]. The melting point differs by 100 °C from that of ours, the NMR data did not fully support postulated structure of **9a**, and the optical rotation was not reported.

3.2. Effects of 17β-*N*-phenylpyrazolyl steroids on $C_{17,20}$-lyase activity

The inhibitory effects of **5a**–**e**, **6a**–**e**, **9a**–**e** and **10a**–**e** on the $C_{17,20}$-lyase activity of rat testicular $P450_{17\alpha}$ were investigated with an *in vitro* radiosubstrate incubation technique. Enzyme incubations with **5c**, **5d**, **6c** and **6e** resulted in conversions identical to those in the control experiments, and hence these compounds did not inhibit rat $C_{17,20}$-lyase *in vitro* even in the rather high concentration of 50 μM (Table 1). Most of the other derivatives exhibited weak inhibition: **5a**, **5d**, **5e**, **6a**, **6b**, **6d**, **9a**, **9b**, **9d** and **10a**–**e** reduced the enzyme activity by 65–95% when applied at 50 μM in the incubates. The *p*-cyano and *p*-methoxy derivatives in the Δ^4-3-oxo series of the 3-pyrazolyl compounds (**9c** and **9e**) displayed higher inhibitory effects. The relative conversions at 50 μM were 34% and 54%, and IC_{50} values for these compounds were 22 μM and 59 μM, respectively.

The tested compounds did not display efficient inhibition of rat testicular $C_{17,20}$-lyase activity *in vitro*. Only 17β-(1-*p*-cyanophenyl-3-pyrazolyl)androst-4-en-3-one (**9c**) and 17β-(1-*p*-methoxyphenyl-3-pyrazolyl)androst-4-en-3-one (**9e**) exerted somewhat higher, but still weak inhibition. The inhibitory potentials of these two compounds were found to be 100–150 times weaker than that of the reference ketoconazole. With the only exception of the 1-phenyl-3-pyrazolyl derivatives (**5a** and **9a**), the Δ^4-3-keto compounds were found to be stronger or at least equally effective $C_{17,20}$-lyase inhibitors than their 3β-hydroxy counterparts. Nevertheless, no systematic difference was observed between the 3- and 5-pyrazolyl regioisomers, and different substituents on the phenyl ring did not influence the inhibition significantly. Investigations are planned to explore other presumed antiandrogenic properties of the new compounds.

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