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Synthesis of D-ring-substituted (5′*R*)- and (5′*S*)-17β-pyrazolinylandrostene epimers and comparison of their potential anticancer activities

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ABSTRACT

Various steroidal benzylidenes were synthetized from pregnenolone with benzaldehyde and *p*-substituted benzaldehydes. The resulting 17β-chalconyl derivatives of pregnenolone were reacted with hydrazine hydrate in acetic acid solution. Regardless of the starting material, the ring-closure reaction afforded (in contrast with the literature data) a mixture of two steroidal pyrazoline epimers. The epimers were critical isomer pairs, which could be separated only in their acetylated form; their structures were investigated by NMR techniques. The *in vitro* inhibition of rat testicular $C_{17,20}$-lyase activity and the antiproliferative effects on four human cancer cell lines were measured, and the results obtained from the two epimer series were compared.

1. Introduction

Steroidal derivatives in which ring D is modified with *exo*-heterocycles exhibit numerous forms of biological activity and are attractive for medicine [1]. A large number of steroid derivatives containing five- or six-membered 17β-*exo*-heterocycles are known to cause the efficient inhibition of 17α-hydroxylase/$C_{17,20}$-lyase ($P450_{17\alpha}$), which can block androgen synthesis at an early stage, and may therefore be useful in the treatment of prostatic carcinoma [2–7]. We recently reported the synthesis of regioisomeric 17β-*N*-phenylpyrazolyl steroids and the inhibitory effect of these compounds on rat testicular $C_{17,20}$-lyase activity *in vitro* [8]. Some steroid compounds are known to exert hormone receptor-independent antiproliferative activity via the inhibition of angiogenesis, tubulin polymerization and the upregulation of apoptotic pathways [9–11]; our novel steroidal pyrazole compounds were therefore also designated for the *in vitro* screening of their activities against a panel of three human cancer cell lines (HeLa, MCF7 and A431). To continue this program, we now set out to synthetize a novel series of phenypyrazolinyl steroid derivatives in which a *C*-phenyl group is present instead of an *N*-phenyl group. Such compounds were recently prepared by Banday et al. by the reactions of pregnenolone with benzaldehyde and *p*-substituted benzaldehydes to give the corresponding benzylidene derivatives, which were finally converted with hydrazine in acetic acid to 17β-(1-acetyl-5-phenyl-3-pyrazolinyl)androst-5-en-3β-ol and its *p*-substituted phenyl derivatives [12]. Only one isomer was characterized. From a theoretical aspect, such a cyclization produces two epimers. Following the synthetic route described by Banday et al., we concentrated on the formation of the two possible epimers in the ring-closure step of the reaction. The epimers were present as a critical isomer pair, which could be separated only in acetylated form. The separation of steroidal epimers is essential prior to the measurement of their biological effects. Stereoselective inhibition against the $P450_{17\alpha}$ was observed among steroidal dihydrooxazines and aziridines [5,13,14]. The antiproliferative activities of the individual epimers may differ considerably, and the epimeric mixture may exert only weak inhibitory effects [15].

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} deg cm^2 g^{-1}. Elementary analysis data were

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determined with a Perkin–Elmer 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) methyl *tert*-butyl ether/hexane (50:50 v/v), (B) methyl *tert*-butyl ether/hexane (70:30 v/v), and (C) methyl *tert*-butyl 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 the preparation of steroidal benzylidenes (**3a**–**f**)

Three grams (75 mmol) NaOH was dissolved in 180 ml of ethanol. 3.16 g (10 mmol) of pregnenolone (**1**) was then added, and the reaction mixture was stirred until a clear solution was obtained. The aromatic aldehyde (**2a**–**f**, 15 mmol) was next added, and the solution was stirred at room temperature for 12 h, and then poured into a solution of 7 ml of cc HCl in ice-cold-water (700 ml). The white precipitate that formed was filtered off, washed with water, and crystallized from acetone/hexane.

2.2.1. (21E)-3β-Hydroxy-21-benzylidenepregn-5-en-20-one (**3a**)

3a (3.54 g, 87%). mp 123–125 C (Ref. [16]: mp 128–131 °C), R_f = 0.30 (ss A); $[\alpha]_D^{20}$ −18 (*c* 1 in $CHCl_3$). (Found C, 82.98; H, 9.85; $C_{28}H_{36}O_2$ requires C, 83.12; H, 8.97%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.64 (s, 3H, 18-H_3), 0.99 (s, 3H, 19-H_3), 2.85 (t, 1H, *J* = 8.5 Hz, 17-H), 3.52 (m, 1H, 3-H), 5.36 (s, 1H, 6-H), 6.78 (d, 1H, *J* = 16.0 Hz, 21-H), 7.38 (overlapping multiplets, 3H, 3′-H, 4′-H, 5′-H), 7.55 (overlapping multiplets, 3H, 2′-H, 6′-H, 22-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.0 (C-18), 19.0 (C-19), 20.7, 22.3, 24.3, 31.2, 31.4, 31.6, 36.1, 36.8, 38.7, 41.8, 44.6, 49.7, 56.8, 61.7, 71.3 (C-3), 121.0 (C-6), 126.4 (C-21), 127.9 and 128.5 (4C, C-2′, C-3′, C-5′ and C-6′), 129.9 (C-4′), 134.4 (C-1′), 140.4 (C-5), 141.1 (C-22), 200.0 (C-20).

2.2.2. (21E)-3β-Hydroxy-21-(p-fluorobenzylidene)pregn-5-en-20-one (**3b**)

3b (3.72 g, 88%). mp 153–156 °C (Ref. [16]: mp 196–198 °C), R_f = 0.35 (ss A); $[\alpha]_D^{20}$ −20 (*c* 1 in $CHCl_3$). (Found C, 79.65; H, 8.57; $C_{28}H_{35}FO_2$ requires C, 79.58; H, 8.35%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.63 (s, 3H, 18-H_3), 0.99 (s, 3H, 19-H_3), 2.83 (t, 1H, *J* = 8.5 Hz, 17-H), 3.52 (m, 1H, 3-H), 5.35 (s, 1H, 6-H), 6.69 (d, 1H, *J* = 16.0 Hz, 21-H), 7.07 (t, 2H, *J* = 8.5 Hz, 3″- and 5″-H), 7.52 (overlapping multiplets, 3H, 2′-, 6′-, and 22-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.4 (C-18), 19.4 (C-19), 21.1, 22.7, 24.6, 31.6, 31.8, 32.0, 36.5, 37.2, 39.1, 42.2, 45.0, 50.1, 57.2, 62.2, 71.7 (C-3), 115.9 (d, 2C, *J* = 21.8 Hz, C-3′ and C-5′), 121.4 (C-6), 126.5 (C-21), 130.1 (d, 2C, *J* = 8.3 Hz, C-2′ and C-6′), 131.0, 140.2 (C-22), 140.7 (C-5), 163.9 (d, 1C, *J* = 250 Hz, C-4″), 200.1 (C-20).

2.2.3. (21E)-3β-Hydroxy-21-(p-chlorobenzylidene)pregn-5-en-20-one (**3c**)

3c (3.83 g, 87%). mp 163–167 °C, R_f = 0.35 (ss A); $[\alpha]_D^{20}$ −17 (*c* 1 in $CHCl_3$). (Found C, 76.85; H, 7.98; $C_{28}H_{35}ClO_2$ requires C, 76.60; H, 8.04%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.63 (s, 3H, 18-H_3), 0.99 (s, 3H, 19-H_3), 2.83 (t, 1H, *J* = 8.5 Hz, 17-H), 3.52 (m, 1H, 3-H), 5.35 (t, 1H, *J* = 2.5 Hz, 6-H), 6.73 (d, 1H, *J* = 16.0 Hz, 21-H), 7.35 and 7.47 (d, 2H, *J* = 8.0 Hz, and d, 2H, *J* = 8.0 Hz, 2′-, 3′-, 5′-, and 6′-H), 7.49 (d, 1H, *J* = 16.0 Hz, 22-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.4 (C-18), 19.4 (C-19), 21.1, 22.7, 24.6, 31.6, 31.8, 32.0, 36.5, 37.2, 39.1, 42.2, 45.0, 50.0, 57.2, 62.2, 71.7 (C-3), 121.4 (C-6), 127.1 (C-21), 129.1 and 129.4 (4C, C-2′, C-3′, C-5′ and C-6′), 133.3, 136.1, 140.0 (C-22), 140.7 (C-5), 200.1 (C-20).

2.2.4. (21E)-3β-Hydroxy-21-(p-bromobenzylidene)pregn-5-en-20-one (**3d**)

3d (4.42 g, 92%). mp 167–170 °C, R_f = 0.32 (ss A); $[\alpha]_D^{20}$ −19 (*c* 1 in $CHCl_3$). (Found C, 69.72; H, 7.18; $C_{28}H_{35}BrO_2$ requires C, 69.56; H, 7.30%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.63 (s, 3H, 18-H_3), 0.99 (s, 3H, 19-H_3), 2.82 (t, 1H, J_1 = 8.5 Hz, 17-H), 3.52 (m, 1H, 3-H), 5.35 (m, 1H, 6-H), 6.75 (d, 1H, *J* = 15.8 Hz, 21-H), 7.40 and 7.51 (d, 2H, *J* = 8.3 Hz, and d, 2H, *J* = 8.3 Hz, 2′-, 3′-, 5′-, 6′-H), 7.46 (d, 1H, *J* = 15.8 Hz, 22-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.4 (C-18), 19.4 (C-19), 21.1, 22.7, 24.6, 31.6, 31.8, 32.0, 36.5, 37.2, 39.1, 42.2, 45.0, 50.0, 57.2, 62.2, 71.7 (C-3), 121.4 (C-6), 124.4, 127.2 (C-21), 129.6 and 132.1 (C-2′, C-3′, C-5′ and C-6′), 133.7, 140.0 (C-22), 140.7 (C-5), 200.1 (C-20).

2.2.5. (21E)-3β-Hydroxy-21-(p-cyanobenzylidene)pregn-5-en-20-one (**3e**)

3e (3.71 g, 86%). mp 199–202 °C, R_f = 0.25 (ss A); $[\alpha]_D^{20}$ −30 (*c* 1 in $CHCl_3$). (Found C, 80.96; H, 8.35; $C_{29}H_{35}NO_2$ requires C, 81.08; H, 8.21%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.64 (s, 3H, 18-H_3), 0.99 (s, 3H, 19-H_3), 2.84 (t, 1H, *J* = 8.5 Hz, 17-H), 3.53 (m, 1H, 3-H), 5.35 (m, 1H, 6-H), 6.82 (d, 1H, *J* = 16.0 Hz, 21-H), 7.50 (d, 1H, *J* = 16.0 Hz, 22-H), 7.62 and 7.67 (d, 2H, *J* = 8.0 Hz, and d, 2H, *J* = 8.0 Hz, 2′-, 3′-, 5′- and 6′-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.5 (C-18), 19.4 (C-19), 21.1, 22.7, 24.6, 31.6, 31.8, 32.0, 36.5, 37.2, 39.2, 42.2, 45.1, 50.0, 57.2, 62.5, 71.7 (C-3), 113.3 (C-4′), 118.4 (CN), 121.3 (C-6), 128.5 and 132.6 (4C, C-2′, C-3′, C-5′ and C-6′), 129.5 (C-21), 138.9 (C-22), 139.2, 140.7 (C-5), 199.9 (C-20).

2.2.6. (21E)-3β-Hydroxy-21-(p-methoxybenzylidene)pregn-5-en-20-one (**3f**)

3f (4.30 g, 99%). mp 180–183 °C (Ref. [16]: mp 200–203 °C), R_f = 0.25 (ss A); $[\alpha]_D^{20}$ +1.5 (*c* 1 in $CHCl_3$). (Found C, 80.22; H, 9.05; $C_{29}H_{38}O_3$ requires C, 80.14; H, 8.81%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.63 (s, 3H, 18-H_3), 0.99 (s, 3H, 19-H_3), 3.52 (m, 1H, 3-H), 3.83 (s, 3H, OCH_3), 5.35 (s, 1H, 6-H), 6.66 (d, 1H, *J* = 15.5 Hz, 21-H), 6.90 (d, 2H, *J* = 8.0 Hz, 3′- and 5′-H), 7.49 (overlapping multiplets, 3H, 2′-, 6′-, 22-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.4 (C-18), 19.4 (C-19), 21.1, 22.7, 24.7, 31.5, 31.8, 32.0, 36.5, 37.2, 39.1, 42.2, 44.9, 50.0, 55.4, 57.1, 61.9, 71.7 (C-3), 114.3 (2C, C-3′3′, C-5′), 121.4 (C-6), 124.6, 127.4, 129.9 (2C, C-2′, C-6′), 140.7 (C-5), 141.3 (C-22), 161.4, 200.3 (C-20).

2.3. General procedure for the preparation of 3β-acetylated pyrazolinylandrost-5-ene derivatives (**4a**–**f** and **5a**–**f**)

The individual compounds **3a**–**f** (3 mmol) were dissolved in 60 ml of acetic acid, and 1.5 ml (30 mmol) of hydrazine hydrate was added. The mixture was refluxed for 2 h and then poured into water (600 ml). The precipitate that formed was filtered off, washed with water, and dried. The crude product was dissolved in a mixture of pyridine (5 ml) and acetic anhydride (5 ml) and the solution was allowed to stand at room temperature for 8 h. The mixture was then diluted with water and the precipitate that separated out was filtered off.

2.3.1. (5′R)- and (5′S)-17β-(1-Acetyl-5-phenyl-3-pyrazolinyl)androst-5-en-3β-yl acetate (**4a** and **5a**)

The acetylated crude product was chromatographed on silica gel with 50% methyl *tert*-butyl ether/hexane to yield pure **4a** (441 mg, 29%), mp 116–120 °C, R_f = 0.42 (ss B); $[\alpha]_D^{20}$ +24 (*c* 1 in $CHCl_3$). (Found C, 76.55; H, 8.34. $C_{32}H_{42}N_2O_3$ requires C, 76.46; H, 8.42%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.66 (s, 3H, 18-H_3), 1.03 (s, 3H, 19-H_3), 2.03 (s, 3H, 3-Ac-CH_3), 2.32 (s, 3H, *N*-Ac-CH_3), 2.64 (dd,

1H, J_1 = 17.9 Hz, J_2 = 4.0 Hz, 4′-H), 3.37 (dd, 1H, J_1 = 17.9 Hz, J_2 = 11.8 Hz, 4′-H), 4.60 (m, 1H, 3-H), 5.41 (overlapping multiplets, 2H, 6-H and 5′-H), 7.15 (d, 2H, J = 7.3 Hz, 2″- and 6″-H), 7.22 (t, 1H, J = 7.3 Hz, 4″-H), 7.30 (t, 2H, J = 7.3 Hz, 3″- and 5″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.2 (C-18), 19.3 (C-19), 20.9, 21.4 (3-Ac-CH_3), 21.9, 24.4, 24.5, 27.7, 31.7, 32.0, 36.6, 37.0, 38.0, 38.3, 44.0, 46.0, 50.0 (C-4′), 51.8, 56.3, 59.1 (C-5′), 73.8 (C-3), 122.3 (C-6), 125.3 and 128.8 (4C, C-2″, C-3″, C-5″ and C-6″), 127.4 (C-4″), 139.7 (C-5), 142.0 (C-1″), 159.3 (C-3′), 168.4 (*N*-Ac-CO), 170.5 (3-Ac-CO). Continued elution resulted in **5a** (896 mg, 59%), mp 140–143 °C, R_f = 0.38 (ss B); $[\alpha]_D^{20}$ −71 (*c* 1 in $CHCl_3$). (Found C, 76.58; H, 8.64. $C_{32}H_{42}N_2O_3$ requires C, 76.46; H, 8.42%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.67 (s, 3H, 18-H_3), 1.00 (s, 3H, 19-H_3), 2.02 (s, 3H, 3-Ac-CH_3), 2.32 (s, 3H, *N*-Ac-CH_3), 2.75 (dd, 1H, J_1 = 18.0 Hz, J_2 = 4.5 Hz, 4′-H), 3.27 (dd, 1H, J_1 = 18.0 Hz, J_2 = 11.5 Hz, 4′-H), 4.59 (m, 1H, 3-H), 5.40 (overlapping multiplets, 2H, 6-H and 5′-H), 7.15 (d, 2H, J = 7.3 Hz, 2″- and 6″-H), 7.23 (t, 1H, J = 7.3 Hz, 4″-H), 7.31 (t, 2H, J = 7.3 Hz, 3″- and 5″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.4 (C-18), 19.3 (C-19), 20.9, 21.4 (3-Ac-CH_3), 21.8, 24.3, 24.6, 27.7, 31.7, 32.0, 36.6, 37.0, 38.0, 38.4, 43.8, 46.2 (C-4′), 49.9, 51.7, 56.4, 59.1 (C-5′), 73.8 (C-3), 122.3 (C-6), 125.3 and 128.8 (4C, C-2″, C-3″, C-5″ and C-6″), 127.4 (C-4″), 139.7 (C-5), 142.3 (C-1″), 159.0 (C-3′), 168.5 (*N*-Ac-CO), 170.5 (3-Ac-CO).

2.3.2. (5′R)- and (5′S)-17β-(1-Acetyl-5-p-fluorophenyl-3-pyrazolinyl)androst-5-en-3β-yl acetate (**4b** and **5b**)

The acetylated crude product was chromatographed on silica gel with 50% methyl *tert*-butyl ether/hexane to yield pure **4b** (276 mg, 18%), mp 180–184 °C, R_f = 0.40 (ss B); $[\alpha]_D^{20}$ +46 (*c* 1 in $CHCl_3$). (Found C, 73.65; H, 8.05. $C_{32}H_{41}FN_2O_3$ requires C, 73.82; H, 7.94%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.66 (s, 3H, 18-H_3), 1.03 (s, 3H, 19-H_3), 2.03 (s, 3H, 3-Ac-CH_3), 2.30 (s, 3H, *N*-Ac-CH_3), 2.61 (dd, 1H, J_1 = 17.8 Hz, J_2 = 3.8 Hz, 4′-H), 3.36 (dd, 1H, J_1 = 17.8 Hz, J_2 = 11.8 Hz, 4′-H), 4.60 (m, 1H, 3-H), 5.38 (overlapping multiplets, 2H, 6-H and 5′-H), 6.98 (t, 2H, J = 8.5 Hz, 3″-, and 5″-H), 7.12 (m, 2H, 2″- and 6″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.2 (C-18), 19.3 (C-19), 20.9, 21.4 (3-Ac-CH_3), 21.9, 24.4, 24.5, 27.7, 31.7, 32.0, 36.6, 37.0, 38.1, 38.3, 44.0, 45.9 (C-4′), 50.0, 51.8, 56.3, 58.4 (C-5′), 73.8 (C-3), 115.6 (d, 2C, J = 21.5 Hz, C-3″ and C-5″), 122.3 (C-6), 127.1 (d, 2C, J = 8.0 Hz, C-2″ and C-6″), 137.8 (C-1″), 139.7 (C-5), 159.3 (C-3′), 162.0 (d, 1C, J = 244 Hz, C-4″), 168.5 (*N*-Ac-CO), 170.5 (3-Ac-CO). Continued elution resulted in **5b** (675 mg, 43%), mp 195–200 °C, R_f = 0.35 (ss B); $[\alpha]_D^{20}$ −54 (*c* 1 in $CHCl_3$). (Found C, 73.72; H, 7.98. $C_{32}H_{41}FN_2O_3$ requires C, 73.82; H, 7.94%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.66 (s, 3H, 18-H_3), 1.01 (s, 3H, 19-H_3), 2.03 (s, 3H, 3-Ac-CH_3), 2.31 (s, 3H, *N*-Ac-CH_3), 2.72 (dd, 1H, J_1 = 18.0 Hz, J_2 = 5.0 Hz, 4′-H), 3.27 (dd, 1H, J_1 = 18.0 Hz, J_2 = 12.0 Hz, 4′-H), 4.60 (m, 1H, 3-H), 5.39 (overlapping multiplets, 2H, 6-H and 5′-H), 6.99 (t, 2H, J = 8.8 Hz, 3″- and 5″-H), 7.13 (m, 2H, 2″- and 6″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.4 (C-18), 19.3 (C-19), 20.9, 21.4 (3-Ac-CH_3), 21.8, 24.3, 24.5, 27.7, 31.7, 32.0, 36.6, 37.0, 38.1, 38.5, 43.9, 46.2 (C-4′), 50.0, 51.7, 56.4, 58.4 (C-5′), 73.8 (C-3), 115.6 (d, 2C, J = 21.5 Hz, C-3″ and C-5″), 122.3 (C-6), 127.1 (d, 2C, J = 8.0 Hz, C-2″ and C-6″), 138.1 (C-1″), 139.7 (C-5), 159.0 (C-3′), 162.0 (d, 1C, J = 244 Hz, C-4″), 168.6 (*N*-Ac-CO), 170.5 (3-Ac-CO).

2.3.3. (5′R)- and (5′S)-17β-(1-Acetyl-5-p-chlorophenyl-3-pyrazolinyl)androst-5-en-3β-yl acetate (**4c** and **5c**)

The acetylated crude product was chromatographed on silica gel with 5% CH_2Cl_2/diisopropyl ether to yield pure **4c** (465 mg, 29%), mp 247–250 °C, R_f = 0.42 (ss B); $[\alpha]_D^{20}$ +22 (*c* 1 in $CHCl_3$). (Found C, 71.62; H, 7.81. $C_{32}H_{41}ClN_2O_3$ requires C, 71.55; H, 7.69%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.66 (s, 3H, 18-H_3), 1.03 (s, 3H, 19-H_3), 2.03 (s, 3H, 3-Ac-CH_3), 2.31 (s, 3H, *N*-Ac-CH_3), 2.60 (dd, 1H, J_1 = 17.9 Hz, J_2 = 4.3 Hz, 4′-H), 3.37 (dd, 1H, J_1 = 17.9 Hz, J_2 = 11.5 Hz, 4′-H), 4.61 (m, 1H, 3-H), 5.37 (overlapping multiplets, 2H, 6-H and 5′-H), 7.09 and 7.27 (d, 2H, J = 8.5 Hz, 2″- and 6″-H, and d, 2H, J = 8.5 Hz, 3″- and 5″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.2 (C-18), 19.3 (C-19), 20.8, 21.4 (3-Ac-CH_3), 21.8, 24.4, 24.5, 27.7, 31.7, 31.9, 36.6, 37.0, 38.0, 38.3, 44.0, 45.8 (C-4′), 49.9, 51.8, 56.2, 58.5 (C-5′), 73.8 (C-3), 122.2 (C-6), 126.8 and 128.9 (4C, C-2″, C-3″, C-5″ and C-6″), 133.1, 139.7 (C-5), 140.5, 159.2 (C-3′), 168.5 (*N*-Ac-CO), 170.5 (3-Ac-CO). Continued elution resulted in **5c** (944 mg, 59%), mp 227–235 °C, R_f = 0.38 (ss B); $[\alpha]_D^{20}$ −50 (*c* 1 in $CHCl_3$). (Found C, 71.60; H, 7.53. $C_{32}H_{41}ClN_2O_3$ requires C, 71.55; H, 7.69%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.65 (s, 3H, 18-H_3), 1.01 (s, 3H, 19-H_3), 2.02 (s, 3H, 3-Ac-CH_3), 2.31 (s, 3H, *N*-Ac-CH_3), 2.70 (dd, 1H, J_1 = 18.3 Hz, J_2 = 4.8 Hz, 4′-H), 3.26 (dd, 1H, J_1 = 18.3 Hz, J_2 = 11.8 Hz, 4′-H), 4.59 (m, 1H, 3-H), 5.37 (overlapping multiplets, 2H, 6-H and 5′-H), 7.09 and 7.28 (d, 2H, J = 8.3 Hz, 2″- and 6″-H, and d, 2H, J = 8.3 Hz, 3″- and 5″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.4 (C-18), 19.3 (C-19), 20.9, 21.4 (3-Ac-CH_3), 21.8, 24.3, 24.5, 27.7, 31.7, 32.0, 36.6, 37.0, 38.0, 38.5, 43.9, 46.1 (C-4′), 49.9, 51.6, 56.4, 58.5 (C-5′), 73.8 (C-3), 122.2 (C-6), 126.7 and 129.0 (4C, C-2″, C-3″, C-5″ and C-6″), 133.1, 139.7 (C-5), 140.8, 159.0 (C-3′), 168.6 (*N*-Ac-CO), 170.5 (3-Ac-CO).

2.3.4. (5′R)- and (5′S)-17β-(1-Acetyl-5-p-bromophenyl-3-pyrazolinyl)androst-5-en-3β-yl acetate (**4d** and **5d**)

The acetylated crude product was chromatographed on silica gel with 50% methyl *tert*-butyl ether/hexane to yield pure **4d** (348 mg, 20%), mp 222–226 °C, R_f = 0.44 (ss B); $[\alpha]_D^{20}$ +17 (*c* 1 in $CHCl_3$). (Found C, 65.92; H, 7.21. $C_{32}H_{41}BrN_2O_3$ requires C, 66.09; H, 7.11%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.65 (s, 3H, 18-H_3), 1.03 (s, 3H, 19-H_3), 2.03 (s, 3H, 3-Ac-CH_3), 2.31 (s, 3H, *N*-Ac-CH_3), 2.59 (dd, 1H, J_1 = 17.8 Hz, J_2 = 4.3 Hz, 4′-H), 3.37 (dd, 1H, J_1 = 18.0 Hz, J_2 = 12.0 Hz, 4′-H), 4.60 (m, 1H, 3-H), 5.36 (overlapping multiplets, 2H, 6-H and 5′-H), 7.03 (d, 2H, J = 8.3 Hz, 2″- and 6″-H), 7.42 (d, 2H, J = 8.3 Hz, 3″- and 5″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.3 (C-18), 19.3 (C-19), 20.9, 21.4 (3-Ac-CH_3), 21.8, 24.4, 24.5, 27.7, 31.7, 32.0, 36.6, 37.0, 38.0, 38.3, 44.0, 45.7 (C-4′), 49.9, 51.8, 56.2, 58.6 (C-5′), 73.8 (C-3), 121.2, 122.3 (C-6), 127.2 and 131.9 (4C, C-2″, C-3″, C-5″ and C-6″), 139.7 (C-5), 141.0, 159.2 (C-3′), 168.5 (*N*-Ac-CO), 170.5 (3-Ac-CO). Continued elution resulted in **5d** (705 mg, 40%), mp 230–234 °C, R_f = 0.38 (ss B); $[\alpha]_D^{20}$ −39 (*c* 1 in $CHCl_3$). (Found C, 66.17; H, 7.28. $C_{32}H_{41}BrN_2O_3$ requires C, 66.09; H, 7.11%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.65 (s, 3H, 18-H_3), 1.01 (s, 3H, 19-H_3), 2.03 (s, 3H, 3-Ac-CH_3), 2.31 (s, 3H, *N*-Ac-CH_3), 2.70 (dd, 1H, J_1 = 18.3 Hz, J_2 = 4.8 Hz, 4′-H), 3.27 (dd, 1H, J_1 = 18.3 Hz, J_2 = 11.8 Hz, 4′-H), 4.59 (m, 1H, 3-H), 5.36 (overlapping multiplets, 2H, 6-H and 5′-H), 7.03 (d, 2H, J = 8.3 Hz, 2″- and 6″-H), 7.43 (d, 2H, J = 8.3 Hz, 3″- and 5″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.5 (C-18), 19.3 (C-19), 20.9, 21.4 (3-Ac-CH_3), 21.8, 24.3, 24.5, 27.7, 31.7, 32.0, 36.6, 37.0, 38.1, 38.5, 43.9, 46.0 (C-4′), 49.9, 51.7, 56.4, 58.6 (C-5′), 73.8 (C-3), 121.2, 122.3 (C-6), 127.1 and 132.0 (4C, C-2″, C-3″, C-5″ and C-6″), 139.7 (C-5), 141.4, 159.0 (C-3′), 168.6 (*N*-Ac-CO), 170.5 (3-Ac-CO).

2.3.5. (5′R)- and (5′S)-17β-(1-Acetyl-5-p-cyanophenyl-3-pyrazolinyl)androst-5-en-3β-yl acetate (**4e** and **5e**)

The acetylated crude product was chromatographed on silica gel with 5% CH_2Cl_2/diisopropyl ether to yield pure **4e** (427 mg, 27%), mp 232–236 °C, R_f = 0.25 (ss B); $[\alpha]_D^{20}$ +24 (*c* 1 in $CHCl_3$). (Found C, 75.31; H, 7.58. $C_{33}H_{41}N_3O_3$ requires C, 75.11; H, 7.83%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.66 (s, 3H, 18-H_3), 1.03 (s, 3H, 19-H_3), 2.03 (s, 3H, 3-Ac-CH_3), 2.32 (s, 3H, *N*-Ac-CH_3), 2.60 (dd, 1H, J_1 = 17.8 Hz, J_2 = 4.0 Hz, 4′-H), 3.42 (dd, 1H, J_1 = 17.8 Hz, J_2 = 12.0 Hz, 4′-H), 4.61 (m, 1H, 3-H), 5.41 (overlapping multiplets, 2H, 6-H and 5′-H), 7.26 and 7.61 (d, 2H, J = 7.0 Hz, 2″- and 6″-H, and d, 2H, J = 7.0 Hz, 3″- and 5″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.3 (C-18), 19.3 (C-19), 20.8, 21.4 (3-Ac-CH_3), 21.8, 24.4, 24.5, 27.7, 31.7, 31.9, 36.6, 37.0, 38.0, 38.3, 44.0, 45.5 (C-4′), 49.9, 51.7, 56.2, 58.8

(C-5′), 73.7 (C-3), 111.4, 118.6, 122.2 (C-6), 126.3 and 132.7 (4C, C-2″, C-3″, C-5″ and C-6″), 139.7 (C-5), 147.1, 159.1 (C-3′), 168.6 (*N*-Ac-CO), 170.5 (3-Ac-CO). Continued elution resulted in **5e** (838 mg, 53%), mp 222–226 °C, R_f = 0.20 (ss B); $[\alpha]_D^{20}$ −46 (*c* 1 in $CHCl_3$). (Found C, 75.25; H, 7.62. $C_{33}H_{41}N_3O_3$ requires C, 75.11; H, 7.83%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.65 (s, 3H, 18-H_3), 1.01 (s, 3H, 19-H_3), 2.03 (s, 3H, 3-Ac-CH_3), 2.32 (s, 3H, *N*-Ac-CH_3), 2.70 (dd, 1H, J_1 = 18.0 Hz, J_2 = 4.5 Hz, 4′-H), 3.32 (dd, 1H, J_1 = 18.0 Hz, J_2 = 12.0 Hz, 4′-H), 4.60 (m, 1H, 3-H), 5.41 (overlapping multiplets, 2H, 6-H and 5′-H), 7.27 and 7.62 (d, 2H, *J* = 8.0 Hz, 2″- and 6″-H, and d, 2H, *J* = 8.0 Hz, 3″- and 5″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.5 (C-18), 19.3 (C-19), 20.9, 21.4 (3-Ac-CH_3), 21.7, 24.3, 24.5, 27.7, 31.7, 32.0, 36.6, 37.0, 38.0, 38.5, 43.9, 45.8 (C-4′), 49.9, 51.6, 56.3, 58.8 (C-5′), 73.7 (C-3), 111.4, 118.6, 122.2 (C-6), 126.2 and 132.8 (4C, C-2″, C-3″, C-5″ and C-6″), 139.7 (C-5), 147.4, 158.9 (C-3′), 168.7 (*N*-Ac-CO), 170.5 (3-Ac-CO).

*2.3.6. (5′R)- and (5′S)-17β-(1-Acetyl-5-p-methoxyphenyl-3-pyrazolinyl)androst-5-en-3β-yl acetate (**4f** and **5f**)*

The acetylated crude product was chromatographed on silica gel with 50% methyl *tert*-butyl ether/hexane to yield pure **4f** (387 mg, 24%), mp 159–161 °C, R_f = 0.33 (ss B); $[\alpha]_D^{20}$ +26 (*c* 1 in $CHCl_3$). (Found C, 74.55; H, 8.41. $C_{33}H_{44}N_2O_4$ requires C, 74.40; H, 8.33%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.66 (s, 3H, 18-H_3), 1.03 (s, 3H, 19-H_3), 2.03 (s, 3H, 3-Ac-CH_3), 2.29 (s, 3H, *N*-Ac-CH_3), 2.62 (dd, 1H, J_1 = 17.8 Hz, J_2 = 4.0 Hz, 4′-H), 3.34 (dd, 1H, J_1 = 17.8 Hz, J_2 = 11.8 Hz, 4′-H), 3.76 (s, 3H, OCH_3), 4.60 (m, 1H, 3-H), 5.36 (overlapping multiplets, 2H, 6-H and 5′-H), 6.83 (d, 2H, *J* = 8.5 Hz, 3″- and 5″-H), 7.08 (d, 2H, *J* = 8.5 Hz, 2″- and 6″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.2 (C-18), 19.3 (C-19), 20.9, 21.4 (3-Ac-CH_3), 21.9, 24.4, 24.5, 27.7, 31.7, 32.0, 36.6, 37.0, 38.1, 38.3, 43.9, 45.9 (C-4′), 50.0, 51.9, 55.2, 56.3, 58.6 (C-5′), 73.8 (C-3), 114.1 and 126.6 (4C, C-2″, C-3″, C-5″ and C-6″), 122.3 (C-6), 134.2, 139.7 (C-5), 158.8, 159.2, 168.4 (*N*-Ac-CO), 170.5 (3-Ac-CO). Continued elution resulted in **5f** (782 mg, 49%), mp 176–178 °C, R_f = 0.27 (ss B); $[\alpha]_D^{20}$ −45 (*c* 1 in $CHCl_3$). (Found C, 74.32; H, 8.25. $C_{33}H_{44}N_2O_4$ requires C, 74.40; H, 8.33%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.68 (s, 3H, 18-H_3), 1.01 (s, 3H, 19-H_3), 2.03 (s, 3H, 3-Ac-CH_3), 2.30 (s, 3H, *N*-Ac-CH_3), 2.74 (dd, 1H, J_1 = 18.0 Hz, J_2 = 4.5 Hz, 4′-H), 3.24 (dd, 1H, J_1 = 18.0 Hz, J_2 = 11.5 Hz, 4′-H), 3.77 (s, 3H, OCH_3), 4.60 (m, 1H, 3-H), 5.36 (overlapping multiplets, 2H, 6-H and 5′-H), 6.83 (d, 2H, *J* = 8.3 Hz, 3″- and 5″-H), 7.08 (d, 2H, *J* = 8.3 Hz, 2″- and 6″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.4 (C-18), 19.3 (C-19), 20.9, 21.4 (3-Ac-CH_3), 21.9, 24.3, 24.5, 27.7, 31.7, 32.0, 36.6, 37.0, 38.1, 38.4, 43.8, 46.2 (C-4′), 50.0, 51.7, 55.2, 56.4, 58.5 (C-5′), 73.8 (C-3), 114.2 and 126.6 (4C, C-2″, C-3″, C-5″ and C-6″), 122.3 (C-6), 134.6, 139.7 (C-5), 158.8, 159.0, 168.5 (*N*-Ac-CO), 170.5 (3-Ac-CO).

*2.4. General procedure for the preparation of 3β-hydroxypyrazolinylandrostene derivatives (**4g–l** and **5g–l**)*

The individual compounds **4a–f** or **5a–f** (100 mg) were dissolved in methanol (15 ml), and $NaOCH_3$ (18 mg, 0.33 mmol) was added. The mixture was allowed to stand at room temperature, and the progress of the reaction was monitored by TLC. After completion of the transformation, the reaction mixture was diluted with water, and neutralized with dilute HCl. The resulting precipitate was filtered off, washed with water and dried at room temperature.

*2.4.1. (5′R)-17β-(1-Acetyl-5-phenyl-3-pyrazolinyl)androst-5-en-3β-ol (**4g**)*

4g (65 mg, 71%). mp 205–209 ° °C, R_f = 0.64 (ss C); $[\alpha]_D^{20}$ +26 (*c* 1 in $CHCl_3$). (Found C, 78.34; H, 8.85; $C_{30}H_{40}N_2O_2$ requires C, 78.22;H, 8.75%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.66 (s, 3H, 18-H_3), 1.02 (s, 3H, 19-H_3), 2.32 (s, 3H, *N*-Ac-CH_3), 2.64 (d, 1H, *J* = 16.0 Hz, 4′-H), 3.37 (dd, 1H, J_1 = 16.0 Hz, J_2 = 12.0 Hz, 4′-H), 3.52 (s, 1H, 3-H), 5.39 (overlapping multiplets, 2H, 6-H és 5′-H), 7.15 (d, 2H, *J* = 7.3 Hz, 2″- és 6″-H), 7.23 (t, 1H, *J* = 7.3 Hz, 4″-H), 7.30 (t, 2H, *J* = 7.3 Hz, 3″- és 5″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.2 (C-18), 19.4 (C-19), 20.9, 21.9 (*N*-Ac-CH_3), 24.4, 24.5, 31.6, 31.7, 32.0, 36.5, 37.3, 38.4, 42.2, 44.0, 45.9, 50.1, 51.9, 56.4, 59.1, 71.7 (C-3), 121.3 (C-6), 125.3 and 128.8 (4C, C-2″, C-3″, C-5″ and C-6″), 127.4 (C-4″), 140.8 (C-5), 142.0 (C-1″), 159.3 (C-3′), 168.5 (*N*-Ac-CO).

*2.4.2. (5′S)-17β-(1-Acetyl-5-phenyl-3-pyrazolinyl)androst-5-en-3β-ol (**5g**)*

5g (80 mg, 87%). mp 194–197 °C, R_f = 0.62 (ss C); $[\alpha]_D^{20}$ −85 (*c* 1 in $CHCl_3$). (Found C, 78.41; H, 8.78; $C_{30}H_{40}N_2O_2$ requires C, 78.22;H, 8.75%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.67 (s, 3H, 18-H_3), 0.99 (s, 3H, 19-H_3), 2.32 (s, 3H, *N*-Ac-CH_3), 2.75 (d, 1H, *J* = 16.0 Hz, 4′-H), 3.27 (dd, 1H, J_1 = 16.0 Hz, J_2 = 11.8 Hz, 4′-H), 3.51 (s, 1H, 3-H), 5.39 (overlapping multiplets, 2H, 6-H and 5′-H), 7.15 (s, 2H, 2″- and 6″-H), 7.23 (t, 1H, *J* = 6.0 Hz, 4″-H), 7.30 (s, 2H, 3″- and 5″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.0 (C-18), 19.0 (C-19), 20.6, 21.5 (*N*-Ac-CH_3), 24.0, 24.2, 31.2, 31.3, 31.6, 36.1, 36.9, 38.1, 41.8, 43.5, 45.8, 49.7, 51.3, 56.1, 58.7, 71.2 (C-3), 120.9 (C-6), 124.9 and 128.4 (4C, C-2″, C-3″, C-5″ and C-6″), 127.0 (C-4″), 140.4 (C-5), 141.9 (C-1″), 158.7 (C-3′), 168.2 (*N*-Ac-CO).

*2.4.3. (5′R)-17β-(1-Acetyl-5-p-fluorophenyl-3-pyrazolinyl)androst-5-en-3β-ol (**4h**)*

4h (70 mg, 76%). mp 269–272 °C, R_f = 0.62 (ss C); $[\alpha]_D^{20}$ +8.5 (*c* 1 in $CHCl_3$). (Found C, 75.37; H, 8.34; $C_{30}H_{39}FN_2O_2$ requires C, 75.28;H, 8.21%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.66 (s, 3H, 18-H_3), 1.02 (s, 3H, 19-H_3), 2.30 (s, 3H, *N*-Ac-CH_3), 2.61 (dd, 1H, J_1 = 18.0 Hz, J_2 = 3.8 Hz, 4′-H), 3.37 (dd, 1H, J_1 = 18.0 Hz, J_2 = 12.0 Hz, 4′-H), 3.51 (m, 1H, 3-H), 5.37 (overlapping multiplets, 2H, 6-H and 5′-H), 6.98 (t, 2H, *J* = 8.5 Hz, 3″-, and 5″-H), 7.12 (m, 2H, 2″- and 6″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.2 (C-18), 19.4 (C-19), 20.9, 21.9 (*N*-Ac-CH_3), 24.4, 24.5, 31.6, 31.7, 32.0, 36.5, 37.3, 38.4, 42.2, 44.0, 45.9, 50.1, 51.9, 56.4, 58.5, 71.6 (C-3), 115.6 (d, 2C, *J* = 21.5 Hz, C-3″ and C-5″), 127.1 (d, 2C, *J* = 7.9 Hz, C-2″ and C-6″), 121.3 (C-6), 137.8 (C-1″), 140.8 (C-5), 159.3 (C-3′), 162.0 (d, 1C, *J* = 244 Hz, C-4″), 168.5 (*N*-Ac-CO).

*2.4.4. (5′S)-17β-(1-Acetyl-5-p-fluorophenyl-3-pyrazolinyl)androst-5-en-3β-ol (**5h**)*

5h (55 mg, 60%). mp 222–225 °C, R_f = 0.58 (ss C); $[\alpha]_D^{20}$ −58 (*c* 1 in $CHCl_3$). (Found C, 75.35; H, 8.30; $C_{30}H_{39}FN_2O_2$ requires C, 75.28;H, 8.21%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.66 (s, 3H, 18-H_3), 0.99 (s, 3H, 19-H_3), 2.30 (s, 3H, *N*-Ac-CH_3), 2.71 (dd, 1H, J_1 = 18.0 Hz, J_2 = 3.5 Hz, 4′-H), 3.26 (dd, 1H, J_1 = 18.0 Hz, J_2 = 11.8 Hz, 4′-H), 3.50 (m, 1H, 3-H), 5.38 (overlapping multiplets, 2H, 6-H and 5′-H), 6.98 (t, 2H, *J* = 8.5 Hz, 3″-, and 5″-H), 7.12 (m, 2H, 2″- and 6″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.4 (C-18), 19.4 (C-19), 21.0, 21.8 (*N*-Ac-CH_3), 24.3, 24.6, 31.6, 31.7, 32.0, 36.5, 37.2, 38.5, 42.2, 43.9, 46.2, 50.0, 51.7, 56.5, 58.4, 71.6 (C-3), 115.6 (d, 2C, *J* = 21.4 Hz, C-3″ and C-5″), 127.0 (d, 2C, *J* = 8.0 Hz, C-2″ and C-6″), 121.3 (C-6), 138.1 (C-1″), 140.8 (C-5), 159.1 (C-3′), 162.0 (d, 1C, *J* = 244 Hz, C-4″), 168.6 (*N*-Ac-CO).

*2.4.5. (5′R)-17β-(1-Acetyl-5-p-chlorophenyl-3-pyrazolinyl)androst-5-en-3β-ol (**4i**)*

4i (73 mg, 80%). mp 247–249 °C, R_f = 0.64 (ss C); $[\alpha]_D^{20}$ +38 (*c* 1 in $CHCl_3$). (Found C, 72.94; H, 8.05; $C_{30}H_{39}ClN_2O_2$ requires C, 72.78; H, 7.94%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.66 (s, 3H, 18-H_3), 1.02 (s, 3H, 19-H_3), 2.31 (s, 3H, *N*-Ac-CH_3), 2.60 (dd, 1H, J_1 = 17.7 Hz, J_2 = 2.8 Hz, 4′-H), 3.37 (dd, 1H, J_1 = 17.7 Hz, J_2 = 12.0 Hz, 4′-H), 3.51 (m, 1H, 3-H), 5.36 (overlapping multiplets, 2H, 6-H and 5′-H), 7.09 and 7.27 (d, 2H, *J* = 8.0 Hz, 2″- and 6″-H, and d, 2H, *J* = 8.0 Hz, 3″- and

5″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.2 (C-18), 19.4 (C-19), 20.9, 21.8 (*N*-Ac-CH_3), 24.4, 24.5, 31.6, 31.7, 32.0, 36.5, 37.3, 38.4, 42.2, 44.0, 45.8, 50.1, 51.8, 56.3, 58.5, 71.6 (C-3), 121.3 (C-6), 126.8 and 128.9 (4C, C-2″, C-3″, C-5″ and C-6″), 133.1, 140.5, 140.8, 159.2 (C-3′), 168.5 (*N*-Ac-CO).

*2.4.6. (5′S)-17β-(1-Acetyl-5-p-chlorophenyl-3-pyrazolinyl)androst-5-en-3β-ol (**5i**)*

5i (69 mg, 75%). mp 227–229 °C, R_f = 0.58 (ss C); $[\alpha]_D^{20}$ −45 (*c* 1 in $CHCl_3$). (Found C, 72.98; H, 7.95; $C_{30}H_{39}ClN_2O_2$ requires C, 72.78; H, 7.94%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.66 (s, 3H, 18-H_3), 1.00 (s, 3H, 19-H_3), 2.31 (s, 3H, *N*-Ac-CH_3), 2.71 (d, 1H, *J* = 17.8 Hz, 4′-H), 3.28 (dd, 1H, J_1 = 17.8 Hz, J_2 = 12.3 Hz, 4′-H), 3.51 (m, 1H, 3-H), 5.37 (overlapping multiplets, 2H, 6-H and 5′-H), 7.09 and 7.28 (d, 2H, *J* = 7.5 Hz, 2″- and 6″-H, and d, 2H, *J* = 7.5 Hz, 3″- and 5″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.1 (C-18), 19.0 (C-19), 20.6, 21.4 (*N*-Ac-CH_3), 24.0, 24.2, 31.2, 31.3, 31.6, 36.1, 36.9, 38.1, 41.8, 43.5, 45.7, 49.7, 51.3, 56.1, 58.1, 71.2 (C-3), 120.9 (C-6), 126.4 and 128.6 (4C, C-2″, C-3″, C-5″ and C-6″), 132.7, 140.4 (2C), 158.7 (C-3′), 168.2 (*N*-Ac-CO).

*2.4.7. (5′R)-17β-(1-Acetyl-5-p-bromophenyl-3-pyrazolinyl)androst-5-en-3β-ol (**4j**)*

4j (68 mg, 73%). mp 212–215 °C, R_f = 0.64 (ss C); $[\alpha]_D^{20}$ +9.4 (*c* 1 in $CHCl_3$). (Found C, 66.54; H, 7.35; $C_{30}H_{39}BrN_2O_2$ requires C, 66.78; H, 7.29%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.65 (s, 3H, 18-H_3), 1.01 (s, 3H, 19-H_3), 2.30 (s, 3H, *N*-Ac-CH_3), 2.59 (dd, 1H, J_1 = 17.5 Hz, J_2 = 3.8 Hz, 4′-H), 3.37 (dd, 1H, J_1 = 17.5 Hz, J_2 = 12.0 Hz, 4′-H), 3.51 (m, 1H, 3-H), 5.35 (overlapping multiplets, 2H, 6-H and 5′-H), 7.02 (d, 2H, *J* = 8.0 Hz, 2″- and 6″-H), 7.42 (d, 2H, *J* = 8.0 Hz, 3″- and 5″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.3 (C-18), 19.4 (C-19), 20.9, 21.8 (*N*-Ac-CH_3), 24.4, 24.5, 31.6, 31.7, 32.0, 36.5, 37.3, 38.3, 42.2, 44.0, 45.7, 50.1, 51.8, 56.3, 58.6, 71.6 (C-3), 121.2, 121.3 (C-6), 127.2 and 131.9 (4C, C-2″, C-3″, C-5″ and C-6″), 140.8 (C-5), 141.0 (C-1″), 159.3 (C-3′), 168.5 (*N*-Ac-CO).

*2.4.8. (5′S)-17β-(1-Acetyl-5-p-bromophenyl-3-pyrazolinyl)androst-5-en-3β-ol (**5j**)*

5j (67 mg, 72%). mp 219–223 °C, R_f = 0.58 (ss C); $[\alpha]_D^{20}$ −46 (*c* 1 in $CHCl_3$). (Found C, 66.62; H, 7.41; $C_{30}H_{39}BrN_2O_2$ requires C, 66.78; H, 7.29%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.65 (s, 3H, 18-H_3), 0.99 (s, 3H, 19-H_3), 2.31 (s, 3H, *N*-Ac-CH_3), 2.70 (dd, 1H, J_1 = 18.2 Hz, J_2 = 4.8 Hz, 4′-H), 3.26 (dd, 1H, J_1 = 18.2 Hz, J_2 = 12.0 Hz, 4′-H), 3.52 (m, 1H, 3-H), 5.36 (overlapping multiplets, 2H, 6-H and 5′-H), 7.03 (d, 2H, *J* = 8.0 Hz, 2″- and 6″-H), 7.43 (d, 2H, *J* = 8.0 Hz, 3″- and 5″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.5 (C-18), 19.4 (C-19), 21.0, 21.8 (*N*-Ac-CH_3), 24.4, 24.6, 31.6, 31.7, 32.0, 36.5, 37.3, 38.5, 42.2, 43.9, 46.0, 50.0, 51.7, 56.5, 58.6, 71.7 (C-3), 121.2 (C-4″), 121.3 (C-6), 127.1 and 132.0 (4C, C-2″, C-3″, C-5″ and C-6″), 140.8 (C-5), 141.3 (C-1″), 159.1 (C-3′), 168.6 (*N*-Ac-CO).

*2.4.9. (5′R)-17β-(1-Acetyl-5-p-cyanophenyl-3-pyrazolinyl)androst-5-en-3β-ol (**4k**)*

4k (80 mg, 87%). mp 193–200 °C, R_f = 0.50 (ss C); $[\alpha]_D^{20}$ +77 (*c* 1 in $CHCl_3$). Found C, 76.82; H, 7.96; $C_{31}H_{39}N_3O_2$ requires C, 76.67;H, 8.09%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.66 (s, 3H, 18-H_3), 1.02 (s, 3H, 19-H_3), 2.32 (s, 3H, *N*-Ac-CH_3), 2.60 (dd, 1H, J_1 = 18.0 Hz, J_2 = 4.5 Hz, 4′-H), 3.42 (dd, 1H, J_1 = 18.0 Hz, J_2 = 12.0 Hz, 4′-H), 3.51 (m, 1H, 3-H), 5.35 (d, 1H, *J* = 5.0 Hz, 6-H), 5.42 (dd, 1H, J_1 = 12.0 Hz, J_2 = 4.5 Hz, 5′-H), 7.26 and 7.61 (d, 2H, *J* = 8.0 Hz, 2″- and 6″-H, and d, 2H, *J* = 8.0 Hz, 3″- and 5″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.3 (C-18), 19.4 (C-19), 20.9, 21.8 (*N*-Ac-CH_3), 24.4, 24.5, 31.5, 31.7, 32.0, 36.5, 37.2, 38.4, 42.2, 44.0, 45.5, 50.0, 51.7, 56.3, 58.8, 71.6 (C-3), 111.4, 118.6, 121.2 (C-6), 126.2 and 132.7 (4C, C-2″, C-3″, C-5″ and C-6″), 140.8 (C-5), 147.1, 159.2 (C-3′), 168.6 (*N*-Ac-CO).

*2.4.10. (5′S)-17β-(1-Acetyl-5-p-cyanophenyl-3-pyrazolinyl)androst-5-en-3β-ol (**5k**)*

5k (78 mg, 85%). mp 209–212 °C, R_f = 0.44 (ss C); $[\alpha]_D^{20}$ −32 (*c* 1 in $CHCl_3$). (Found C, 76.78; H, 8.15; $C_{31}H_{39}N_3O_2$ requires C, 76.67;H, 8.09%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.65 (s, 3H, 18-H_3), 0.99 (s, 3H, 19-H_3), 2.32 (s, 3H, *N*-Ac-CH_3), 2.70 (dd, 1H, J_1 = 18.3 Hz, J_2 = 4.7 Hz, 4′-H), 3.32 (dd, 1H, J_1 = 18.3 Hz, J_2 = 11.8 Hz, 4′-H), 3.50 (m, 1H, 3-H), 5.35 (m, 1H, 6-H), 5.42 (dd, 1H, J_1 = 11.8 Hz, J_2 = 4.7 Hz, 5′-H), 7.27 and 7.62 (d, 2H, *J* = 8.0 Hz, 2″- and 6″-H, and d, 2H, *J* = 8.0 Hz, 3″- and 5″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.5 (C-18), 19.4 (C-19), 20.9, 21.7 (*N*-Ac-CH_3), 24.3, 24.5, 31.5, 31.7, 32.0, 36.5, 37.2, 38.5, 42.2, 43.9, 45.8, 50.0, 51.6, 56.4, 58.8, 71.5 (C-3), 111.3, 118.6, 121.2 (C-6), 126.2 and 132.8 (4C, C-2″, C-3″, C-5″ and C-6″), 140.8 (C-5), 147.4, 159.0 (C-3′), 168.8 (*N*-Ac-CO).

*2.4.11. (5′R)-17β-(1-Acetyl-5-p-methoxyphenyl-3-pyrazolinyl)androst-5-en-3β-ol (**4l**)*

4l (75 mg, 81%). mp 224–230 °C, R_f = 0.52 (ss C); $[\alpha]_D^{20}$ +6.4 (*c* 1 in $CHCl_3$). (Found C, 75.92; H, 8.51; $C_{31}H_{42}N_2O_3$ requires C, 75.88; H, 8.63%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.66 (s, 3H, 18-H_3), 1.01 (s, 3H, 19-H_3), 2.30 (s, 3H, *N*-Ac-CH_3), 2.62 (dd, 1H, J_1 = 18.0 Hz, J_2 = 3.5 Hz, 4′-H), 3.34 (dd, 1H, J_1 = 18.0 Hz, J_2 = 11.8 Hz, 4′-H), 3.51 (m, 1H, 3-H), 3.77 (s, 3H, OCH_3), 5.36 (overlapping multiplets, 2H, 6-H and 5′-H), 6.83 (d, 2H, *J* = 8.5 Hz, 3″- and 5″-H), 7.08 (d, 2H, *J* = 8.5 Hz, 2″- and 6″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.2 (C-18), 19.4 (C-19), 20.9, 21.9 (*N*-Ac-CH_3), 24.4, 24.5, 31.6, 31.7, 32.0, 36.5, 37.3, 38.3, 42.2, 43.9, 45.9, 50.1, 51.9, 55.2, 56.4, 58.6, 71.6 (C-3), 114.1 and 126.6 (4C, C-2″, C-3″, C-5″ and C-6″), 121.3 (C-6), 134.2, 140.8 (C-5), 158.8, 159.3, 168.4 (*N*-Ac-CO).

*2.4.12. (5′S)-17β-(1-Acetyl-5-p-methoxyphenyl-3-pyrazolinyl)androst-5-en-3β-ol (**5l**)*

5l (83 mg, 90%). mp 211–215 °C, R_f = 0.50 (ss C); $[\alpha]_D^{20}$ −48 (*c* 1 in $CHCl_3$). (Found C, 75.73; H, 8.71; $C_{31}H_{42}N_2O_3$ requires C, 75.88; H, 8.63%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.68 (s, 3H, 18-H_3), 0.99 (s, 3H, 19-H_3), 2.30 (s, 3H, *N*-Ac-CH_3), 2.74 (dd, 1H, J_1 = 18.0 Hz, J_2 = 4.5 Hz, 4′-H), 3.24 (dd, 1H, J_1 = 18.0 Hz, J_2 = 11.5 Hz, 4′-H), 3.49 (m, 1H, 3-H), 3.77 (s, 3H, OCH_3), 5.36 (overlapping multiplets, 2H, 6-H and 5′-H), 6.83 (d, 2H, *J* = 8.5 Hz, 3″- and 5″-H), 7.08 (d, 2H, *J* = 8.5 Hz, 2″- and 6″-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.4 (C-18), 19.4 (C-19), 21.0, 21.9 (*N*-Ac-CH_3), 24.4, 24.6, 31.6, 31.7, 32.0, 36.5, 37.2, 38.5, 42.2, 43.9, 46.2, 50.1, 51.7, 55.2, 56.5, 58.5, 71.6 (C-3), 114.2 and 126.6 (4C, C-2″, C-3″, C-5″ and C-6″), 121.3 (C-6), 134.5, 140.8 (C-5), 158.8, 159.1, 168.5 (*N*-Ac-CO).

2.5. Determination of $C_{17,20}$-lyase inhibition

Inhibitory effects exerted on the $C_{17,20}$-lyase activity were determined by an *in vitro* radiosubstrate incubation method described in earlier publications [5–8], with some modifications.

In brief, adult Wistar rat testicular tissue was homogenized with an Ultra-Turrax in 0.1 M HEPES buffer (pH = 7.3) containing 1 mM EDTA and 1 mM dithiotreitol. Aliquots of this homogenate were incubated in 200 μl final volume at 37 °C for 20 min in the presence of 0.1 mM NADPH. 1 μM [^{3}H]17-hydroxyprogesterone was added to the incubate in 20 μl of a 25 v/v% propylene glycol solution. Test compounds were applied at 50 μM and introduced in 10 μl of DMSO. (These organic solvent contents did not reduce the enzyme activity substantially.) Control incubates without test substances, and incubates with the reference compound ketoconazole were also prepared in every series. Following incubation, the androst-4-ene-3,17-dione formed and the 17-hydroxyprogesterone remaining were isolated through extraction and TLC. $C_{17,20}$-lyase activity was calculated from the radioactivity of the androst-4-ene-3,17-dione obtained. At least two experiments were

performed with each test compound and the standard deviations of the mean enzyme activity results were within ± 10%.

2.6. Determination of antiproliferative effects

Human cancer cell lines were purchased from ECACC (Salisbury, UK). HeLa (cervix adenocarcinoma), MCF7 (breast adenocarcinoma), A2780 (ovarian carcinoma) and A431 (skin epidermoid carcinoma) cells were cultivated in minimal essential medium supplemented with 10% fetal bovine serum, 1% non-essential amino acids and an antibiotic–antimycotic mixture.

Near-confluent cancer cells were seeded onto a 96-well microplate (5000/well) and attached to the bottom of the well overnight. On the second day, 200 μl of new medium containing the tested compound (at 10 or 30 μM) was added. After incubation for 72 h at 37 °C in humidified air containing 5% CO_2, the living cells were assayed by the addition of 20 μl of 5 mg/ml MTT solution. MTT was converted by intact mitochondrial reductase and precipitated as blue crystals during a 4-h contact period. The medium was then removed and the precipitated crystals were dissolved in 100 μl of DMSO during a 60-min period of shaking at 25 °C. Finally, the reduced MTT was assayed at 545 nm, using a microplate reader; wells with untreated cells were utilized as controls [17]. All *in vitro* experiments were carried out on two microplates with at least five parallel wells. Cisplatin was used as positive control. Stock solutions of the tested substances (10 mM) were prepared with DMSO. The DMSO content of the medium (0.1% or 0.3%) did not have any significant effect on the cell proliferation.

3. Results and discussion

3.1. Synthetic studies

The reactions between α,β-unsaturated ketones and hydrazines are widely used for the preparation of substituted 2-pyrazolines [18].

When an appropriately substituted starting material is used, one or two new stereogenic centers are formed during the ring-closure, and as a result the reaction can lead to a diastereomeric mixture of pyrazoline products [18]. However, in some cases the ring closure results in only a single diastereomer [19]. Amr et al. found that the reaction of (16*E*)-3β-trifluoroacetoxy-16-benzylidene-5α-androstan-17-one with hydrazine hydrate in glacial acetic acid afforded a single D-ring fused pyrazoline diastereomer, (16*R*,5′*S*)-3β-trifluoroacetoxy-5α-androstano[17,16-*c*]-1′*H*-5′-(*p*-methoxyphenyl)-*N*-acetylpyrazoline [19]. In this reaction, a fused pyrazoline ring is formed from a hydrazine derivative and an α,β-unsaturated ring ketone, and the stereochemistry of the ring closure can therefore be influenced by the conformation of the starting material. There are very few examples in the literature where the stereochemistry of the reaction between an open-chain unsaturated ketone and hydrazine was studied [20].

Banday et al. synthetized a series of steroidal pyrazolines by reacting 21-benzylidenepreg-5-ene derivatives (**3a**, **3b** and **3f**) [12] with hydrazine hydrate in glacial acetic acid. This reaction leads to a formation of a new stereogenic center at position 5′; in principle, therefore a mixture of 5′*R* and 5′*S* epimers can be obtained. However, the separation of these two possible isomers and a comparison of their physical and antiproliferative properties have not yet been described.

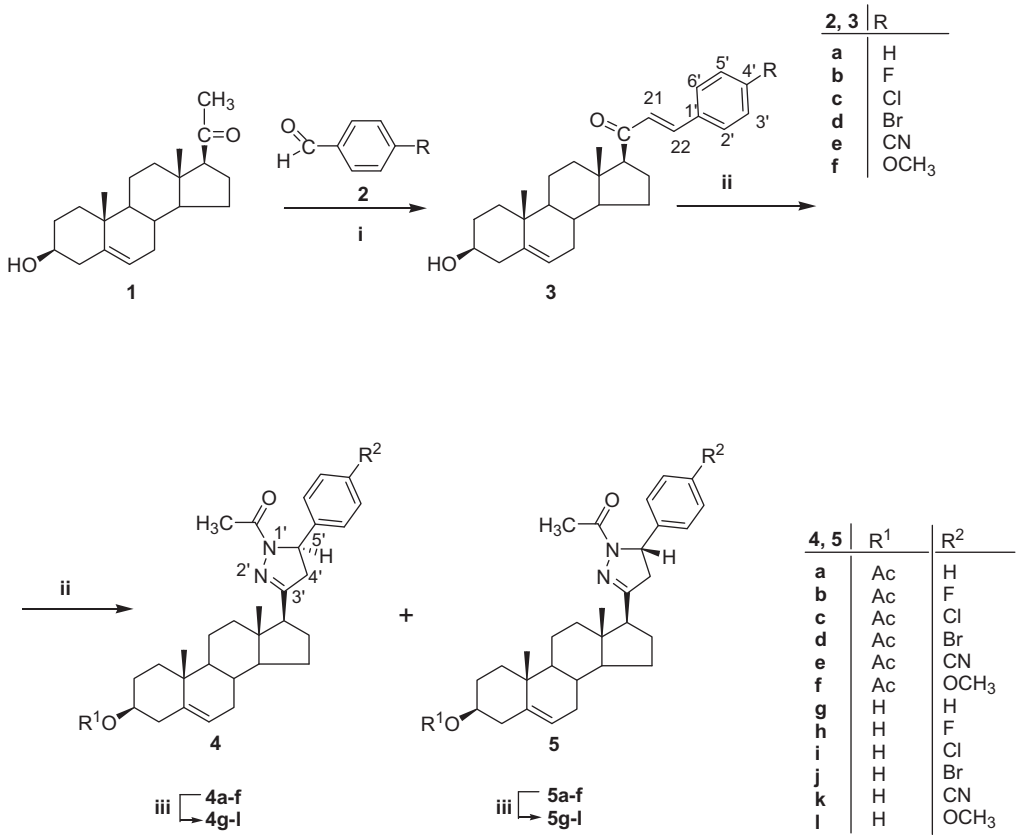
The benzylidene products (**3a**–**f**) were synthetized by the aldol condensation of pregnenolone (**1**) with various benzaldehyde derivatives (**2a**–**f**) (Scheme 1). After crystallization, the purity of the condensed products was tested with TLC. The reaction led to a single product, the starting material was completely consumed, and no further purification was needed. The condensation formed a new double bond at position 21. The ^{1}H NMR spectra of the crystallized products revealed that the reaction resulted only in the *E* isomers, as described by Catsoulacos and Stassinopoulou for (21*E*)-3β-acetoxy-21-benzylidenepregn-5-en-20-one [21]. It has also been reported that the aldol condensation of 3β,7α,11α-trihydroxypregn-5-en-20-one with benzaldehyde gives the *E* isomer as the sole product [22].

To obtain pyrazolinylandrostene derivatives and to study the stereochemistry of their formation, we reacted (21*E*)-3β-hydroxybenzylidenepregn-5-en-20-one (**3a**) with hydrazine hydrate in acetic acid. The consumption of the starting material was monitored by TLC. We found that the reaction took 2 h to complete under reflux, and the TLC indicated two products. The spots were distinguishable only when methyl *tert*-butyl ether was used as eluent. The more polar of these two spots was the more intense, and the ^{1}H NMR spectra of the crude mixtures revealed that the product ratio was 1:2. Two products appeared in the ring-closure reactions of all the other benzylidene derivatives (**3b**–**f**) and their ratio was independent of the substituent in the *para* position of the phenyl group. For easier separation, the product mixture was acetylated. For all of the substituted pyrazolines, the two products could be fully separated by column chromatography. After separation, 2D NMR demonstrated that the minor products were the 5′*R* epimers (**4a**–**f**), while the major products were the 5′*S* epimers (**5a**–**f**) of the corresponding phenylpyrazolinylandrostene derivatives. After separation of the acetylated pyrazoline products (**4a**–**f** and **5a**–**f**), they were hydrolyzed by the method of Zemplen to obtain the 3β-hydroxy derivatives (**4g**–**l** and **5g**–**l**), which are presumably more effective in the $C_{17,20}$-lyase inhibition [2,23,24] and in antiproliferative measurements [25]. Their NMR spectra were also taken, and it was proved that the pyrazoline ring with the *N*-acetyl group remained unchanged under the mild conditions of deacetylation.

The optical rotations of the pyrazolinyl compounds (**4a**–**l** and **5a**–**l**) in $CHCl_3$ presented a characteristic picture. The $[\alpha]_D^{20}$ values of **4a**–**l** were always positive (between +6.4 and +77), while those of **5a**–**l** were negative (between −85 and −32).

The structures of the newly synthetized steroids were determined by NMR spectroscopy. The ^{1}H NMR spectra of the steroidal benzylidenes (**3a**–**f**) contained two characteristic peaks at around 6.7 and 7.5 ppm, which can be assigned to the coupling protons of the double bond at position 21. Their coupling constant was 16 Hz, indicating their *trans* position relative to each other, and the geometry of the double bond is therefore *E*. One of these two peaks overlaps with the signals of the phenyl group (δ = 6.89–7.68 ppm) in the spectra of **3a**, **3b**, and **3f**. The 3-H multiplet is to be found at ∼3.5 ppm in the spectra of all the 3β-hydroxy products (**3a**–**f**, **4g**–**l** and **5g**–**l**). In the ^{13}C NMR spectra of **3a**–**f**, the signals of the phenyl group (δ = 114–164 ppm) and C21 (δ = 124–130) and C22 (δ = 139–141) are separate from the aliphatic region, and the C20 carbonyl group resonates at ∼200 ppm.

In the ^{1}H NMR spectra of the newly synthetized pyrazolines (**4a**–**f** and **5a**–**f**), the 22- and the 21-(sp^2-)H signals have disappeared and there are three new signals. Two of them are located between 2.59 and 3.42 ppm, while the third one overlaps with the 6-H signal at ∼5.7 ppm. These signals forming an AMX spin system can be assigned to the protons of the pyrazoline ring; their chemical shifts are in good agreement with those of other, non-steroidal 2-pyrazoline derivatives [26,27]. It can be concluded that the two protons at higher fields (δ = 2.59–3.37) are the 4′-CH_2 protons, and their chemical shifts in the two epimer series differ in a characteristic manner. One of these signals is situated at ∼2.61 in the spectra of the 5′*R* epimeric series, and at ∼2.72 in the 5′*S* series, while the other is at ∼3.37 in the 5′*R* series and ∼3.27 in the 5′*S* series. Other than that, there are very few differences in the spectra



Scheme 1. Reagents and conditions: (i) ethanol, NaOH, rt; (ii) acetic acid, hydrazine hydrate, reflux/pyridine, acetic anhydride, rt; (iii) methanol, $NaOCH_3$, rt.

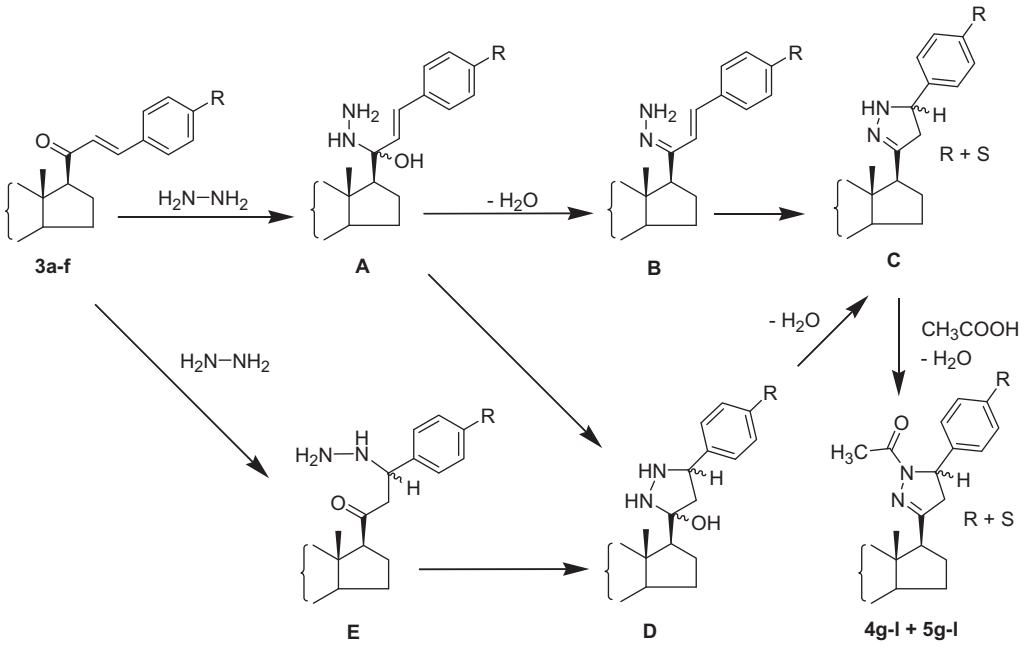
of the two series; minor changes can be observed in the aliphatic region. The third pyrazoline proton is located at ~5.7 ppm in both epimeric series. From the ^{1}H NMR spectra of **4a–f** and **5a–f**, it can be concluded that *N*-acetylation also takes place because of the presence of the glacial acetic acid. In the spectra of **4a–f** and **5a–f**, the singlet that appears at 2.3 ppm can be assigned to the methyl group of the *N*-acetyl function. Acetylation with acetic anhydride/pyridine resulted in a shift of the 3-H signal toward lower fields, ~4.5 ppm, and the 3-Ac-CH_3 resonated at around δ = 2.02. In the ^{13}C NMR spectra of compounds **4a–f** and **5a–f**, the characteristic signals of the pyrazoline ring were at ~46, 59 and 159 ppm, while the *N*-Ac-CH_3 resonated at δ = ~168.6 ppm, and the 3-Ac-CH_3 at ~170.5 ppm. The ^{1}H and ^{13}C NMR spectra of **4g–l** and **5g–l** presented a similar picture to that for **4a–f** and **5a–f**, except for the lack of the signals of the 3-Ac-CH_3 group.

Spectral data on the phenylpyrazolinylandrostene compound and its *p*-fluoro and *p*-methoxy derivatives have already been published [12]. A comparison of the spectra of the major products (**5g**, **5h** and **5l**) with those reported by Banday et al. reveals several differences. Our measurements indicated that H_a, and H_m in **5g** resonate at 2.75 and 3.27, while those in **5h** do so at 2.71 and 3.26, and those in **5l** at 2.74 and 3.24, respectively. In the literature data, three multiplets are described in that region for each compound: 2.65, 2.79 and 3.26 for the phenylpyrazolinylandrostene compound, and 2.76, 3.26 and 3.35 for its *p*-fluoro and 2.53–2.73, 3.20 and 3.29 for its *p*-methoxy derivative.

The ^{1}H NMR peaks of **4c** and **5c** were fully assigned with the aid of the H,H COSY and HSQC spectra. NOESY techniques were then used to determine the absolute configuration of C-5′. The NOESY spectra of the two isolated products are very similar, but do reveal some characteristic differences. The main difference is that the 12β-H signal in the spectrum of **4c** gives a cross-peak with one of the two 4′-CH_2 peaks, which can be found at lower fields. In the case of **5c**, 12β-H gives a cross-peak with the 4′-H signal which resonates at higher fields. It was previously established from the assignment that in both epimers the 4′-H that resonates at higher fields is on the same side of the pyrazoline ring as the phenyl group. By identifying the positions of the two 4′-H protons relative to the steroidal skeleton (cross-peak with 12β-H), the absolute configuration at the neighboring 5′ position was ascertained.

For the formation of the epimers **4g–l** and **5g–l**, three mechanistic pathways may be suggested (**Scheme 2**). According to a widely accepted interpretation (**A**–**B**–**C**) [18,19,21], the nucleophilic attack of the hydrazine nitrogen takes place on the carbonyl group of the enone, and intermediate **A** is formed containing a chiral carbon atom (C-20). In the case of the present molecules, the direction of the nucleophilic attack may be affected by the steric hindrance of the angular 18-CH_3, and one side of the carbonyl group therefore becomes more favorable. Then with the elimination of one molecule of water, a hydrazone **B** is formed, with the loss of the C-20 asymmetric center. Intramolecular Michael addition of the NH_2 group of **B** to the double bond leads to pyrazoline **C**. This step is probably not affected by the steric effect of the angular 18-CH_3, as it is several bonds away from it, and this should therefore result in an approximately 1:1 ratio of the epimers. The acetylation of **C** by the solvent leads to the formation of the *N*-acetyl products **4g–l** and **5g–l**.

Another possible mechanistic pathway (**A**–**D**–**C**) starts with the formation of **A**, but without the elimination of water a ring-closure step takes place next, furnishing a hydroxypyrazolidine intermediate **D**. The formation of a similar intermediate was reported in the reaction between β-dicarbonyls and hydrazine derivatives, which is isolable in some cases [28]. As the asymmetric center at C-20 is still present in the ring closure step, its configuration determines



Scheme 2. Presumed mechanisms for the formation of epimers **4g–l**, **5g–l.**

the configuration of the new chiral carbon atom (C-5′). As the two epimers of intermediate **A** may not be present in 1:1 ratio because of the steric effects mentioned above, the 5′*S* and 5′*R* epimers of **C** (and therefore **4g–l** and **5g–l**) may not form in equal quantities, which is in agreement with our experimental findings.

According to some statements in the literature [18], the Michael addition of the hydrazine derivate can be regarded as the first step, but this mechanism (**E**–**D**–**C**) needs special experimental conditions (addition of piperidine), which were not present in our reactions.

As concerns the three potential mechanisms, path **A**–**D**–**C** appears to be the most probable, as it can explain the unequal amounts of the two epimers (**4g–l**, **5g–l**).

3.2. $C_{17,20}$-lyase inhibition tests

The inhibitory effects of compounds **4g–l** and **5g–l** on rat testicular $C_{17,20}$-lyase were investigated with an *in vitro* radioincubation technique. At the applied concentration of 50 μM, compound **4g** and **5g** with the non-substituted phenyl group on the heterocycle decreased the enzyme activity by 72 ± 3% and 92 ± 4%, respectively. Enzyme incubations with the other test compounds resulted in conversions identical to those in the control experiments; hence these compounds did not inhibit rat $C_{17,20}$-lyase *in vitro* even in the relatively high concentration of 50 μM.

The attachment of pyrazolyl groups to a steroid skeleton at C-17 may produce potent inhibitors of $P450_{17\alpha}$ [2,3] and extension of the 17β heterocyclic side-chain with various substituted aromatic rings may improve the inhibitory effect [7]. Our tested steroidal phenylpyrazolines, however, did not exert efficient $C_{17,20}$-lyase inhibition. Nevertheless, the stereoselectivity of the inhibition could be demonstrated in our experiments too, as the phenyl-substituted 5′*R* isomer **4g** exhibited a higher inhibitory effect than that of the 5′*S* isomer counterpart **5g** in the rat testicular $C_{17,20}$-lyase test.

3.3. Antiproliferative measurements

Although the antiproliferative potencies of the currently reported compounds are not comparable with that of the reference compound cisplatin, many of them do exhibit pronounced activity *in vitro* (Table 1). Skin cancer cells (A431) seemed to be commonly less sensitive than cell lines of gynecological origin.

Since substantial differences were found in the *in vitro* results on the epimer pairs, the configuration at position 5′ has a crucial impact on the activities of the synthetized compounds. In general, the 5′*S* epimers were more potent than their 5′*R* counterparts, the difference proving most substantial in compounds **h** and **l**. **5h** and **5l** exhibited >90% inhibition of cancer cell growth in all four cell lines used, whereas the growth inhibition by **4h** did not exceed 50%, and **4l** was also less effective. The differences between the epimer pairs were not so pronounced in the case of analogs **g** and **k**,

Table 1
Antiproliferative effects of the synthetized compounds.

Product	μM	Growth inhibition% (±SEM)							
		HeLa		MCF7		A2780		A431	
4g	10	>20		22.0	(±1.1)	40.3	(±0.9)	>20	
	30	55.6	(±0.8)	75.3	(±0.5)	92.9	(±0.9)	>20	
4h	10	39.8	(±0.8)	23.3	(±1.6)	25.4	(±0.6)	27.6	(±0.6)
	30	41.5	(±0.5)	42.4	(±0.6)	45.2	(±0.5)	>20	
4i	10	21.3	(±2.1)	50.3	(±0.4)	69.3	(±1.9)	>20	
	30	98.0	(±0.2)	95.4	(±0.4)	96.1	(±1.0)	93.9	(±0.7)
4j	10	>20		47.2	(±2.1)	54.8	(±2.8)	>20	
	30	98.5	(±0.2)	95.4	(±0.5)	97.7	(±0.1)	94.1	(±0.5)
4k	10	27.1	(±1.7)	31.9	(±1.1)	53.0	(±1.2)	>20	
	30	31.9	(±2.0)	41.3	(±1.2)	54.5	(±2.7)	>20	
4l	10	21.4	(±2.7)	30.8	(±1.5)	46.6	(±1.1)	>20	
	30	48.9	(±1.5)	67.1	(±2.2)	81.4	(±1.0)	21.5	(±1.2)
5g	10	22.3	(±1.7)	54.1	(±1.6)	53.2	(±1.9)	>20	
	30	97.8	(±0.3)	96.2	(±0.2)	96.2	(±0.2)	91.9	(±0.4)
5h	10	>20		>20		38.7	(±1.4)	21.9	(±1.9)
	30	97.1	(±0.5)	95.6	(±0.5)	96.1	(±0.2)	91.8	(±1.0)
5i	10	>20		25.8	(±1.6)	52.3	(±1.1)	>20	
	30	97.8	(±0.2)	96.6	(±0.1)	96.0	(±0.2)	92.1	(±0.4)
5j	10	>20		37.9	(±2.7)	54.0	(±1.6)	>20	
	30	97.2	(±0.2)	96.1	(±0.3)	95.7	(±0.3)	93.1	(±0.2)
5k	10	20.7	(±1.6)	46.2	(±0.6)	60.4	(±0.7)	>20	
	30	31.48	(±2.3)	81.8	(±1.7)	89.2	(±0.4)	>20	
5l	10	>20		42.1	(±2.6)	61.5	(±2.0)	>20	
	30	97.7	(±0.2)	96.6	(±0.1)	96.0	(±0.2)	91.2	(±1.2
Cisplatin	10	42.6	(±2.3)	53.0	(±2.3)	83.6	(±1.2)	88.6	(±0.5)
	30	99.9	(±0.3)	86.9	(±1.3)	95.0	(±0.3)	90.2	(±1.8)

while the efficacies of compounds **i** and **j** seemed to be independent of the configuration at position 5′.

A large body of evidence demonstrates that *exo*-heterocyclic compounds with a steroidal skeleton possess valuable pharmacological properties, including antiproliferative activity [25,29–31]. The current results allow the conclusion that the configuration of the *exo* ring should be considered in the design of drugs and in the selection of agents for further development.

Acknowledgments

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