SYNTHESIS, X-RAY CRYSTAL STRUCTURE AND ANTIESTROGENIC ACTIVITY OF 17-METHYL-16,17-SECOESTRA-1,3,5(10)-TRIENE DERIVATIVES

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Starting from 3-(benzyloxy)-16-(hydroxyimino)-estra-1,3,5(10)-trien-17-one (**1**) several 17-methyl-16,17-secoestratriene derivatives were synthesized. In the first step of synthesis, hydroxyimino ketone **1** was transformed into 3-(benzyloxy)-16-(hydroxyimino)-17α-methyl-estra-1,3,5(10)-trien-17β-ol (**2**), the Beckmann fragmentation of which gave 3-(benzyloxy)-17-methyl-17-oxo-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**3a**). Reduction of **3a** with sodium borohydride yielded (17*S*)-3-(benzyloxy)-17-hydroxy-17-methyl-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**4a**), whose configuration at the newly formed chiral center was established by X-ray structural analysis. Catalytic hydrogenation of compound **3a** under different reaction conditions yielded 3-hydroxy-17-methyl-17-oxo-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**3b**) and 16-amino-17-methyl-16,17-secoestra-1,3,5(10)-triene-3,17-diol (**6b**). Biological tests in vivo of compounds **3b** and **6b** showed their moderate antiestrogenic activity.

Keywords: 17-Substituted 16,17-secosteroids; Steroids; Antiestrogenic activity; Aromatase inhibition; X-ray analysis; Oximes; Nitriles.

With the aim of preparing potential antiestrogens, we synthesized in our previous works[1–4] various 17-substituted 16,17-secoestratriene-16-nitrile derivatives. Some of these compounds showed a moderate antiestrogenic activity in biological in vivo studies[2,4]. Thus, 3-hydroxy-17-oxo-16,17-secoestra-1,3,5(10)-triene-16-nitrile exibited 16% of antiestrogenic activity and

corresponding 3,17-diol exibited 21% of antagonistic effect[2]. On the other hand, 17-chloro and 17-bromo derivatives of 16,17-secoestratriene-16-nitrile exibited stronger antiestrogenic effect (33 and 32%, respectively)[4]. In this work we continue our investigations of the influence of diverse functional groups in the positions C-16 and C-17 of 16,17-secoestratriene derivatives on biological response of the molecule. Accordingly, we synthesized 17-methyl-17-oxo-16,17-secoestra-1,3,5(10)-triene-16-nitrile as well as 17-hydroxy and 16-amino-17-hydroxy derivatives. Also, we wanted to examine the potential inhibitory activity of 16-cyano-17-methyl-16,17-secoestratriene derivative **3b** against aromatase, having in mind that in our previous work[5] the corresponding 3-oxo-4-ene-, as well as 3-oxo-1,4-diene- and 17-methyl-3-oxo-16,17-seco-1,4,6-triene-16-nitrile derivatives of androstane, showed a high degree of antiaromatase activity.

EXPERIMENTAL

Melting points were determined using a Büchi SMP 20 apparatus and are uncorrected. Infrared spectra (wavenumbers in cm^{-1}) were recorded in KBr pellets on a Nexus 670 SP-IR spectrometer. NMR spectra were taken on a Bruker AC 250E spectrometer operating at 250 MHz (^1H) and 62.5 MHz (^{13}C) with tetramethylsilane as the internal standard. Chemical shifts are given in ppm (δ-scale); coupling constants (*J*) are given in Hz. Mass spectra were recorded on a Finnigan MAT 8230 instrument, using chemical ionization (isobutane) or electron impact (70 eV) technique; the first number denotes the *m/z* value, the ion abundances are given in parentheses. All reagents used were of analytical grade.

3-(Benzyloxy)-16-(hydroxyimino)-17α-methylestra-1,3,5(10)-trien-17β-ol (**2**)

To methylmagnesium iodide, prepared from methyl iodide (4.8 ml, 77 mmol) and magnesium (1.25 g. 51.3 mmol) in absolute ether (80 ml), a solution of compound **1** (2.0 g, 5.13 mmol) in absolute tetrahydrofuran (80 ml) was added dropwise at room temperature during 1.5 h. The reaction mixture was stirred vigorously at the same temperature for another 2 h, then poured into water (500 ml) and left standing for 2–3 days, to allow the organic solvent to evaporate. The cake formed on the water surface was separated by filtration and washed with a small volume of EtOAc. After drying, the crude product was purified by flash chromatography (benzene–EtOAc, 1:1), affording pure compound **2** (1.45 g, 70%), whose recrystallization from methanol gave a product, m.p. 179–180 °C. IR: 3396, 3032, 2978, 1609, 1499, 1282, 1233, 1045, 901. ^1H NMR ($CDCl_3$): 0.91 s, 3 H (H-18); 1.42 s, 3 H (CH_3-C_{17}); 2.68 dd, 1 H, *J*(15a,15b) = 18.9, *J*(15a,14) = 7.3 (H-15a); 2.92 m, 2 H (H-6); 5.06 s, 2 H (OC**H**$_2$$C_6H_5$); 5.42 bs, 2 H (NOH and C_{17}-OH); 6.72 d, 1 H, *J*(2,4) = 2.7 (H-4); 6.80 dd, 1 H, *J*(1,2) = 8.5, *J*(2,4) = 2.7 (H-2); 7.25 d, 1 H, *J*(1,2) = 8.5 (H-1); 7.40 m, 5 H (C_6**H**$_5$$CH_2$). ^{13}C NMR ($CDCl_3$): 13.72 (C-18); 22.96 (CH_3-C_{17}); 25.88, 26.65, 27.56, 29.57, 30.84, 38.37, 43.73, 45.22, 46.23 (C-15); 69.84 (CH_2-Ph); 81.41 (C-17); 112.21, 114.78, 126.10, 127.35, 127.77, 128.45 (Ar CH); 132.40, 137.17, 137.69 (Ar C); 156.73 (C-3); 169.51 (C=NOH). MS: 406 (M^+ + 1, 100); 388 (M^+ + 1 – H_2O, 8). For $C_{26}H_{31}NO_3 \cdot CH_3OH$ (437.6) calculated: 74.14% C, 8.01% H, 3.20% N; found: 74.38% C, 7.91% H, 3.12% N.

3-(Benzyloxy)-17-methyl-17-oxo-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**3a**)

A solution of compound **2** (0.98 g, 2.42 mmol) and acetic anhydride (12 ml, 0.13 mol) in absolute pyridine (24 ml) was stirred at 100 °C for 2 h. The reaction mixture was then poured into water (100 ml) and its pH adjusted to 1 with 6 M HCl. The precipitate formed was separated by filtration and washed with water. Drying of the crude product gave 0.83 g (88.5%) of **3a**. Recrystallization from methanol afforded 0.76 g (81%) of white needle-like crystals, m.p. 128–129 °C. IR: 3030, 2909, 2238, 1693, 1604, 1500, 1286, 1027. ^{1}H NMR ($CDCl_3$): 1.33 s, 3 H (H-18); 2.23 s, 3 H (CH_3-C_{17}); 2.53 dd, 1 H, *J*(15a,15b) = 17.8, *J*(15a,14) = 5.3 (H-15a); 2.91 m, 2 H (H-6); 5.06 s, 2 H ($OC\mathbf{H}_2C_6H_5$); 6.76 d, 1 H, *J*(2,4) = 2.7 (H-4); 6.82 dd, 1 H, *J*(1,2) = 8.5, *J*(2,4) = 2.7 (H-2); 7.20 d, 1 H, *J*(1,2) = 8.5 (H-1); 7.30–7.48 m, 5 H ($C_6\mathbf{H}_5CH_2$). ^{13}C NMR ($CDCl_3$): 15.49 (C-18); 18.09 (C-15); 25.36 (CH_3-C_{17}); 25.44, 26.70, 29.74, 36.49, 39.15, 40.43, 42.43, 52.01 (C-13); 69.91 (CH_2Ph); 112.70, 114.49, 119.11 (CN); 126.27, 127.38, 127.84, 128.51, 131.29, 137.11, 137.49, 156.99 (C-3); 213.23 (C=O). MS: 444 (M^+ + i-Bu – 1, 100); 388 (M^+ + 1, 52). For $C_{26}H_{29}NO_2$ (387.5) calculated: 80.58% C, 7.54% H, 3.61% N; found: 80.50% C, 7.49% H, 4.28% N.

3-Hydroxy-17-methyl-17-oxo-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**3b**)

Compound **3a** (0.15 g, 0.39 mmol) was dissolved in a mixture of methanol and methylene chloride (1:1, 4 ml). To the solution was added 10% Pd/C (0.015 g) and the suspension stirred in a hydrogen atmosphere at room temperature for 12 h. The reaction mixture was then filtered, the precipitate washed with methylene chloride and the solvent removed by evaporation to dryness. The crude reaction product was purified by flash chromatography (benzene–EtOAc, 6:1), affording 0.97 g (83%) of compound **3b** in the form of white crystals, which after recrystallization from EtOAc had m.p.186–187 °C. IR: 3370, 3018, 2252, 1697, 1609, 1224, 1108, 866. ^{1}H NMR ($CDCl_3$): 1.33 s, 3 H (H-18); 2.23 s, 3 H (CH_3-C_{17}); 2.55 dd, 1 H, *J*(15a,15b) = 17.8, *J*(15a,14) = 5.3 (H-15a); 2.90 m, 2 H (H-6); 6.59 d, 1 H, *J*(2,4) = 2.7 (H-4); 6.67 dd, 1 H, *J*(1,2) = 8.5, *J*(2,4) = 2.7 (H-2); 7.13 d, 1 H, *J*(1,2) = 8.5 (H-1). ^{13}C NMR ($CDCl_3$): 15.51 (C-18); 18.10 (C-15); 25.40 (CH_3-C_{17}); 25.46, 26.66, 29.57, 36.49, 39.12, 40.35, 42.39, 52.04, 113.07, 115.10, 126.45 (Ar CH); 119.13 (CN); 130.88 (C-10); 137.65 (C-5); 153.87 (C-3); 213.60 (C=O). MS: 297 (M^+, 40); 279 ($M^+ - H_2O$, 6); 254 ($M^+ - CH_3CO$, 19); 43 (100). For $C_{19}H_{23}NO_2$ (297.4) calculated: 76.73% C, 7.79% H, 4.71% N; found: 77.38% C, 7.35% H, 5.19% N.

(17*S*)-3-(Benzyloxy)-17-hydroxy-17-methyl-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**4a**) and (17a*R*)-3-(Benzyloxy)-17a-methyl-17a-homo-17-oxaestra-1,3,5(10)-trien-16-one (**5a**)

To a solution of compound **3a** (0.22 g, 0.57 mmol) in methanol (12 ml) sodium borohydride (0.25 g, 6.61 mmol) was added portionwise at room temperature. The reaction mixture was stirred at boiling temperature for 1.5 h, then poured into water (50 ml) and extracted with methylene chloride (3 × 20 ml). The combined extracts were washed with water, dried and the solvent was removed. The remaining mixture of products was separated by flash chromatography (benzene–EtOAc, 6:1). This gave 0.141 g (64%) of compound **4a**, m.p. 113–114 °C and 0.021 g (9%) of compound **5a**, m.p. 177–179 °C.

Compound **4a**: IR: 3440, 3032, 2241, 1608, 1500, 1236, 1025. ^{1}H NMR ($CDCl_3$): 0.97 s, 3 H (H-18); 1.26 d, 3 H, *J* = 6.3 (CH_3-C_{17}); 2.46 dd, 1 H, *J*(15a,15b) = 17.8, *J*(15a,14) = 3.6 (H-15a); 2.66 dd, 1 H, *J*(15a,15b) = 17.8, *J*(15b,14) = 5.3 (H-15b); 2.91 m, 2 H (H-6); 3.80 q,

1 H, *J* = 6.3 (H-17); 5.06 s, 2 H (OC**H**$_2$C$_6$H$_5$); 6.76 d, 1 H, *J*(2,4) = 2.7 (H-4); 6.82 dd, 1 H, *J*(1,2) = 8.5, *J*(2,4) = 2.7 (H-2); 7.20 d, 1 H, *J*(1,2) = 8.5 (H-1); 7.30–7.48 m, 5 H (C$_6$**H**$_5$CH$_2$). ^{13}C NMR ($CDCl_3$): 14.92 (C-15); 16.99 (C-18); 17.76 (CH_3-C_{17}); 25.92, 27.09, 29.95, 31.23, 39.52, 40.39, 40.50 (C-13); 42.69, 69.88 (CH_2Ph); 72.41 (C-17); 112.57, 114.34, 126.31, 127.32, 127.79, 128.47 (Ar CH); 120.04 (CN); 132.12, 137.16, 137.57 (Ar C); 156.81 (C-3). For $C_{26}H_{31}NO_2$ (389.5) calculated: 80.17% C, 8.02% H, 3.60% N; found: 80.19% C, 8.28% H, 3.88% N.

Compound **5a**: IR: 1720, 1609, 1498, 1246, 1022. ^{1}H NMR ($CDCl_3$): 0.91 s, 3 H (H-18); 1.29 d, 2 H, *J* = 6.4 (CH_3-C_{17}); 2.90 m, 3 H (H-6, H-15a); 4.20 q, 1 H, *J* = 6.4 (H-17); 5.05 s, 2 H (OC**H**$_2$C$_6$H$_5$); 6.76 d, 1 H, *J*(2,4) = 2.7 (H-4); 6.82 dd, 1 H, *J*(1,2) = 8.5, *J*(2,4) = 2.7 (H-2); 7.20 d, 1 H, *J*(1,2) = 8.5 (H-1); 7.30–7.50 m, 5 H (C$_6$**H**$_5$CH$_2$). ^{13}C NMR ($CDCl_3$): 10.60, 14.80, 17.86, 25.36, 29.66, 31.59, 35.32, 36.55, 39.25, 42.52, 44.79, 69.92 (CH_2Ph); 86.24 (C-17); 112.59, 114.58, 126.13, 127.39, 127.86, 128.52 (Ar CH); 131.74, 137.13, 137.51 (Ar C); 156.94 (C-3); 170.90 (C=O). MS: 390 (M^+, 20); 297 (12); 256 (5); 91 (100).

(17*S*)-3,17-Dihydroxy-17-methyl-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**4b**)

From compound **4a** (0.14 g, 0.36 mmol) in the presence of 10% Pd/C (0.014 g) under the reaction conditions given for compound **3b**, crude **4b** (0.113 g) was obtained in the form of colorless oil. Its purification by flash chromatography (benzene–EtOAc, 4:1) gave 0.067 g (62%) of white crystals, m.p. 183 °C. IR: 3449, 2246, 1612, 1500, 1287, 1120, 928. ^{1}H NMR (acetone-d_6): 0.92 s, 3 H (H-18); 1.16 d, 3 H, *J*(17,1′) = 6.3 (CH_3-C_{17}); 2.58 dd, 1 H, *J*(15a,15b) = 17.8, *J*(15a,14) = 4.1 (H-15a); 2.75 dd, 1 H, *J*(15a,15b) = 17.8, *J*(15b,14) = 5.0 (H-15b); 2.83 m, 2 H (H-6); 3.73 m, 1 H (H-17); 3.88 d, 1 H, *J*(17,OH) = 4.9 (C_{17}-OH); 6.55 d, 1 H, *J*(2,4) = 2.7 (H-4); 6.61 dd, 1 H, *J*(1,2) = 8.5, *J*(2,4) = 2.7 (H-2); 7.11 d, 1 H, *J*(1,2) = 8.5 (H-1); 8.13 s, 1 H (C_3-OH). ^{13}C NMR (acetone-d_6): 15.25 (C-15); 17.35 (C-18); 17.87 (CH_3-C_{17}); 27.03, 27.94, 28.88, 32.22, 41.00, 41.18, 41.52, 43.62, 72.28 (C-17); 113.75, 115.58, 127.14 (Ar CH); 121.00 (CN); 131.55 (C-10); 138.10 (C-5); 156.00 (C-3). MS: 299 (M^+, 9); 284 (M^+ – CH_3, 5); 214 (20), 43 (100). For $C_{19}H_{25}NO_2 \cdot 0.5H_2O$ (308.4) calculated: 73.99% C, 8.50% H, 4.54% N; found: 73.59% C, 8.70% H, 5.05% N.

16-Amino-17-methyl-16,17-secoestra-1,3,5(10)-triene-3,17-diol (**6b**)

Compound **3a** (0.2 g, 0.52 mmol) was dissolved in a mixture of methanol and methylene chloride (1:1, 4 ml). To this solution 10% Pd/C (0.05 g) was added and the suspension was stirred in the hydrogen atmosphere at room temperature for 24 h. After that the reaction mixture was filtered, the precipitate washed with methylene chloride and the solvent removed. The resulting crude mixture was purified by flash chromatography (CH_2Cl_2–MeOH, 1:1), affording pure compound **6b** (0.14 g, 89%) in the form of white crystals, m.p. 265 °C. IR: 3431, 2940, 2861, 1585, 1286, 1252, 1126, 870. ^{1}H NMR (DMSO-d_6): 0.80 s, 3 H (H-18); 0.90 d, 3 H, *J*(17,1′) = 6.4 (CH_3-C_{17}); 1.82 s, 1 H (OH); 2.37 q, 1 H, *J*(17,1′) = 6.4 (H-17); 3.16 s, 2 H (NH); 6.42 d, 1 H, *J*(2,4) = 2.7 (H-4); 6.50 dd, 1 H, *J*(1,2) = 8.5, *J*(2,4) = 2.7 (H-2); 7.03 d, 1 H, *J*(1,2) = 8.5 (H-1). ^{13}C NMR (DMSO-d_6): 11.88 (C-18); 14.53 (CH_3-C_{17}); 23.44, 25.65, 29.46, 36.17, 42.89, 46.12 (C-16); 48.87, 61.49 (C-17); 112.79, 114.65, 125.89 (Ar CH); 130.53 (C-10); 137.07 (C-5); 154.97 (C-3). MS: 285 (M^+ – H_2O, 23); 270 (M^+ – H_2O – CH_3, 37); 58 (100). For $C_{19}H_{29}NO_2$ (303.4) calculated: 75.15% C, 9.63% H, 4.61% N; found: 75.56% C, 9.77% H, 4.95% N.

X-ray Crystal Studies of **4b** and **5a**

Diffraction data (Table I) for compounds **4b** and **5a** were collected on a CCD Bruker Smart Apex diffractometer at 173 and 110 K, respectively, using MoKα radiation (λ = 0.71073 Å). Both structures were solved by direct methods (SHELXS97)[6] and refined by full-matrix least-squares procedures (SHELXL97)[6]. Non-hydrogen atoms were refined anisotropically. All hydrogen atoms were identified on difference electron density maps and isotropically refined. Lorentz and polarization corrections were applied to the data. No absorption correction was made.

TABLE I
Crystallographic data, data collection and structure refinement for compounds **4b** and **5a**

Parameter	Compound **4b**	Compound **5a**
Formula	$C_{19}H_{25}NO_2$	$C_{26}H_{30}O_3$
M_w	299.4	390.5
Crystal size, mm^3	0.51 × 0.50 × 0.81	0.20 × 0.30 × 0.50
Crystal description	colorless prism	colorless prism
Crystal system, space group	orthorhombic, $P2_12_12_1$	triclinic, *P1*
a, Å; α, °	8.218(2); 90	6.852(2); 91.903(3)
b, Å; β, °	9.567(3); 90	8.744(2); 101.881(3)
c, Å; γ, °	21.383(6); 90	9.740(2); 112.525(3)
V, $Å^3$; *Z*	1681.0(8); 4	523.38(8); 1
D_c, g/cm^{-3}	1.183	1.24
F(000), μ(MoKα), mm^{-1}	647.9; 0.076	210.0; 0.079
θ range, °; data completeness, %	2.3–33.1; 100	2.2–33.0; 100
Range of *h*, *k*, *l*	–12/9, –14/14, –32/32	–10/10, –12/12, –14/14
No. of unique diffractions	5813	6909
No. of observed diffractions[a]	5199	6305
No. of parameters	311	382
R, *wR* for observed diffractions, %	3.5, 9.4	3.5, 9.4
R, *wR* for all data, %	4.0, 9.8	4.0, 9.7
GOF for all data	0.846	1.018
$(\Delta/\sigma)_{max}$	0.001	0.001
Residual electron density, e $Å^{-3}$	0.360, –0.161	0.359, –0.167

[a] Diffractions with $F_o > 4\sigma(F_o)$.

CCDC 244805 (for **4b**) and 244804 (for **5a**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge, CB2 1EZ, UK; fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk).

Biological Tests

Chemicals. Antiestradiol serum No. 244 was kindly supplied by Dr G. D. Niswender (Colorado State University, CO, U.S.A.); pregnant mares serum gonadotrophin (PMSG) was obtained from the Veterinary Institute Subotica (Serbia and Montenegro), [1,2,6,7-$^3H_4$]-estradiol from New England Nuclear (Belgium), NADPH and aminoglutethimide (AG) from Sigma (St Louis, MO, U.S.A.).

Uterotrophic assay. Immature Wistar strain female rats (21–23 days old) were randomly divided into groups of six to eight animals each. The animals were treated by subcutaneous injection once a day with 0.1 ml of a solution of the test compound in olive oil for 3 days, either solely or in combination with estradiol benzoate (EB). The control group obtained the vehicle only. The total administered amounts of tested compounds were 5 or 25 mg/kg body weight, whereas the EB dose was 0.03 mg/kg body weight. The animals were killed on the fourth day. The adhering fat was removed from uteri and blotted dry after expulsion of uterine fluid and the wet weight was recorded.

Per cent agonist and antagonist activity in immature rat uterine weight assays were calculated from the ratio of values recorded in treated and control animals thus

$$\% \text{ agonism} = (C - A) \times 100/(B - A)$$

and

$$\% \text{ antagonism} = (B - D) \times 100/(B - A),$$

where *A*, *B*, *C* and *D* are uterine wet weights, corrected for differences in body weight, i.e. (mg/100 g body weight) for the vehicle alone, EB, test compound alone, or test compound plus EB groups, respectively.

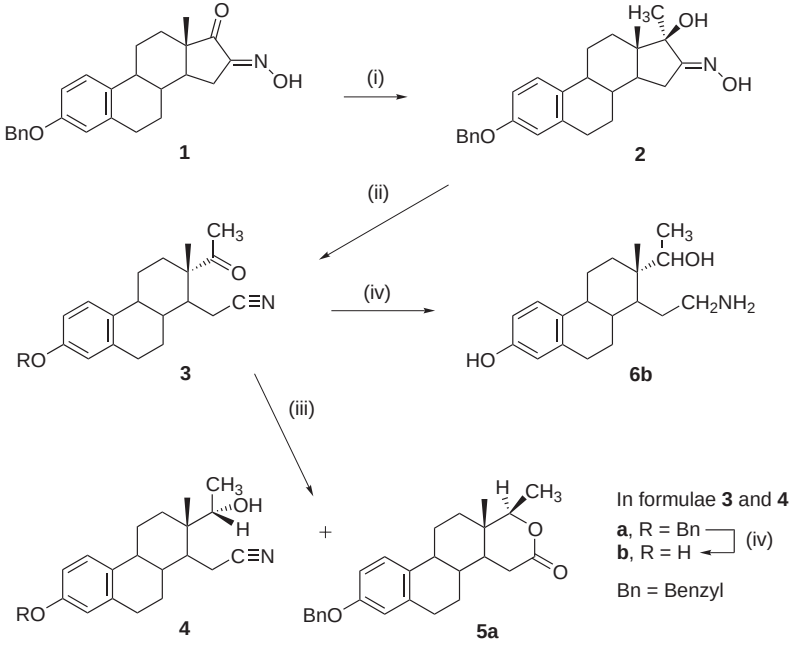
Animals, treatments and assays. Preparation of denucleated ovarian fraction from PMSG pretreated rats and determination of aromatase activity in ovarian homogenate was carried out as described previously[3,5].

For preliminary assessment of potential antiaromatase activity of compound **3b**, the compound was added in three concentrations (10, 20 and 50 μM) to the incubation mixture containing 500 nM of testosterone as a substrate. (saturated concentration; the estimated K_m for testosterone was 49 nM and V_{max} 5.76 pmol/min/mg protein).

RESULTS AND DISCUSSION

The starting compound, 16-(hydroxyimino) derivative of 3-(benzyloxy)-estrone (**1**; Scheme 1), was synthesized in two synthetic steps, starting from estrone[2]. Regio- and stereospecific addition of methylmagnesium iodide to

the C-17 carbonyl group of hydroxyimino ketone **1** yielded the corresponding 17β-hydroxy-17α-methyl derivative **2**, the fragmentation of which with acetic anhydride in pyridine gave 16,17-seco derivative **3a**.



(i) CH_3MgI, ether, THF, then NH_4Cl, H_2O, r.t.; (ii) Ac_2O, Py, 100 ^0C;
(iii) $NaBH_4$, MeOH, reflux; (iv) H_2, 10% Pd/C, MeOH, CH_2Cl_2, r.t.

SCHEME 1

Reduction of compound **3a** with sodium borohydride gave the hydroxy derivative **4a** as main reaction product (yield 64%). Configuration at the newly formed chiral center C-17 was established by X-ray analysis of compound **4b** (Fig. 1), which was obtained from **4a** after deprotection of the 3-hydroxy function, under conditions of catalytic hydrogenolysis. As a by-product of the reduction of compound **3a** with $NaBH_4$, lactone **5a** was isolated in a yield of 9%. The structure of compound **5a** was established on the basis of spectroscopic data and X-ray analysis (Fig. 1).

Reduction of compound **3a** under conditions of catalytic hydrogenation with 10% Pd/C as a catalyst (weight ratio **3a**:10% Pd/C 4:1) for 24 h, yielded 3,17-dihydroxy-16-amino derivative **6b**. When the ratio **3a**:10% Pd/C was 10:1 and the reaction time 12 h, only removal of the benzyl group took place, resulting in compound **3b**.

The estrogenic and antiestrogenic effects of compounds **3b**, **4b** and **6b** were tested on female rats using the uterotrophic and antiuterotrophic methods[8]. The differences in weights of uteri of treated and control animals served for the calculation of the agonistic and antagonistic effects[9] presented in Table II.

It is evident from Table II that compounds **3b** and **6b** caused identical decrease in estrogenic activity and a moderate antiestrogenic effect, but this activities is stronger that in earlier synthesized compounds (see introduction part of the paper).

Also, according to our results, **3b** expressed antiaromatase activity when applied in high concentrations (20 and 50 μM). However, this activity is much lower compared with our previous data obtained with androstane derivatives modified in ring D [5].

TABLE II
Agonistic and antagonistic effects of compounds **3b**, **4b** and **6b**

Compound	Dose mg/kg	*n*	Agonistic effect %, mean ± SEM	*n*	Antagonistic effect %, mean ± SEM
3b	5	6	–4.0 ± 1.31	8	37.6 ± 5.51
	25	7	12.3 ± 1.51	6	32.9 ± 0.95
4b	5	7	–	7	29.0 ± 4.41
6b	5	6	–9.7 ± 0.62	7	38.1 ± 1.58
	25	6	–1.5 ± 0.29	7	39.8 ± 1.16

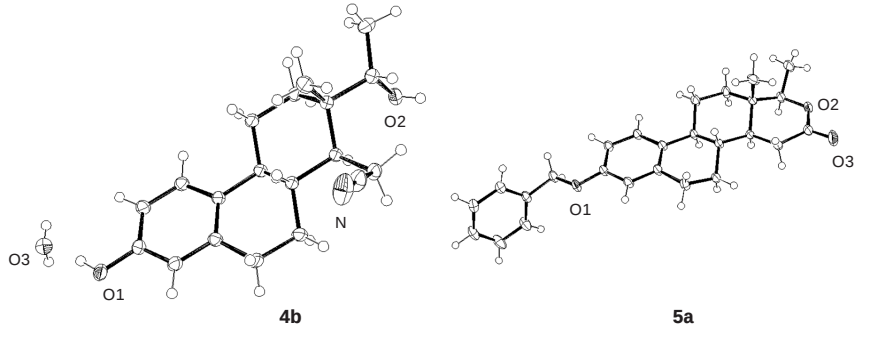


FIG. 1
Perspective view[7] of the molecular structure of compounds **4b** and **5a**

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