SYNTHESIS AND BIOLOGICAL EVALUATION OF 17-[4-(2-AMINOETHOXY)PHENYL]-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**), 3-(benzyloxy)-17-{4-[2-(dimethylamino)ethoxy]phenyl}-17-oxo-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**3a**) was synthesized. Reduction of **3a** with sodium borohydride yielded secocyano alcohol **4a**, as well as the secoamino alcohol **5a** when reduction was performed with sodium borohydride in the presence of cobalt(II) salt. Deprotection of the C-3 hydroxy group in compounds **3a**–**5a** by catalytic hydrogenolysis resulted in the corresponding 3-hydroxy derivatives **3b**–**5b**. Compounds **3b**–**5b** were tested on residual estrogenic and potential antiestrogenic activities.

Keywords: Steroids; 16,17-Secosteroids; Estra-1,3,5(10)-triene derivatives; Nitriles; Estrogenic activity; Antiestrogenic activity.

Antiestrogens are a group of compounds which block, completely or partly, the action of estrogens on estrogen target tissues. Tamoxifen (TAM; Fig. 1), a well-known nonsteroidal antiestrogen, first described by Harper and Walpole[1], has been employed in the course of three decades for the treatment of estrogen-receptor (ER)-positive tumors[2]. However, TAM is not only a pure antagonist of the human ER but also retains a weak agonistic activity for the same receptor. Because of that, breast cancer patients chronically treated with TAM often experience relapse of the cancer[3].

As shown in Fig. 1, TAM ({4-[2-(dimethylamino)ethoxy]phenyl}-1,2-diphenylbut-1-ene) bears the 4-[2-(dimethylamino)ethoxy]phenyl function. There are reports on the synthesis of estratrienes with a [(dimethylamino)-ethoxy]phenyl side chain in the 7α (RU 45144)[4] or 11β (RU 39411)[5] posi-

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tion. These compounds are potent antiestrogens, but they display, like TAM, partial estrogenic activity.

Previously we synthesized[6] 16-amino- and 16-cyano-17-methyl-16,17-seco-estratriene derivatives which showed moderate antiestrogenic activity.

The objective of this work was to synthesize a 16,17-secoestra-1,3,5(10)-triene derivative with 4-[2-(dimethylamino)ethoxy]phenyl group in the position 17, in order to investigate its antiestrogenic activity. Namely, when the molecular formulas of TAM and compound **3b** (Fig. 1) overlap so that the ring A of the steroid skeleton overlies one benzene ring of TAM, then 4-[2-(dimethylamino)ethoxy]phenyl grouping in both the molecules stretch in the same direction (Fig. 2). Hence, it seemed plausible to suppose that the molecules of similar spatial arrangement might also have similar biological activities.

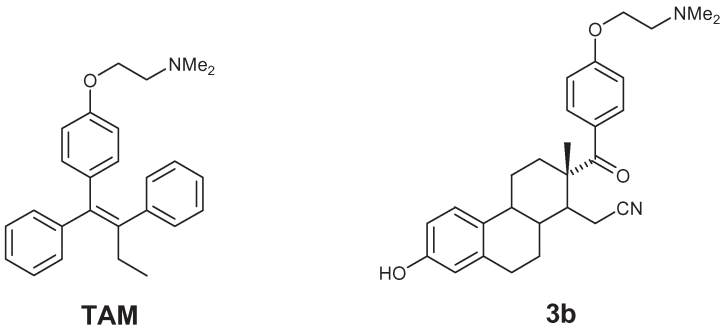


FIG. 1
Structures of TAM and synthesized compound **3b**

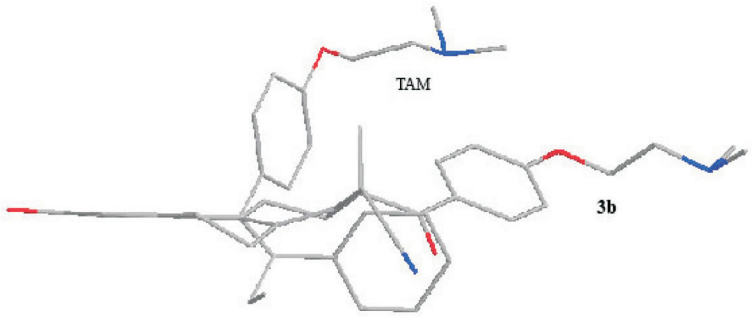


FIG. 2
The overlap of TAM and compound **3b** in the projection normal to the longitudinal axis of the molecule **3b** and C_{13}–C_{18} bond

Besides, we have also synthesized 17-{4-[2-(dimethylamino)ethoxy]phenyl}-17-hydroxy derivative **4b** as well as the corresponding 16-amino-17-{4-[2-(dimethylamino)ethoxy]phenyl}-17-hydroxy derivative **5b** and investigated their residual estrogenic and potential antiestrogenic activities.

EXPERIMENTAL

General Procedure

Melting points were determined using a Büchi SMP 20 apparatus and are uncorrected. IR spectra (in cm^{-1}) were recorded 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) and are reported in ppm downfield from a tetramethylsilane internal standard; 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 *m/z* value, and the ion abundances are given in parentheses. All the reagents used were of analytical grade. All solutions were dried over anhydrous Na_2SO_4.

3-(Benzyloxy)-17α-{4-[2-(dimethylamino)ethoxy]phenyl}-16-(hydroxyimino)estra-1,3,5(10)-triene-17β-ol (**2**)

To a freshly prepared suspension of {4-[2-(dimethylamino)ethoxy]phenyl}magnesium bromide in absolute tetrahydrofuran (40 ml), which was prepared from [2-(4-bromophenoxy)ethyl]-dimethylamine (14.64 g, 0.06 mol) and magnesium (1.44 g, 0.06 mol), a solution of compound **1** (1 g, 2.5 mmol) in absolute tetrahydrofuran (60 ml) was added at room temperature during 30 min. The reaction mixture was stirred at the same temperature for another hour and then quenched with 15% NH_4Cl (120 ml). Organic and water layers were separated, and the organic layer was evaporated. The residual oil was dissolved in CH_2Cl_2 (60 ml) and 2 M HCl (40 ml) was added. Two-phase mixture was stirred for 30 min. The precipitate formed was separated by filtration and treated with 2 M NaOH (40 ml). The suspension formed was extracted with CH_2Cl_2 (3 × 30 ml). The combined extracts were washed with water and saturated aqueous sodium chloride. After drying and removing the solvent, the crude product was purified by flash chromatography (CH_2Cl_2–MeOH, 1:1), affording compound **2** (0.8 g, 56%) as yellowish oil. IR: 3406 (17β-OH); 3212 (N–OH); 1608, 1504 (ArC–C); 1244 (C–O). ^1H NMR ($CDCl_3$): 1.00 s, 3 H (H-18); 2.37 s, 6 H ($(CH_3)_2N$); 2.78 t, 2 H, J = 6.2 (CH_2N); 4.13 t, 2 H, J = 6.2 (CH_2O); 5.05 s, 2 H (CH_2, Bn); 6.75–7.45 m, 12 H (Ar H). ^{13}C NMR ($CDCl_3$): 14.46 (C-18); 26.19 (CH_2); 27.63 (CH_2); 28.35 (CH_2); 29.78 (CH_2); 33.17 (CH_2); 38.45 (CH); 43.21 (CH); 44.06 (CH); 45.76 (2 CH_3); 47.76 (C-15); 58.11 (CH_2N); 65.54 (CH_2O); 69.98 (CH_2, Bn); 85.74 (C-17); 112.31, 113.29, 114.80, 126.24, 127.53, 127.93, 128.62, 129.26 (ArCH); 132.71, 135.51, 137.33, 137.85, 156.78, 157.83 (Ar C); 168.21 (C=NOH). MS: 554 (M^+, 2); 536 ($M^+ - H_2O$, 2); 463 (M^+ – Bn, 0.3); 58 [$(CH_3)_2N^+=CH_2$, 100]. For $C_{35}H_{42}N_2O_4 \cdot 2H_2O$ (591.1) calculated: 71.12% C, 7.90% H, 4.74% N; found: 71.54% C, 7.40% H, 5.37% N.

3-(Benzyloxy)-17-{4-[2-(dimethylamino)ethoxy]phenyl}-17-oxo-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**3a**)

To a solution of compound **2** (0.82 g, 1.48 mmol) in absolute pyridine (16 ml), tosyl chloride (2.8 g, 14.7 mmol) was added. The reaction mixture was allowed to stand at room temperature for 18 h, then poured into cold 2 M HCl (100 ml) and extracted with CH_2Cl_2 (2 × 30 ml). The combined extracts were washed with water, dried, and the solvent was removed. The residual oil was purified by flash chromatography (CH_2Cl_2–MeOH, 1:1). This gave 0.66 g (83.5%) of compound **3a** as amorphous solid. IR: 2243 (C≡N); 1656 (C=O); 1600, 1506 (ArC–C); 1237 (C–O); 1172. ^{1}H NMR ($CDCl_3$): 1.49 s, 3 H (H-18); 2.37 s, 6 H ($(CH_3)_2N$); 2.78 t, 2 H, *J* = 6.3 (CH_2N); 4.13 t, 2 H, *J* = 6.3 (CH_2O); 5.05 s, 2 H (CH_2, Bn); 6.78 d, 1 H, *J* = 2.5 (H-4); 6.84 dd, 1 H, *J*(2,1) = 8.5, *J*(2,4) = 2.5 (H-2); 6.93 d, 2 H, *J* = 7.8 (Ar H); 7.20 d, 1 H, *J* = 8.5 (H-1); 7.37–7.50 m, 5 H (Ar H, Bn); 7.80 d, 2 H, *J* = 7.8 (Ar H). ^{13}C NMR ($CDCl_3$): 17.15 (C-18); 18.48 (C-15); 25.72 (CH_2); 26.80 (CH_2); 29.79 (CH_2); 37.69 (CH_2); 39.41 (CH); 41.87 (CH); 42.35 (CH); 45.88 (2 CH_3); 52.15 (C-13); 58.07 (CH_2N); 66.14 (CH_2O); 69.92 (CH_2, Bn); 112.70, 113.99, 114.51, 126.30, 127.40, 127.85, 128.52, 130.70 (Ar CH); 119.39 (C≡N); 130.37, 131.40, 137.13, 137.56, 156.99, 161.49 (Ar C); 206.29 (C=O). MS: 536 (M^+, 5); 58 [$(CH_3)_2N^+=CH_2$, 100].

3-(Benzyloxy)-17-{4-[2-(dimethylamino)ethoxy]phenyl}-17-hydroxy-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**4a**)

To a stirred solution of compound **3a** (0.218 g, 0.4 mmol) in methanol (2 ml), sodium borohydride (0.038 g, 1 mmol) was added portionwise at room temperature. When the addition was complete, stirring at the same temperature was continued for one hour, then the content was poured into water (10 ml) and extracted with CH_2Cl_2 (3 × 10 ml). The combined extracts were washed with water, dried, and the solvent was removed. The residual mixture was separated by flash chromatography (CH_2Cl_2–MeOH, 1:1). This gave 0.132 g (60.3%) of compound **4a** as white amorphous solid. IR: 3441, 2241 (C≡N); 1608, 1504 (ArC–C); 1238 (C–O); 1034. ^{1}H NMR ($CDCl_3$): 0.92 s, 3 H (H-18); 2.32 s, 6 H ($(CH_3)_2N$); 2.72 t, 2 H, *J* = 6.2 (CH_2N); 4.03 t, 2 H, *J* = 6.3 (CH_2O); 4.60 s, 1 H (H-17); 5.04 s, 2 H (CH_2, Bn); 6.75 d, 1 H, *J* = 2.5 (H-4); 6.80 dd, 1 H, *J*(2,1) = 8.5, *J*(2,4) = 2.5 (H-2); 6.88 d, 2 H, *J* = 7.8 (Ar H); 7.17 d, 1 H, *J* = 8.5 (H-1); 7.23 d, 2 H, *J* = 7.8 (Ar H); 7.35–7.45 m, 5 H (Ar H, Bn). ^{13}C NMR ($CDCl_3$): 15.24 (C-15); 17.00 (C-18); 25.66 (CH_2); 27.07 (CH_2); 29.93 (CH_2); 31.86 (CH_2); 39.80 (CH); 40.79 (CH); 41.01 (C-13); 42.47 (CH); 45.63 (2 CH_3); 57.96 (CH_2N); 65.65 (CH_2O); 69.77 (CH_2, Bn); 78.78 (C-17); 112.43, 113.48, 114.24, 126.23, 127.28, 127.69, 128.38, 129.04 (Ar CH); 120.17 (C≡N); 132.18, 133.56, 137.10, 137.46, 156.68, 157.95 (Ar C).

3-(Benzyloxy)-17-{4-[2-(dimethylamino)ethoxy]phenyl}-17-hydroxy-16,17-secoestra-1,3,5(10)-triene-16-amine (**5a**)

To a solution of compound **4a** (0.3 g, 0.56 mmol) in methanol (6 ml), cobalt(II) chloride hexahydrate (0.27 g, 1.12 mmol) and sodium borohydride (0.242 g, 6.37 mmol) were added. The black suspension was stirred at reflux for 2 h. Water (20 ml) and 6 M HCl were poured into the reaction mixture, which was stirred till the black precipitate dissolved. The solution was extracted with ether (3 × 10 ml), the aqueous layer was made alkaline with concentrated ammonium hydroxide solution and then extracted with CH_2Cl_2 (3 × 10 ml). The combined CH_2Cl_2 extracts were washed with saturated aqueous sodium chloride solution, dried, and

evaporated. The residue was purified by flash chromatography (CH_2Cl_2–MeOH, 1:1), affording 0.10 g (33%) of compound **5a** in the form of white crystals, which, after recrystallization from methanol, had m.p. 138 °C. IR: 3443, 1609, 1505 (ArC–C); 1238 (C–O); 1030. ^{1}H NMR ($CDCl_3$): 0.93 s, 3 H (H-18); 2.37 s, 6 H (($CH_3)_2$N); 2.75 t, 2 H, *J* = 6.2 (CH_2N); 3.38 s, 1 H (H-17); 4.10 t, 2 H, *J* = 6.3 (CH_2O); 5.05 s, 2 H (CH_2, Bn); 6.78 m, 2 H (H-2 and H-4); 6.88 d, 2 H, *J* = 7.8 (Ar H); 7.20 d, 1 H, *J* = 8.5 (H-1); 7.32 d, 2 H, *J* = 7.8 (Ar H); 7.35–7.45 m, 5 H (Ar H, Bn). ^{13}C NMR ($CDCl_3$): 12.64 (C-18); 24.56 (CH_2); 25.81 (CH_2); 26.08 (CH_2); 30.04 (CH_2); 37.41 (CH_2); 37.82 (C-13); 38.82 (CH); 43.42 (CH); 45.82 (2 CH_3); 48.31 (C-16); 50.32 (CH); 58.25 (CH_2N); 65.80 (CH_2O); 69.80 (CH_2, Bn); 72.49 (C-17); 112.19, 113.32, 114.38, 126.03, 127.31, 127.70, 128.41, 129.61 (Ar CH); 133.21, 133.58, 137.23, 137.81, 156.60, 157.77 (Ar C). MS: 525 (M^+ + 1 – H_2O, 100), 524 (M^+ – H_2O, 28). For $C_{35}H_{46}N_2O_3$ (542.7) calculated: 77.45% C, 8.54% H, 5.16% N; found: 77.52% C, 8.83% H, 5.22% N.

Deprotecting C-3 Hydroxy Group in Compounds **3a**–**5a**. General Procedure

To an ethanolic solution (40 ml) of compound **3a**–**5a** (1 mmol), 10% Pd/C (10% relative to the steroid) was added. The suspension was stirred in hydrogen atmosphere at room temperature for 12 h. The reaction mixture was then filtered and the solvent evaporated to dryness. The crude reaction product was purified by column chromatography (silica gel, 0.04–0.062 mm; CH_2Cl_2–MeOH, 100:1 for compound **3b** or CH_2Cl_2–MeOH, 1:1 for compounds **4b** and **5b**).

17-{4-[2-(Dimethylamino)ethoxy]phenyl}-3-hydroxy-17-oxo-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**3b**): White amorphous solid. Yield 94%. IR: 3435 (OH); 2243 (C≡N); 1655 (C=O); 1601, 1506 (ArC–C); 1238 (C–O); 1 173. ^{1}H NMR ($CDCl_3$): 1.49 s, 3 H (H-18); 2.37 s, 6 H (($CH_3)_2$N); 2.78 t, 2 H, *J* = 6.3 (CH_2N); 4.13 t, 2 H, *J* = 6.3 (CH_2O); 6.59 d, 1 H, *J* = 2.6 (H-4); 6.65 dd, 1 H, *J*(2,1) = 8.5, *J*(2,4) = 2.6 (H-2); 6.92 d, 2 H, *J* = 7.8 (Ar H); 7.13 d, 1 H, *J* = 8.5 (H-1); 7.77 d, 2 H, *J* = 7.8 (Ar H). ^{13}C NMR ($CDCl_3$): 17.21 (C-18); 18.52 (C-15); 25.79 (CH_2); 26.79 (CH_2); 29.63 (CH_2); 37.73 (CH_2); 39.44 (CH); 41.86 (CH); 42.36 (CH); 45.86 (2 CH_3); 52.19 (C-13); 58.05 (CH_2N); 66.05 (CH_2O); 113.09, 114.02, 115.23, 126.49, 130.72 (Ar CH); 119.41 (C≡N); 130.43, 131.03, 137.80, 153.93, 161.45 (Ar C); 206.37 (C=O). MS: 446 (M^+, 6); 58 [$(CH_3)_2N^+$=CH_2, 100]. For $C_{28}H_{34}N_2O_3$·0.5H_2O (455.6) calculated: 73.82% C, 7.74% H, 6.15% N; found: 74.37% C, 7.49% H, 6.16% N.

17-{4-[2-(Dimethylamino)ethoxy]phenyl}-3,17-dihydroxy-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**4b**): Colorless oil. Yield 80%. IR: 3407, 2243 (C≡N); 1610, 1508 (ArC–C); 1238 (C–O); 1177. ^{1}H NMR ($CDCl_3$): 0.89 s, 3 H (H-18); 2.35 s, 6 H (($CH_3)_2$N); 2.75 m, 6 H (3 CH_2); 4.05 t, 2 H, *J* = 6.3 (CH_2O); 4.55 s, 1 H (H-17); 6.52 d, 1 H, *J* = 2.5 (H-4); 6.61 dd, 1 H, *J*(2,1) = 8.5, *J*(2,4) = 2.5 (H-2); 6.80 d, 2 H, *J* = 7.8 (Ar H); 7.06 d, 1 H, *J* = 8.5 (H-1); 7.21 d, 2 H, *J* = 7.8 (Ar H). ^{13}C NMR ($CDCl_3$): 15.32 (C-15); 17.10 (C-18); 25.78 (CH_2); 27.15 (CH_2); 29.85 (CH_2); 31.87 (CH_2); 39.87 (CH); 40.75 (CH); 41.14 (C-13); 42.52 (CH); 45.58 (2 CH_3); 57.96 (CH_2N); 65.47 (CH_2O); 78.98 (C-17); 113.30, 113.61, 115.28, 126.27, 129.05 (Ar CH); 120.21 (C≡N); 131.11, 133.35, 137.55, 154.42, 157.98 (Ar C). MS: 285 (M^+ – $C_{10}H_{14}NO$, 8); 256 (M^+ – $C_{11}H_{15}NO_2$, 3).

17-{4-[2-(Dimethylamino)ethoxy]phenyl}-3,17-dihydroxy-16,17-secoestra-1,3,5(10)-triene-16-amine (**5b**): White crystals, m.p. 199–201 °C. Yield 93%. IR: 3434, 1609, 1509 (ArC–C); 1242 (C–O). ^{1}H NMR ($CDCl_3$): 0.90 s, 3 H (H-18); 2.37 s, 6 H (($CH_3)_2$N); 2.80 m, 6 H (3 CH_2); 3.38 s, 1 H (H-17); 4.10 t, 2 H, *J* = 6.3 (CH_2O); 6.55 m, 2 H (H-2 and H-4); 6.80 d, 2 H, *J* = 7.8 (Ar H); 7.06 d, 1 H, *J* = 8.5 (H-1); 7.20 d, 2 H, *J* = 7.8 (Ar H). ^{13}C NMR ($CDCl_3$):

12.54 (C-18); 23.93 (CH_2); 25.80 (CH_2); 26.15 (CH_2); 29.89 (CH_2); 37.32 (CH_2); 37.83 (C-13); 38.82 (CH); 43.37 (CH); 45.65 (2 CH_3); 47.69 (C-16); 48.31 (C-16); 50.00 (CH); 58.08 (CH_2N); 65.55 (CH_2O); 71.99 (C-17); 113.09, 113.56, 115.29, 126.09, 129.73 (Ar CH); 131.87, 137.83, 154.38, 157.85 (Ar C). MS: 452 (M^+, 42); 451 ($M^+ - 1$, 39). For $C_{28}H_{40}N_2O_3 \cdot H_2O$ (470.7) calculated: 71.45% C, 9.00% H, 5.95% N; found: 71.28% C, 9.12% H, 6.28% N.

Biological Tests

All experiments approved by the local ethical committee of the University of Novi Sad and were conducted in accordance with the principles and procedures of the NIH Guide for Care and Use of Laboratory Animals.

Uterotrophic and antiuterotrophic assays. Immature Wistar strain female rats (21–23 days old) were randomly divided into groups with six to eight animals each. Animals were treated by subcutaneous injection once a day for three consecutive days with 0.1 ml of a solution of the test compound in olive oil, either alone or in combination with estradiol 3-benzoate (EB). The control group obtained the vehicle only. The total administered amount of compounds **4b** and **5b** were 25 mg/kg of body weight (b.w.), while in the case of compound **3b** and TAM (used for comparison) these amounts were 5 and 25 mg/kg b.w., respectively, whereas the EB dose was 0.03 mg/kg b.w. The animals were killed 24 h after the last injection. The adhering fat was removed from the uteri and blotted dry after expulsion of uterine fluid and the wet weight was recorded.

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

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

and

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

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

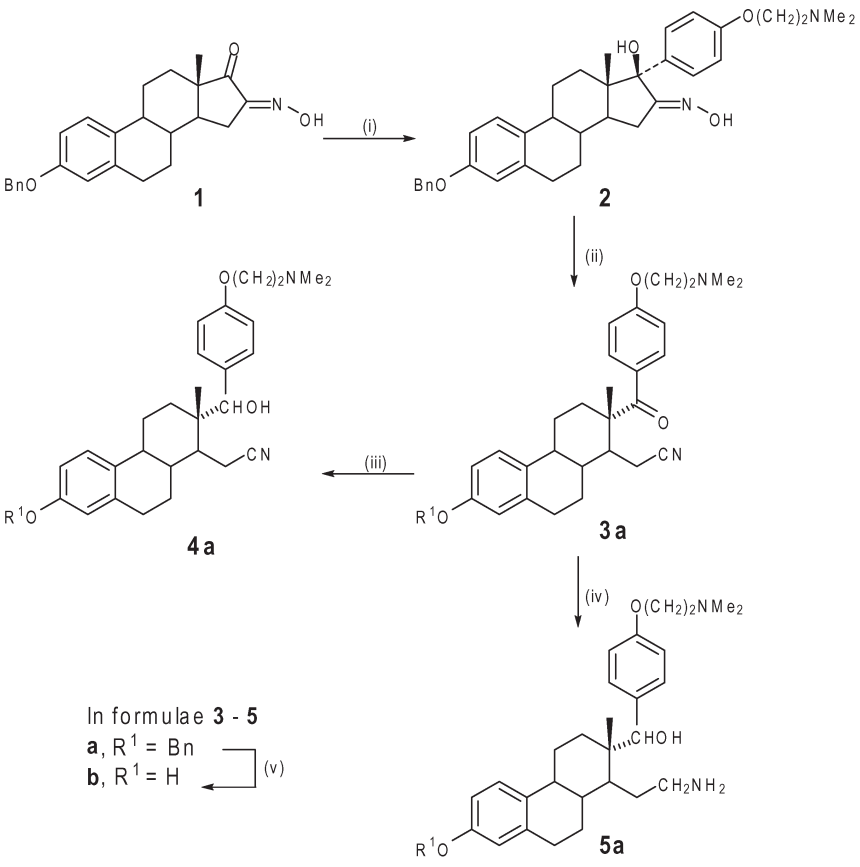
RESULTS AND DISCUSSION

The starting compound, 16-(hydroxyimino) derivative of 3-(benzyloxy)-estrone (**1**; Scheme 1), was synthesized in two steps, starting from estrone[7]. Regio- and stereospecific addition of Grignard reagent derived from [2-(4-bromophenoxy)ethyl]dimethylamine[8] to the C-17 carbonyl group of hydroxyimino ketone **1** yielded the corresponding 17β-hydroxy derivative **2**, the Beckmann fragmentation of which with TsCl in pyridine gave 16-cyano-17-oxo-16,17-seco derivative **3a**.

Reduction of compound **3a** with $NaBH_4$ gave the secocyano alcohol **4a**. The action of $NaBH_4$ on compound **3a** in the presence of cobalt(II) chloride, caused not only the reduction of the keto group but also of the cyano

function, affording amino alcohol **5a**. Deprotection of the 3-hydroxy function in compounds **3a**–**5a** was performed by hydrogenolysis at low hydrogen pressure and in the presence of 10% Pd/C as catalyst, whereby the corresponding 3-hydroxy derivatives **3b**–**5b** were obtained.

The estrogenic and antiestrogenic effects of compounds **3b**–**5b** were tested on female rats using the uterotrophic and antiuterotrophic methods[9]. The differences in weights of the uteri of treated and control animals served for the calculation of the agonistic and antagonistic effects[10] presented in Table I.



SCHEME 1

(i) {4-[2-(dimethylamino)ethoxy]phenyl}magnesium bromide, THF, r.t., 1.5 h, then 15% NH_4Cl; (ii) TsCl, Py, r.t., 18 h; (iii) $NaBH_4$, MeOH, r.t., 1 h; (iv) $NaBH_4$, $CoCl_2$, MeOH, reflux, 2 h, then 6 M HCl, H_2O; (v) H_2, 10% Pd/C, EtOH, r.t., 12 h

TABLE I
Agonistic and antagonistic effects of tested compounds and TAM

Compound	Dose, mg/kg b.w.	Agonistic effect, %	Antagonistic effect, %
3b	5	–	20.1
	25	–	12.4
4b	25	34.3	6.7
5b	25	–2.3	19.2
TAM	5	39.8	62.4

As can be seen from the table, compound **3b** in a lower dose, exhibited a certain antagonistic effect (20.1%), which was lower compared with TAM (62.8%). With increasing dose, this effect decreased and at a dose of 25 mg/kg b.w. it was only 12.4%. Applying the same dose (25 mg/kg b.w.) of compounds **3b**–**5b**, the highest antagonistic effect was shown by the amino alcohol **5b** (19.2%). Compound **5b** lost completely its estrogenic activity, and even showed a mild synergistic action (agonistic effect –2.3%).

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