SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME A,D-RING MODIFIED 16,17-SECOANDROSTANE DERIVATIVES

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Starting from 3β-hydroxy-17-oxo-16,17-secoandrost-5-ene-16-nitrile (**1**), the new 16,17-secoandrostane derivatives **2**–**11** were synthesized. Protection of the 17-oxo function of compound **1** with ethylene glycol yielded compounds **2** and **3**. The Oppenauer oxidation of **2** or oxidation with H_2O_2 in alkaline conditions gave the respective compounds **4** and **10.** Epoxidation of compound **4** yielded a mixture of 4α,5α- and 4β,5β-epoxides **5** and **6** and a mixture of 4α,5α- and 4β,5β-epoxy-carboxamides **7** and **8**. Opening of the oxirane ring of a mixture of compounds **5** and **6** with formic acid afforded the 4-hydroxy derivative **9**. Anti-aromatase activity and in vitro cytotoxicity for three tumor cell lines (human breast adenocarcinoma ER+, MCF-7 as well as human breast adenocarcinoma ER-, MDA-MB-231, and prostate cancer, PC3) of selected compounds were evaluated. Compounds **2**, **4**, **9**, and **10** showed a strong cytotoxicity for PC3 cells.

Keywords: 16,17-Secosteroids; 4-En-3-one steroids; Androstane derivatives; Anti-aromatase activity; In vitro cytotoxicity.

The enzyme responsible for converting androgens to estrogens is P-450 aromatase. Aromatase is expressed primarily in gonads such as ovaries, whereas human aromatase was detected in some other peripheral tissues including adipose tissue[1], bone[2], muscle[1], skin[3], aorta[4], and placenta[5]. On the other hand, it is also well known that pathological tissues such as breast[6] and prostate[7] cancer express increased amounts of intratumoral aromatase. Inhibition of aromatase has been an important strategy for breast cancer treatment in postmenopausal women. Three generations of aromatase inhibitors have been developed, including first-generation

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aminoglutethimide, second-generation fadrozole and formestane, and third-generation letrozole, anastrozole and exemestane[8].

Our previous works[9,10] concerning the inhibitory character of some androst-5-ene derivatives showed that the conjugated 4-en-3-one system as well as the 16,17-seco fragment in the steroid molecule caused a significant inhibition of the aromatase enzyme. Bearing in mind that the formestane (4-hydroxyandrost-4-ene-3,17-dione) with its 4-hydroxy-4-en-3-one system is a strong aromatase inhibitor[8], the aim of this work was to synthesize some 16,17-secoaldehydes with the 4-en-3-one and 4-hydroxy-4-en-3-one systems, test their anti-aromatase activity, and compare it with that of the corresponding 16,17-seco-17-methyl derivatives[10]. The cytotoxic activity of the synthesized compounds against MCF-7, MDA-MB-231 and PC3 cell lines was also examined.

EXPERIMENTAL

General Procedure

Melting points were determined using a Büchi SMP 20 apparatus and are uncorrected. IR spectra were recorded on a NEXUS 670 SP-IR spectrometer (wavenumbers in cm^{-1}). 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 (δ-scale) downfield from the 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 the *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.

13-(1,3-Dioxolan-2-yl)-3β-hydroxy-16,17-seco-17-norandrost-5-ene-16-nitrile (**2**)

Compound **1** (0.50 g, 1.67 mmol) was dissolved in ethylene glycol (12 ml) and 4-toluenesulfonic acid (0.03 g, 0.14 mmol) was added. The reaction mixture was kept at 37–40 °C for 40 min, and then poured into water (50 ml), and $NaHCO_3$ was added to adjust pH 8. The separated precipitate of crude compound **2** was collected, recrystallized from dichloromethane–hexane, affording the pure compound **2** (0.43 g, 75%; m.p. 151 °C). IR: 3439 (OH); 2242 (C≡N); 1634 (C=C); 1469, 1224 (C–O); 1103, 1067, 1029 (C–O from $O(CH_2)_2O$); 985, 950. ^1H NMR ($CDCl_3$): 1.01 s, 3 H (H-19); 1.04 s, 3 H (H-18); 2.69 m, 2 H (H-15); 3.53 m, 1 H (H-3); 3.80–3.99 m, 4 H ($O(CH_2)_2O$); 4.51 s, 1 H (H-17); 5.36 d, 1 H, J = 5.2 (H-6). ^{13}C NMR ($CDCl_3$): 13.84 (C-18); 16.31 (CH_2); 19.22 (C-19); 19.55 (CH_2); 31.46 (CH_2); 31.78 (CH_2); 32.68 (CH); 33.42 (CH_2); 36.73 (Cq); 36.90 (CH_2); 40.19 (Cq); 41.90 (CH_2); 43.51 (CH); 49.09 (CH); 64.52 and 65.31 ($O(CH_2)_2O$); 71.56 (C-3); 110.09 (C-17); 120.06 (C≡N); 120.53 (C-6); 140.42 (C-5). MS: 345 (2, M^+); 91 (4); 73 (100). For $C_{21}H_{31}NO_3 \cdot 0.25H_2O$ (350.0) calculated: 72.07% C, 8.93% H, 4.00% N; found: 72.24% C, 9.19% H, 3.81% N.

13-(1,3-Dioxolan-2-yl)-3β-(2-hydroxyethoxy)-16,17-seco-17-norandrost-5-ene-16-nitrile (**3**)

Compound **1** (3.41 g, 11.3 mmol) was dissolved in ethylene glycol (80 ml) and 4-toluenesulfonic acid (0.18 g, 0.965 mmol) was added. The reaction mixture was stirred at 45–50 °C for 1 h, and at 75–80 °C for another 1 h, then poured into water (50 ml) and pH was adjusted to 8 with $NaHCO_3$. The crude precipitate was purified by column chromatography (silica gel, 0.04–0.063 mm; petroleum ether–ethyl acetate 1:1) affording the pure compound **3** (1.14 g, 29%; m.p. 150 °C after recrystallization from dichloromethane–hexane) and compound **2** in a yield of 13% (0.51 g; m.p. 151 °C). IR: 3477 (OH); 2243 (C≡N); 1635 (C=C); 1468, 1219 (C–O); 1151, 1066, 1028 (C–O from $O(CH_2)_2O$); 984, 950. ^{1}H NMR ($CDCl_3$): 1.01 s, 3 H (H-19); 1.04 s, 3 H (H-18); 2.68 m, 2 H (H-15); 3.21 m, 1 H (H-3); 3.59 m, 2 H and 3.72 m, 2 H (OCH_2CH_2OH); 3.80–4.03 m, 4 H ($O(CH_2)_2O$); 4.51 s, 1 H (H-17); 5.35 d, 1 H, J = 5.0 (H-6). ^{13}C NMR ($CDCl_3$): 13.84 (C-18); 16.32 (CH_2); 19.19 (C-19); 19.53 (CH_2); 29.69 (Cq); 31.81 (CH_2); 32.70 (CH); 33.42 (CH_2); 36.82 (CH_2); 37.08 (Cq); 38.72 (CH_2); 40.18 (CH_2); 43.51 (CH); 49.13 (CH); 62.10 and 68.97 (OCH_2CH_2OH); 64.53 and 65.31 ($O(CH_2)_2O$); 79.20 (C-3); 110.08 (C-17); 120.08 (C≡N); 120.58 (C-6); 140.37 (C-5). MS: 348 (64); 286 (69); 255 (58); 99 (100). For $C_{23}H_{35}NO_4 \cdot 0.5H_2O$ (398.5) calculated: 69.32% C, 8.85% H, 3.51% N; found: 68.98% C, 8.89% H, 3.35% N.

13-(1,3-Dioxolan-2-yl)-3-oxo-16,17-seco-17-norandrost-4-ene-16-nitrile (**4**)

Compound **2** (0.13 g, 0.37 mmol), aluminum isopropoxide (0.12 g, 0.59 mmol) and cyclohexanone (2.5 ml) were refluxed at constant stirring for 8.5 h. When the reaction was complete, cyclohexanone was removed by steam distillation. The reaction mixture was then extracted with dichloromethane (4 × 10 ml). The combined extracts were dried, the solvent was removed, and the crude product purified by column chromatography (silica gel, 0.063–0.2 mm; toluene–ethyl acetate 4:1). The obtained compound **4** (0.06 g, 45%) was recrystallized from dichloromethane–hexane, affording white crystals (m.p. 142–144 °C). IR: 2945, 2242 (C≡N); 1670 (C=O); 1615 (C=C); 1468, 1273, 1231, 1189, 1130, 1093, 1072, 1032 (C–O from $O(CH_2)_2O$). ^{1}H NMR ($CDCl_3$): 1.04 s, 3 H (H-19); 1.20 s, 3 H (H-18); 2.85 m, 2 H (H-15); 3.80–3.99 m, 4 H ($O(CH_2)_2O$); 4.51 s, 1 H (H-17); 5.76 s, 1 H (H-4). ^{13}C NMR ($CDCl_3$): 13.88 (C-18); 16.27 (CH_2); 17.54 (C-19); 19.70 (CH_2); 31.22 (CH_2); 32.46 (CH_2); 33.64 (CH_2); 33.82 (CH_2); 35.48 (CH_2); 36.21 (CH); 38.59 (Cq); 40.14 (Cq); 42.41 (CH); 52.66 (CH); 64.53 and 65.31 ($O(CH_2)_2O$); 109.82 (C-17); 119.82 (C≡N); 123.78 (C-4); 169.83 (C-5); 199.24 (C=O). MS: 343 (2, M^+); 303 (11); 73 (100). For $C_{21}H_{29}NO_3 \cdot 0.5H_2O$ (352.5) calculated: 71.56% C, 8.29% H, 3.97% N; found: 71.12% C, 8.51% H, 3.96% N.

13-(1,3-Dioxolan-2-yl)-4α,5α- and 4β,5β-epoxy-3-oxo-16,17-seco-17-norandrostane-16-nitrile (**5**) and (**6**) and 13-(1,3-Dioxolan-2-yl)-4α,5α- and 4β,5β-epoxy-3-oxo-16,17-seco-17-norandrostan-16-amide (**7**) and (**8**)

Aqueous 30% H_2O_2 (3.0 ml, 27.6 mmol) and 4 M NaOH (1.5 ml) were added to a solution of compound **4** (0.405 g, 1.17 mmol) in methanol (16 ml) and the mixture was stirred at 0 °C for 1 h and then at 10 °C for 23 h in the dark. The reaction mixture was poured into water (30 ml) and extracted with ethyl acetate (5 × 20 ml). The combined extracts were dried and the solvent was evaporated. The solid product was separated by column chromatography (silica gel, 0.04–0.063 mm; toluene–ethyl acetate 15:1), giving a mixture of compounds **5** and **6** (0.108 g, 25.5%; the ratio **5**:**6** was 1:11). The pure compound **6** was obtained after re-

peated column chromatography (silica gel, 0.04–0.063 mm) as colorless oil. Further elution with ethyl acetate gave a mixture of **7** and **8** (0.094 g, 21%; the ratio **7**:**8** was 1:5). The pure compound **8** was obtained after repeated column chromatography (silica gel, 0.04–0.063 mm) as a colorless oil.

Compound **6**: IR: 2945, 2241 (C≡N); 1708 (C=O); 1454, 1268, 1134, 1100, 1037 (C–O from $O(CH_2)_2O$); 987. ^{1}H NMR ($CDCl_3$): 1.05 s, 3 H (H-18); 1.19 s, 3 H (H-19); 2.70 m, 2 H (H-15); 3.01 s, 1 H (H-4α); 3.90 m, 4 H ($O(CH_2)_2O$); 4.50 s, 1 H (H-17). ^{13}C NMR ($CDCl_3$): 14.04 (C-18); 16.36 (CH_2); 18.86 (C-19); 20.17 (CH_2); 25.87 (CH_2); 29.38 (CH_2); 29.80 (CH_2); 32.49 (CH_2); 33.56 (CH_2); 35.70 (CH); 37.14 (Cq); 40.30 (Cq); 42.41 (CH); 45.50 (CH); 62.47 (CH); 64.58 and 65.33 ($O(CH_2)_2O$); 69.77 (C-5); 109.73 (C-17); 119.80 (C≡N); 206.33 (C=O).

Compound **8**: IR: 3353 (NH_2); 2945, 2877, 1704 (C=O); 1674 ($CONH_2$); 1454, 1403, 1323, 1250 (C–O); 1101, 1037 (C–O from $O(CH_2)_2O$); 980. ^{1}H NMR ($CDCl_3$): 0.91 s, 3 H (H-18); 1.12 s, 3 H (H-19); 2.79 d, 2 H, J = 14.04 (H-15); 2.97 s, 1 H (H-4α); 3.92 m, 4 H ($O(CH_2)_2O$); 4.70 s, 1 H (H-17); 5.65 bs, 1 H ($CONH_2$); 6.13 bs, 1 H ($CONH_2$). ^{13}C NMR ($CDCl_3$): 14.40 (C-18); 18.87 (C-19); 20.19 (CH_2); 25.86 (CH_2); 29.52 (CH_2); 29.67 (CH_2); 31.38 (CH_2); 32.54 (CH_2); 36.87 (CH_2); 37.11 (Cq); 38.46 (CH); 39.95 (Cq); 44.01 (CH); 45.69 (CH); 62.51 (C-4); 64.81 and 65.42 ($O(CH_2)_2O$); 70.03 (C-5); 109.33 (C-17); 176.65 ($CONH_2$); 206.48 (C=O).

4-Hydroxy-3,17-dioxo-16,17-secoandrost-4-ene-16-nitrile (**9**)

A mixture of compounds **5** and **6** (0.341 g, 0.95 mmol) was refluxed in formic acid (7.5 ml) for 105 min. The reaction mixture was then cooled, poured into water (20 ml) and a 5% solution of $NaHCO_3$ was added to pH 8. Extraction with ethyl acetate (4 × 20 ml) gave the crude product as a brown oil. The pure compound **9** was obtained after silica gel column chromatography (0.063–0.2 mm; toluene–ethyl acetate 11:1) in a yield of 16% (47.5 mg) as a yellow oil. IR: 3419 (OH); 2244 (C≡N); 1723 (HC=O); 1668 (C=O); 1642 (C=C); 1386, 1169 (C–O). ^{1}H NMR ($CDCl_3$): 1.22 s, 3 H (H-19); 1.26 s, 3 H (H-18); 3.10 m, 2 H (H-15); 6.15 bs, 1 H (OH); 9.37 s, 1 H (H-17). ^{13}C NMR ($CDCl_3$): 13.41 (C-19); 17.30 (C-18); 17.67 (CH_2); 18.96 (CH_2); 22.61 (CH_2); 30.05 (CH_2); 31.67 (CH_2); 32.95 (CH_2); 33.04 (CH_2); 34.65 (CH); 37.81 (Cq); 40.21 (CH); 49.84 (Cq); 52.81 (CH); 118.50 (C≡N); 137.82 (C-5); 141.30 (C-4); 193.36 (C-3); 204.77 (C-17).

13-(1,3-Dioxolan-2-yl)-3β-hydroxy-16,17-seco-17-norandrost-5-en-16-amide (**10**)

Compound **2** (0.075 g, 0.22 mmol) was dissolved in methanol and 4 M NaOH (0.4 ml) and 30% H_2O_2 (0.8 ml, 7.36 mmol) were added. The reaction mixture was stirred at room temperature for 24 h. The reaction mixture was poured into water (5 ml) and the crude product was filtered off. Recrystallization from methanol–dichloromethane afforded the pure compound **10** (0.0584 g, 74%) as white crystals (m.p. 220–224 °C). IR: 3434 (OH); 1658 ($CONH_2$); 1619 (C=C); 1214 (C–O); 1134, 1103, 1065 (C–O from $O(CH_2)_2O$). ^{1}H NMR ($CDCl_3$): 0.91 s, 3 H (H-18); 0.99 s, 3 H (H-19); 2.78 d, 2 H, J = 14.05 (H-15); 3.53 m, 1 H (H-3); 3.92 m, 4 H ($O(CH_2)_2O$); 4.69 s, 1 H (H-17); 5.24 bs, 1 H ($CONH_2$); 5.36 s, 1 H (H-6); 6.13 bs, 1 H ($CONH_2$). ^{13}C NMR ($CDCl_3$): 14.18 (C-18); 19.30 (C-19); 19.55 (CH_2); 20.69 (CH_2); 31.37 (CH_2); 31.49 (CH_2); 35.62 (CH); 36.99 (CH_2); 37.16 (CH_2); 39.85 (Cq); 41.99 (CH_2); 44.73 (CH); 49.46 (CH); 54.29 (Cq); 64.73 and 65.37 ($O(CH_2)_2O$); 71.68 (C-3); 109.65 (C-17); 121.45 (C-6); 139.96 (C-5); 176.80 ($CONH_2$).

Biological Tests

All experiments were 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.

Anti-Aromatase Activity

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 supplied by the Veterinary Institute Subotica (Serbia). [1,2,6,7-^{3}H(N)] Estradiol was obtained from New England Nuclear (Belgium). NADPH and testosterone were from Sigma (St. Louis, MO, U.S.A.). All other chemicals were of analytical reagent grade.

Animals (female rats), treatment, and assays. Preparation of denucleated ovarian fraction from PMSG-pretreated rats and the determination of aromatase activity in ovarian homogenate was carried out as described previously[10].

To measure aromatase activity of the synthesized compounds **4**, **9**, **11**, **12**, and formestane, purified denucleated fraction of ovaries from PMSG-pretreated female rats was incubated in the environment with subsaturated (50 nM) concentration of substrate testosterone and NADPH (1 mM) and absence (control) or presence of tested compounds (1 μM) or formestane (1 μM). Estradiol level was determined by RIA.

Cytotoxic Activity

Cell lines. Three human tumor cell lines and one human non-tumor cell line were used in the study: human breast adenocarcinoma ER+, MCF-7, human breast adenocarcinoma ER-, MDA-MB-231, prostate cancer PC3, and normal fetal lung fibroblasts, MRC-5.

The cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 4.5% of glucose. Media were supplemented with 10% of fetal calf serum (FCS, NIVNS) and antibiotics: 100 IU/ml of penicillin and 100 mg/ml of streptomycin (ICN, Galenika). All cell lines were cultured in flasks (Costar, 25 cm^2) at 37 °C in the 100% humidity atmosphere and 5% of CO_2. Only viable cells were used in the assay. Viability was determined by dye exclusion assay with Trypan Blue.

Cytotoxicity assay. Cytotoxicity was evaluated by colorimetric Sulforhodamine B (SRB) assay according to Skehan et al.[11]. Briefly, single cell suspension was plated into 96-well microtitre plates (Costar, flat bottom): 5×10^3 cells (MCF-7, MDA-MB-231, PC3, MRC-5) per 180 ml of medium. Plates were pre-incubated for 24 h at 37 °C and in 5% of CO_2. Tested substances at concentrations ranging from 10^{-8} to 10^{-4} M were added to all wells except for the controls. After the incubation (48 h/37 °C/5% CO_2) SRB assay was carried out as follows: 50 μl of 80% trichloroacetic acid (TCA) were added to all wells; 1 h later the plates were washed with distilled water, and 75 μl of 0.4% SRB were added to all wells; 30 min later the plates were washed with citric acid (1%) and dried at room temperature. Finally, 200 μl of 10 mM Tris (pH 10.5) were added to all wells. Absorbance (A) was measured on the microplate reader (Multiscan MCC340, Labsystems) at 540/690 nm. The wells without cells, containing complete medium only, served as blank.

Cytotoxicity was calculated according to the formula:

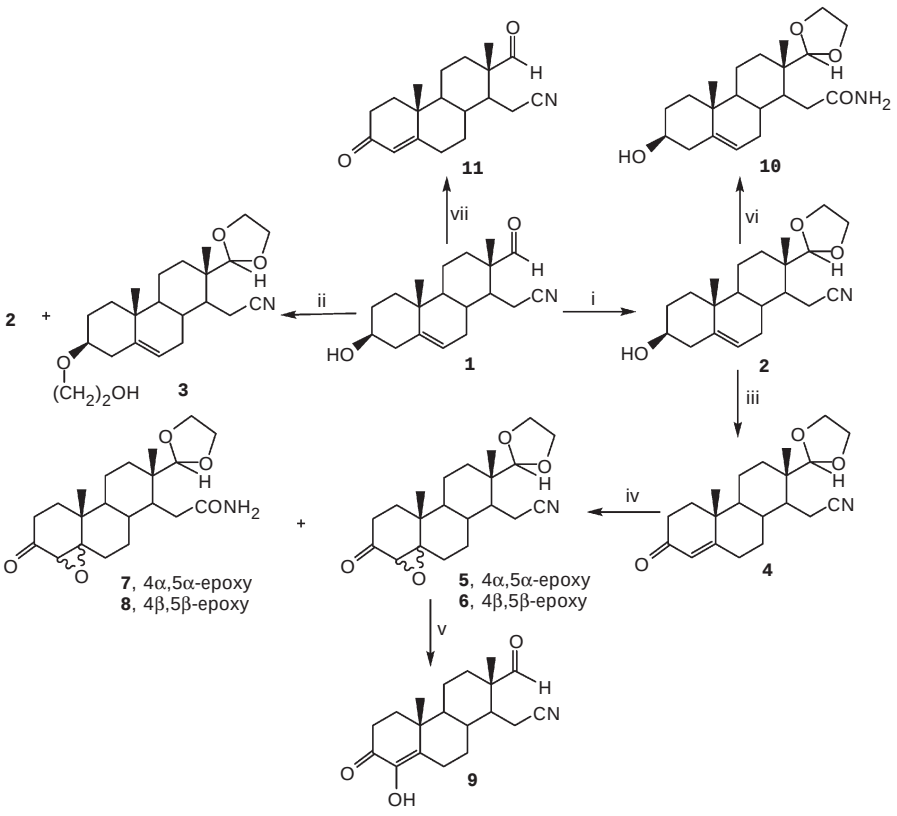
$$(1 - A_{\text{test}} / A_{\text{control}}) \times 100$$

and expressed as a percentage of cytotoxicity (CI, %).

Data analysis. Two independent experiments were performed in quadruplicate for each concentration of the compound. IC_{50} value defines the dose of compound that inhibits cell growth by 50%. The IC_{50} of compounds was determined by median effect analysis.

RESULTS AND DISCUSSION

Compound **1**, 3β-hydroxy-17-oxo-16,17-secoandrost-5-ene-16-nitrile, the starting compound for the synthesis of the corresponding D-seco derivatives **2**–**11**, was synthesized earlier[12]. The synthesis of compounds **2**–**11** is outlined in Scheme 1.



SCHEME 1

(i) $(CH_2)_2(OH)_2$, TsOH, 37–40 °C, 40 min; (ii) ethylene glycol, TsOH, 45–50 °C, 1 h → 75–80 °C, 1 h; (iii) cyclohexanone, Al(*i*-PrO)$_3$, reflux, 8.5 h; (iv) 30% H_2O_2, 4 M NaOH, MeOH, 0 °C, 1 h → 10 °C, 23 h; (v) HCOOH, reflux, 105 min; (vi) 30% H_2O_2, 4 M NaOH, MeOH, r.t., 24 h; (vii) cyclohexanone, Al(*t*-BuO)$_3$, xylene, reflux, 1 h

Compound **1** reacted with ethylene glycol on the treatment of the reaction mixture with 4-toluenesulfonic acid at 37–40 °C, affording 17-(ethylenedioxy) derivative **2**, or a mixture of compounds **3** and **2** when the reaction mixture was kept at 45–50 °C for 1 h and then at 75–80 °C for 1 h. The Oppenauer oxidation of compound **2** with cyclohexanone gave the 3-oxo-4-ene derivative **4** which was reacted with 30% H_2O_2 in alkaline medium to yield epoxides **5** and **6**. After repeated column chromatography, only 4β,5β-epoxide **6** was obtained as a pure compound. In the same reaction mixture, besides compounds **5** and **6**, a mixture of 4,5-epoxy-16-amides **7** and **8** was obtained. After repeated column chromatography, only the 4β,5β-epoxide **8** was obtained as a pure compound. Treatment of the mixture of 4α,5α- and 4β,5β-epoxides **5** and **6** with formic acid at reflux for 105 min afforded 4-hydroxy-3,17-dioxo-16,17-secoandrost-4-ene-16-nitrile (**9**). The 16-amide **10** was obtained from **2** with 30% H_2O_2 under alkaline conditions at room temperature for 24 h. The Oppenauer oxidation of compound **1** with cyclohexanone and Al(*t*-BuO)$_3$ afforded 3-oxo-4-ene derivative **11**, synthesized in our previous work[13].

Compounds **4** and **9** were tested for the anti-aromatase activity. To study the effect of the 4-hydroxy group with this type of D-seco compounds, we compared the inhibitory activity of these compounds with that of compound **11**, as well as with formestane as a reference compound (Fig. 1).

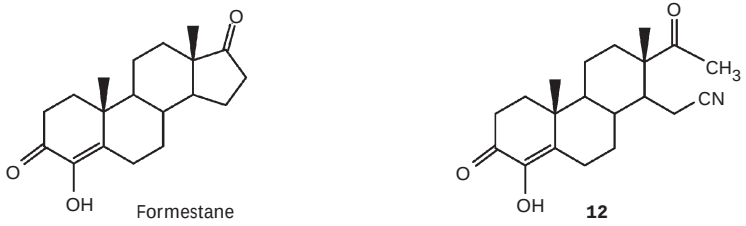


FIG. 1
Structure of formestane and compound **12** [14]

As can be seen from Table I (results are presented as percentage of inhibition versus control), compounds **4**, **9**, and **11** at a concentration of 1 μM and subsaturated concentration of testosterone showed a significantly lower inhibition against aromatase compared with formestane. The percentage of aromatase inhibition is identical for compounds **4** and **11** (18.6%), and significantly higher in the case of compound **9** (27.6%). This indicates that the introduction of the 1,3-dioxolane ring does not influence

the inhibitory activity and the 4-hydroxy group exerts a certain positive effect. In our previous work[14] we synthesized compound **12** (Fig. 1), with an acetyl group at the C-13 instead of the formyl group (compound **9**). The percentage of aromatase inhibition for this compound of 45.6% shows that the methyl group at the C-17 position of the 16,17-seco system contributes to the increase in the inhibitory activity.

TABLE I
Inhibitory effects of androst-4-ene derivatives **4**, **9**, **11** and **12**, and formestane on the aromatase activity in the denucleated fraction of ovaries from PMSG-pretreated rats

Compound (1μM)	Aromatase activity[a] (of inhibitors vs control), %
4	18.6 ± 9.7
9	27.6 ± 8.5
11	18.6 ± 3.6*
12	45.6 ± 2.3**
Formestane	99.2 ± 2.0**

[a] Numbers represent mean ± SEM of 10–20 replicates. Significance: *$p < 0.05$, **$p < 0.005$ vs control (Mann–Whitney non-parametric test).

TABLE II
In vitro cytotoxicity of 16,17-secoandrostane derivatives **2**, **4**, **6** and **8**–**10**, and formestane

Compound	IC_{50}, μM			
	MCF-7	MDA-MB-231	PC3	MRC-5
2	>100	56.0	6.3	>100
4	39.3	>100	2.2	>100
6	>100	>100	>100	>100
8	>100	>100	>100	>100
9	>100	>100	5.6	>100
10	33.7	>100	5.8	>100
Formestane	>100	55.5	48.4	>100

The cytotoxic activity of synthesized compounds **2**, **4**, **6**, and **8**–**10** against MCF-7, MDA-MB-231, PC3, and MRC-5 was evaluated. In vitro cytotoxicity was evaluated after 48-h cell treatment by the SRB assay[11]. Formestane served as the reference compound. The results are presented in Table II.

The results show that compounds **2, 4, 9,** and **10** exhibited a strong cytotoxic activity against PC3 prostate carcinoma cells, the corresponding IC_{50} values being in the range from 2.2 to 6.3 μM. Compound **2** exhibited the lowest activity. Similar activity was also observed with compound **10**, possessing the 16-amide instead of the 16-nitrile function. On the other hand, the presence of the conjugated 4-en-3-one system (compound **4**) increased cytotoxicity. The loss of the conjugation by introducing the 4β,5β-epoxide function leads to the loss of cytotoxicity (compounds **6** and **8**). Compound **9** is selective against the PC3 cells. A satisfactory cytotoxicity against MCF-7 was shown only by compounds **10** and **4**, and against MDA-MB-231 only by compound **2**.

In conclusion, it can be said that the synthesized 16,17-seco derivatives of the androstane series with the 16-nitrile or 16-amide groups in the 4-en-3-one or 3β-hydroxy-5-ene systems of the A ring (compounds **2**, **4**, **9**, and **10**) represent potent cytotoxic agents against the PC3 cell line.

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