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SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF SOME A- AND B-MODIFIED D-HOMO LACTONE ANDROSTANE DERIVATIVES

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*An efficient synthesis of several A- and B-modified D-homo lactone androstane derivatives from 3β-hydroxy-17-oxa-D-homoandrost-5-en-16-one (**1**) is reported. 17-Oxa-D-homoandrost-4-ene-3,16-dione (**2**), obtained by the Oppenauer oxidation of compound **1**, was converted via the unstable intermediate 3,16-dioxo-4,17-dioxa-D-homoandrostane-5α-carboxaldehyde (**3**) to 17-oxa-D-homo-3,5-seco-4-norandrostan-5-one-3-carboxylic acid (**4**), which was also obtained directly from compound **2**. Compound **1** was acetylated to give 17-oxa-D-homoandrost-5-en-16-on-3β-yl acetate (**5**) which was then oxidized with chromium(VI)-oxide in 50% acetic acid or with meta-chlorperbenzoic acid and chromium(VI)-oxide to yield compounds **6**-**8** and 5α-hydroxy-17-oxa-D-homoandrostane-6,16-dion-3β-yl acetate (**9**), respectively. The oximination of compound **9** gave a mixture of 6(E)-hydroximino-5α-hydroxy-17-oxa-D-homoandrostan-16-on-3β-yl acetate (**10**) and 6(Z)-hydroximino-5α-hydroxy-17-oxa-D-homoandrostan-16-on-3β-yl acetate (**11**), the hydrolysis of which gave 6(E)-hydroximino-3β,5α-dihydroxy-17-oxa-D-homoandrostan-16-one (**12**) and 6(Z)-hydroximino-3β,5α-dihydroxy-17-oxa-D-homoandrostan-16-one (**13**). 6-Nitrile-17-oxa-5,6-seco-D-homoandrostane-5,16-dion-3β-yl acetate (**14**) was obtained under the Beckmann fragmentation of compounds **10** and **11**. Only pure and stable compounds (**1**, **2**, **4**, **5**, **9** and **14**) were tested in vitro on six malignant cell lines (MCF-7, MDA-MB-231, PC-3, HeLa, HT-29, K562) and one non-tumor MRC-5 cell line. Significant antiproliferative activity against MDA-MB-231 cells showed compounds **1**, **5** and **9**, while compound **2** exhibited a strong antiproliferative activity. Only compound **14** showed weak antiproliferative activity against MCF-7 cells. All tested compounds were not toxic on MRC-5 cells, whereas Doxorubicin was highly toxic on these cells.*

KEY WORDS: androstane derivatives, D-homo lactones, A- and B-seco derivatives, antiproliferative activity

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INTRODUCTION

Steroids are an important class of compounds which have been reported to exhibit useful biological activity such as anticancer, antibacterial and antiandrogenic activities (1-4). The introduction of heteroatoms or the replacement of one or more carbon atoms in the steroidal molecule by a heteroatom impacts the physiological properties of these compounds and often result in alterations of their biological activities. The steroidal nucleus with a lactone function may cause extensive changes in the biological activity (5-8), as for example D-homo lactones of androstane series express antiproliferative and antiaromatase activities (9-12). Testolactone is moderately specific first generation inhibitor of human aromatase activity and thereby may contribute to the prevention of hormone dependent tumors such as breast cancer (13), prostatic hyperplasia and prostate cancer (14).

In our previous works (15-17) we have synthesized a number of D-homo lactone androstane derivatives with antitumor activity and in the present study we synthesized some A- and B-seco D-homo androstane derivatives and determined their antiproliferative activity *in vitro* against six different human cancer cell lines: PC-3 (AR- prostate cancer), MCF-7 (ER+ breast adenocarcinoma), MDA-MB-231 (ER- breast adenocarcinoma), HeLa (cervical cancer), HT-29 (colon cancer) and K562 (chronic myelogenous leukemia) and non-cancerous cell line (MRC-5).

EXPERIMENTAL

Reagents and instruments

The following reagents were used for the synthesis: aluminium *tert*-butoxide (Sigma-Aldrich, Switzerland) *meta*-chlorperbenzoic acid (MCPBA; Sigma-Aldrich, Germany), chromium(VI)-oxide (Kemika, Croatia), benzyltriethylammonium chloride (BTEAC; Merck, Germany), hydroxylamine-hydrochloride (Sigma-Aldrich, Germany). All reagents used were of analytical grade. Other chemicals and solvents used were of the highest analytical grade, obtained from Lach-Ner (Czech Republic).

Melting points were determined using an Electrothermal 9100 apparatus and are uncorrected. Infrared spectra (wave numbers in cm^{-1}) were recorded in KBr pellets (for crystals) on a NEXUS 670 SP-IR spectrometer. NMR spectra were recorded using a Bruker AC 250E spectrometer operating at 250 MHz (proton) and 62.5 MHz (carbon) with tetramethylsilane as the internal standard. Chemical shifts are given in ppm (δ-scale). High resolution mass spectra (HRMS) were recorded on a 6210 Time-of-Flight LC/MS Agilent Technologies (ESI+) instrument. Chromatographic separations were performed on silica gel columns (Kieselgel 60, 0.063-0.20 mm; Merck, Germany). Antiproliferative activity was determined using a microplate reader (Multiscan MCC340, Labsystems).

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Synthesis of A- and B-modified D-homo lactone

3,16-Dioxo-4,17-dioxa-D-homoandrostane-5α-carboxaldehyde (3). 17-Oxa-D-homoandrost-4-ene-3,16-dione (**2**, 0.266 g, 0.88 mmol) was dissolved in dichloromethane (26 ml) at 0°C and MCPBA (0.947 g, 5.50 mmol) was added. The reaction mixture was stirred at 0°C for 3 h, followed by stirring at 10°C for 20 h. Then, the reaction mixture was cooled to 0°C, and a new amount of MCPBA (0.312 g, 1.81 mmol) and dichloromethane (9 ml) was added. The reaction mixture was stirred at 0°C for 3 h, followed by stirring at 10°C for 46 h. When the reaction was completed, water (40 ml) was added, then separated from the dichloromethane. This solution was washed successively with aqueous 10% $Na_2S_2O_3$ (20 ml), then with 5% $NaHCO_3$ (20 ml), and brine (20 ml), dried, and evaporated. The crude product (0.687 g) was purified by column chromatography (21 g silica gel, hexane-ethyl acetate 1:3), giving pure compound **3** (0.078 g, 26.6%) in the form of oil. IR ν_{max} (film) cm^{-1}: 2944, 1731, 1466, 1404, 1382, 1241, 1190, 1108, 1079, 1040. ^1H NMR ($CDCl_3$): 1.12 (3H, s, H-18); 1.20 (3H, s, H-19); 2.61 (1H, m, H-2a); 2.73 (1H, m, H-2b); 2.75 (2H, m, H-15); 3.90 (1H, d, J = 10.8 Hz, H-17a); 4.00 (1H,d, J = 10.9 Hz, H-17b); 9.94 (1H, s, CHO). ^{13}C NMR ($CDCl_3$): 14.67 (C-18); 14.95; 15.04; 19.62 (C-19); 19.71; 23.70; 26.73; 27.01; 32.23; 32.37; 35.41; 38.81; 43.92; 46.89; 50.14; 80.69 (C-5); 80.70 (C-17); 170.32 (C_{16}=O); 178.71 (C_3=O); 199.93 (CHO).

17-Oxa-D-homo-3,5-seco-4-norandrostan-5-one-3-carboxylic acid (4). Compound **3** (0.3 g, 0.99 mmol) was dissolved in dichloromethane (30 ml) at 0°C and MCPBA (1.064 g, 6.15 mmol) was added. The reaction mixture was stirred at 0°C for 3 h, followed by stirring at 10°C for 115 h. When the reaction was completed, water (40 ml) was added, then separated from the dichloromethane. This solution was washed successively with aqueous 10% $Na_2S_2O_3$ (20 ml), then with 5% $NaHCO_3$ (20 ml), and brine (20 ml), dried, and evaporated. The crude product (0.4025 g) was purified by column chromatography (15 g silica gel, hexane-ethyl acetate 1:4), giving pure compound **4** (0.171 g, 53.6%) in the form of oil. IR ν_{max} (film) cm^{-1}: 3500, 2944, 1731, 1454, 1407, 1383, 1243, 1196, 1117, 1040. ^1H NMR ($CDCl_3$): 1.08 (3H, s, H-18); 1.15 (3H, s, H-19); 2.56 (1H, m, H-2a); 2.75 (1-H, m, H-2b); 2.81 (2H, m, H-15); 3.92 (1H, d, J = 10.8 Hz, H-17a); 4.02 (1H, d, J = 10.9 Hz, H-17b). ^{13}C NMR ($CDCl_3$): 14.99 (C-18); 20.27 (C-19); 19.71; 28.79; 29.27; 32.26; 34.03; 37.32; 43.99; 47.00; 50.22; 80.71 (C-17); 170.24 (C_{16}=O); 178.84 (COOH); 213.55 (C_5=O). MS (*m/z*) %: 335 (7); 323 (100, M^++1); 305 (85). HRMS TOF (*m/z*) for $C_{18}H_{26}O_5$: $[M+H]^+$ calcd. 323.18585, found 323.18540.

17-Oxa-D-homoandrost-5-en-16-on-3β-yl acetate (5) was synthesized from compound **1** in our previous work (15).

3β-Acetoxy-17-oxa-D-homo-5,6-secoandrostan-5-one-6-carboxylic acid (6), 3β-acetoxy-5-hydroxy-17-oxa-D-homo-5,6-secoandrost-4-ene-6-carboxylic acid (7) and 17-oxa-D-homoandrost-6-ene-7,16-dion-3β-yl acetate (8). Compound **5** (0.566 g, 1.63 mmol) was dissolved in 50% acetic acid (6.5 ml) and a solution of chromium(VI)-oxide (1.56 g, 15.6 mmol) in 50% acetic acid (7.8 ml) was added. The reaction mixture was stirred for 3 h at 60°C, quenched with methanol (4 ml) and evaporated. The reaction mixture was diluted with water (10 ml) and extracted with ethyl acetate (5 x 5 ml). The combined organic extract was dried and the solvent evaporated to yield a crude product (0.158 g), which was purified by column chromatography (6 g silica gel, hexane-ethyl

acetate 1:1 and 1:2). The mixture of compounds **6** and **7** (0.043 g, 6.5%) was obtained in teh form of oil and pure compound **8** as white crystals (0.076 g, 12.9%, mp 213-214°C, lit. (18) mp 213-214°C) after recrystallization from hexane-ethyl acetate. Spectral data for mixture of **6** and **7**: IR ν_{max} (film) cm^{-1}: 3500, 2966, 1732, 1406, 1382, 1245, 1137, 925, 755. ^{1}H NMR (Py-d_5): 0.90 and 0.92 (6H, 2s, 2H-18); 1.15 and 1.20 (6H, 2s, 2H-19); 1.92 and 1.99 (6H, 2s, 2CH_3 from 2OAc); 8.538 and 8.541 (2H from 2COOH). ^{13}C NMR (Py-d_5): 15.90 (C-18); 19.41 and 19.97 (2C-19); 22.47; 23.00; 24.53; 24.81; 26.76; 33.48; 34.06; 34.10; 35.11; 35.65; 35.71; 36.29; 36.55; 37.39; 38.21; 42.13; 43.07; 43.54; 44.43; 45.21; 49.69; 54.28; 75.73 (C-3); 82.07 (C-17); 130.42; 148.33; 171.50 and 171.63 (2C=O from 2OAc); 171.76 (C_{16}=O); 176.36 and 176.81 (2C=O from 2COOH); 217.27 (C_5=O). MS (*m/z*) %: 393 (3, M^+-1); 357 (15); 335 (100); 317 (68). Spectral data for **8** (18): IR ν_{max} (KBr) cm^{-1}: 2950, 1732, 1672, 1467, 1380, 1243, 1183, 1039, 754. ^{1}H NMR ($CDCl_3$): 1.03 (3H, s, H-18); 1.27 (3H, s, H-19); 2.07 (3H, s, CH_3 from OAc); 3.75 (1H,dd, J_1 = 5.6 Hz, J_2 = 18.9 Hz, H-15a); 3.97 (2H, s, 2H-17); 4.72 (1H, m, H-3); 5.75 (1H, d, J = 1.4 Hz, H-6). ^{13}C NMR ($CDCl_3$): 15.15 (C-18); 16.96 (C-19); 19.04; 21.20; 25.17; 27.14; 31.86; 33.19; 33.86; 35.76; 37.76; 44.91; 48.77; 71.96 (C-3); 80.13 (C-17); 117.64; 120.66 (C-6); 163.87 (C-3); 170.49 (C_{16}=O); 187.70 (C=O from OAc); 199.94 (C_7=O).

5α-Hydroxy-17-oxa-D-homoandrostan-6,16-dion-3β-yl acetate (9) was obtained from compound **5** and **6(*E*)-hydroximino-5α-hydroxy-17-oxa-D-homoandrostan-16-on-3β-yl acetate (10)** and **6(*Z*)-hydroximino-5α-hydroxy-17-oxa-D-homoandrostan-16-on-3β-yl acetate (11)** were obtained from compound **9**, and also described in our previous paper (15).

6(*E*)-Hydroximino-3β,5α-dihydroxy-17-oxa-D-homoandrostan-16-one (12) and 6(*Z*)-hydroximino-3β,5α-dihydroxy-17-oxa-D-homoandrostan-16-one (13). The mixture of compounds **10** and **11 (**0.190 g, 0.48 mmol) was dissolved in a mixture of chloroform-ethyl acetate (1:1, 4 ml) and solution of sodium hydroxide (0.5 ml, 4 M) and benzyltriethylammonium chloride (BTEAC, 0.005 g, 0.022 mmol) was added. The reaction mixture was stirred at reflux for 1 h. When the reaction was complete, the mixture was poured into water (5 ml), acidified with HCl (1:1) to the pH 1 and extracted with dichloromethane (4 x 5 ml). The combined organic extract was dried (anh. Na_2SO_4) and the solvent was evaporated to yield a crude product (0.180 g). After the purification by column chromatography (5 g silica gel, hexane-ethyl acetate 1:1 and 1:3) a mixture of **12** and **13** (0.043 g, 25.3%) was obtained in the form of white crystals (mp 241-243°C) after the recrystallization from hexane Against ER+ human breast adenocarcinoma cell line (MCF-7)-ethyl acetate. Spectral data for the mixture of **12** and **13**: IR ν_{max} (KBr) cm^{-1}: 3365, 2943, 1713, 1556, 1473, 1406, 1382, 1319, 1249, 1196, 1161, 1030, 978, 755. ^{1}H NMR (Py-d_5): 0.83 i 0.87 (6H, 2s, 2H-18); 0.85 and 0.89 (6H, 2s, 2H-19); 3.83 (1H,d, J = 10.0 Hz, H-17a); 3.85 (1H, d, J = 10.0 Hz, H-17b); 4.65 and 4.70 (2H, 2m, 2H-3); 6.67 and 7.36 (2H, 2bs, 2x5α-OH); 12.60 (1H, s, =NOH). ^{13}C NMR (Py-d_5): 15.52 (C-18); 16.36 (C-19); 21.68; 31.88; 33.10; 33.37; 33.65; 34.32; 35.92; 37.18; 38.97; 39.28; 41.58; 42.94; 43.96; 45.20; 45.70; 46.08; 68.13; 66.48 (C-3); 77.89 (C-17); 81.34; 82.32; 82.46; 162.10 (C_6=NOH); 171.55 (C_{16}=O).

6-Nitrile-17-oxa-5,6-seco-D-homoandrostane-5,16-dion-3β-yl acetate (14) was synthesized earlier (15) from the mixture of compounds **10** and **11**.

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Determination of antiproliferative activity

The antiproliferative activity was measured against six different human cancer cell lines: MCF-7 (ER+ breast adenocarcinoma), MDA-MB-231 (ER- breast adenocarcinoma), PC-3 (AR- prostate cancer), HeLa (cervical cancer), HT-29 (colon cancer) and K562 (chronic myelogenous leukemia) and one human non-cancerous cell line (normal fetal lung fibroblasts MRC-5). The cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 4.5% of glucose. The media were supplemented with 10% of fetal calf serum (FCS, NIVNS) and antibiotics: 100 IU/ml of penicillin and 100 lg/ml of streptomycin (ICN Galenika). All cell lines were cultured in flasks (Costar, 25 cm^2) at 37^{o}C in a 100% humidity atmosphere supplemented with 5% of CO_2. Only viable cells were used in the assays, and the cell viability was determined by the dye exclusion assay using trypan blue.

The cytotoxicity was evaluated using a colorimetric sulforhodamine B (SRB) assay (19). Briefly, single cell suspensions were plated onto 96-well microtiter plates (Costar, flat bottom): 5 x 10^3 cells per 180 ml of the medium. The plates were pre-incubated for 24 h at 37^{o}C, 5% CO_2. The test substances (**1**, **2**, **4**, **5**, **9** or **14**) were added to all wells (except for controls) at the concentrations ranging from 10^{-8} to 10^{-4} mol/L. After the incubation period (48 h/37^{o}C/5% CO_2), the SRB assay was carried out as follows: 50 μL of 80% trichloroacetic acid (TCA) was added to all wells; after one-hour incubation the plates were washed with distilled water, and 75 μL of 0.4% SRB was added to all wells; 30 minutes later the plates were washed with citric acid (1%) and dried at room temperature. Finally, 200 μL of 10 mmol Tris (pH = 10.5) base was added to all wells. The absorbance (λ 540/690nm) was measured using a microplate reader. The wells containing complete medium only were used as blanks.

The cytotoxicity was calculated according to the formula:

$$\left(\frac{1-A_{test}}{A_{control}}\right)100$$

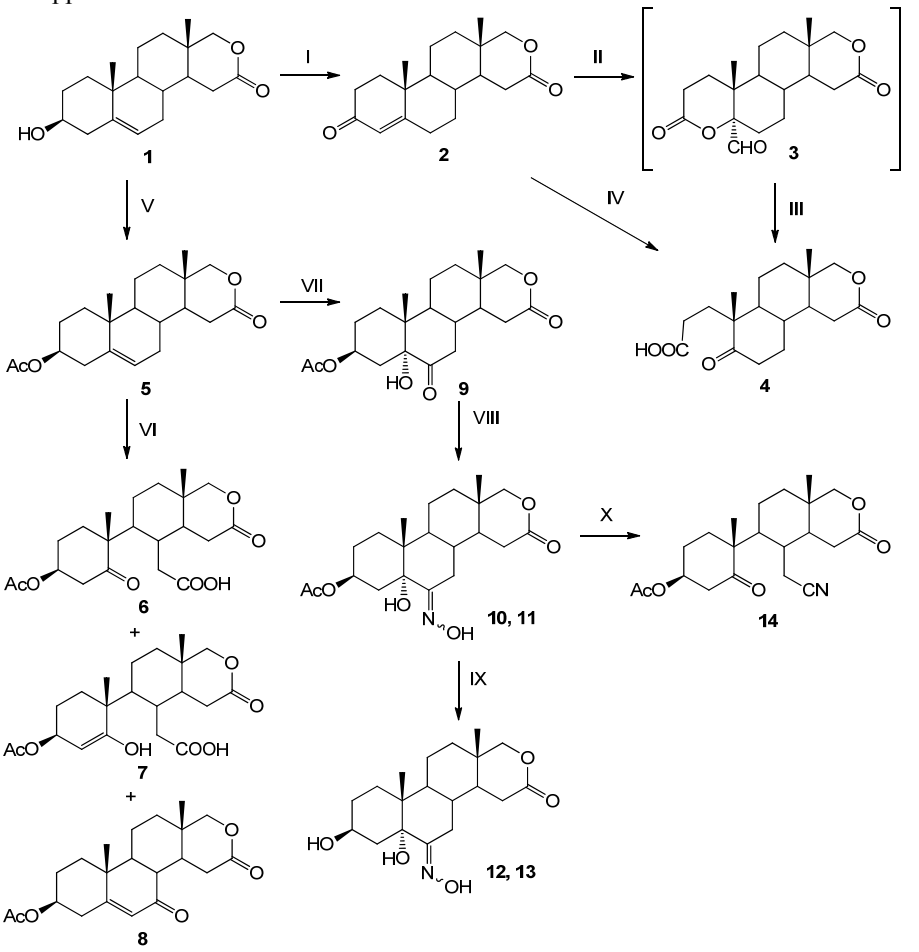
and expressed as percent cytotoxicity (CI%).

RESULTS AND DISCUSSION

Chemistry

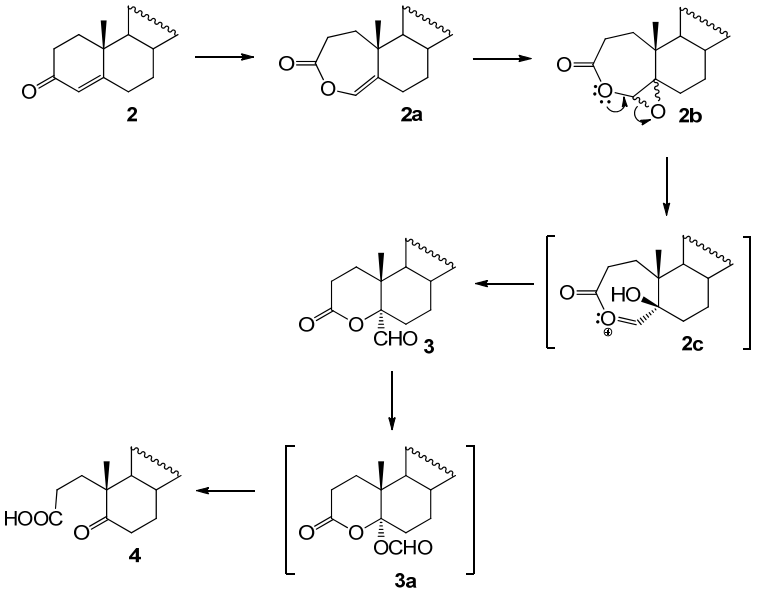
Scheme 1 outlines the synthetic procedures used to obtain compounds **2**-**14**. The starting compound 3β-hydroxy-17-oxa-D-homoandrost-5-en-16-one (**1**) was synthesized from dehydroepiandrosterone applying known procedure (20). The *Oppenauer* oxidation of **1** yielded 17-oxa-D-homoandrost-4-ene-3,16-dione (**2**) (20). The corresponding 17-oxa-D-homo-3,5-seco-4-norandrostan-5-one-3-carboxylic acid (**4**) was obtained by oxidation of compound **2** with MCPBA at room temperature after 90 days *via* 3,16-dioxo-4,17-dioxa-D-homoandrostan-5α-carboxaldehyde (**3**). Alternatively, the A-seco keto acid **4** was obtained directly by the oxidation of **2** with MCPBA at 0^{o}C for 3 h and then for 115 h at 10^{o}C.

The structures of compounds **3** and **4** were confirmed by NMR spectroscopy. The ^{1}H NMR spectra of **3** shows a signal at 9.94 ppm for formyl proton; similarly, the ^{13}C NMR spectra contain characteristic signal for the CHO function at 199.93 ppm. The presence of the aldehydo-A-lactone moiety was confirmed by comparing spectroscopic data of **3** with the reported values for closely related steroidal compounds (7, 21-23). In the ^{13}C NMR spectrum of compound **4** the signals at 178.84 ppm for the COOH and C_5=O function at 213.55 ppm were confirmed.



Scheme 1. Reagents: I) cyclohexanone, Al-(*t*-BuO)$_3$, reflux, 12 h, then HCl (1:1); II) MCPBA, CH_2Cl_2, 0°C, 6 h→ 10°C, 66 h, III) rt, 90 days; IV) MCPBA, CH_2Cl_2, 0°C, 3 h → 10°C, 115 h; V) Ac_2O, abs. Py, rt, 24 h, then HCl (1:1); VI) CrO_3, 50% CH_3COOH, 60°C, 3 h; VII) MCPBA, CH_2Cl_2, 0°C, 90 min → rt, 60 min, then CrO_3, water, rt, 70 min; VIII) $NH_2OH{\cdot}HCl$, CH_3COONa, C_2H_5OH, 60°C, 1 h; IX) BTEAC, NaOH, chloroform, ethyl acetate, reflux, 1 h, then HCl (1:1); X) Ac_2O, abs. Py, 70°C, 2 h, then at 0°C HCl (1:1)

The possible oxidation sequence (Scheme 2) is supported by the following observations: compound **2** reacted with MCPBA to give lactone **2a** and epoxy lactone **2b** which was converted *via* **2c** to the aldehydo-A-lactone **3**. Compound **3** was then transformed *via* formate **3a** to the A-seco acid **4**. Similarly, *Gorodetsky* and co-workers (24) described the mechanism of the oxidation of testosterone acetate with perbenzoic acid, in which 5α-carboxaldehyde was obtained.



Scheme 2. A proposed mechanism for the formation of compound **4**

One of the aims of present study was also to obtain B-seco D-homo lactones, using a modified procedure described in the literature (25, 26). When compound **5** (15, 18, 27) reacted with CrO_3 in 50% acetic acid at 60°C for 3 h, compounds **6-8** were obtained. The compounds **6** and **7** could not be separated by column chromatography. The NMR spectral data suggested that compounds **6** and **7** are present in the resulting mixture. In the ^{1}H NMR spectra, two signals at 8.538 and 8.541 ppm were indicative of the presence of two H atoms from two COOH groups (in **6** and **7**). Also, in the ^{13}C NMR two signals at 176.36 and 176.81 ppm were detected for two C atoms from two COOH groups. The structure of compound **8** was confirmed by comparing its spectral data with the reported values for **8** which was obtained earlier (18). Compounds **9-11** were synthesized in our previous work (15,16).

In this work, we have investigated the effects of applying BTEAC reagent, which was described in the literature (28), for obtaining the B-seco-cyano compounds. We have also expected to obtain compound **14**, but in our case when compounds **10** and **11** reacted with BTEAC in alkaline condition at reflux for 1 h (and then acidified) only a mixture of compounds **12** and **13** (with 3β-OH function) was obtained. Namely, compound **14** was

still obtained under the *Beckmann* fragmentation as we have described in our previous paper (15), and in this work we have investigated its antiproliferative activity against five human tumor cell lines and one non-tumor cell line.

The ^{1}H NMR spectra of a mixture of **12** and **13** was characterized by the broad signals at 4.65 and 4.70 ppm for two 3α-protons. In the ^{1}H NMR spectra, the signals for hydroxyl protons at 6.67 ppm, 7.36 ppm and 12.60 ppm were exchangeable with the deuterium of heavy water. Moreover, no signals were observed at 171.66 and 171.87 ppm from the two 3β-acetoxy functions in compounds **12** and **13** by ^{13}C NMR, while the presence a broad IR band at 3365 cm^{-1} confirmed the presence of hydroxyl groups in compounds **12** and **13**.

Antiproliferative activity

In this work we examined the effect of chemical modifications on the antiproliferative activity of the synthesized compounds. Starting compounds **1** (9), **2** (9), **5** (15), **9** (15) and the novel, pure and stable compounds **4** and **14** were evaluated for antiproliferative activity against PC-3 (AR- prostate cancer), MCF-7 (ER+ breast adenocarcinoma), MDA-MB-231 (ER- breast adenocarcinoma), HeLa (cervical cancer), HT-29 (colon cancer) and K562 (chronic myelogenous leukemia), as well as control non-cancerous MRC-5 (normal fetal lung fibroblast) cells. Antiproliferative activity was evaluated *in vitro* using the SRB assay (19), following 48 h treatment with test compounds. The results were compared with the non-selective antiproliferative drug Doxorubicin as well as a clinically approved androstane derivative (Formestane) used in the treatment of ER+ breast cancer, and are presented in Table 1 as IC_{50} values (μM).

Table 1. Antiproliferative activity against a panel of human cancer cell lines

Compounds	*IC_{50} (μM)*						
	MCF-7	MDA-MB-231	PC-3	HeLa	HT-29	K562	MRC-5[c]
1 (9)	> 100	20.20	89.00	-	-	-	> 100
2 (9)	>100	9.30	>100	-	-	-	>100
4	>100	>100	>100	>100	>100	-	>100
5 (15)	>100	23.54	78.31	>100	61.16	>100	>100
9 (15)	>100	15.67	66.87	>100	>100	>100	>100
14	44.99	85.26	>100	83.45	>100	-	>100
Formestane[a]	>100	55.5	48.36	5.55	>100	>100	>100
Doxorubicin[b]	0.75	0.12	95.61	1.17	0.32	0.36	0.12

[a]Control steroidal compound in clinical use for treatment of breast cancer. [b]Control antiproliferative compound. [c]Normal control cells.

The unstable compound **3** was not tested on antiproliferative activity, and neither were the mixture of compounds **6**-**8** and **10**-**13**, which could not be separated. All of the tested compounds were non-toxic on normal MRC-5 cells, whereas the control compound Doxorubicin was very toxic on these cells. As can be seen, a notable antiproliferative activity was observed only against MCF-7 (ER+ breast adenocarcinoma) and MDA-MB-231 (ER- breast adenocarcinoma) cells. The starting compound **1** showed a moderate

antiproliferative activity (IC_{50} 20.20 μM), while compound **2** displayed the strongest activity (IC_{50} 9.30 μM), which suggests the importance of the 3-keto-4-ene system. Compound **5**, with a 3β-acetoxy group, showed also a moderate antiproliferative activity (IC_{50} 23.54 μM), but lower compared with the starting compound **1** with a 3β-hydroxyl group. Compound **4** (IC_{50} >100) with an A-seco system was not toxic on the MDA-MB-231 cells, compared to starting compounds **1** and **2**. Along the same lines, compound **9**, which contains a 5α-hydroxy-6-keto system displayed also moderate antiproliferative activity (IC_{50} 15.67 μM), which was slightly higher than the activity of compounds **1** and **5**. Only compound **14**, with a B-seco system, showed weak (IC_{50} 44.99 μM) antiproliferative activity against MCF-7 cells, which was still higher than the activity of the starting compound **9** (IC_{50} >100 μM).

CONCLUSION

Here we report a convenient and efficient scheme for the synthesis of A- and B-seco D-homo lactone androstane derivatives. Also, we have investigated the effects of chemical transformations of the synthesized compounds on their antiproliferative activity. The antiproliferative activity was tested only on pure and stable compounds **1**, **2**, **4**, **5**, **9** and **14**. The analysis of the results revealed that only compounds **1**, **2**, **5** and **9** displayed significant antiproliferative activity against an ER- human breast adenocarcinoma cell line (MDA-MB-231). Compound **9**, with a 5α-hydroxy-6-keto system, showed a moderate antiproliferative activity (IC_{50} 15.67 μM), which was stronger than the activity of compounds **1** (IC_{50} 20.20 μM) and **5** (IC_{50} 23.54 μM). Only compound **2**, with a 3-keto-4-ene system, showed a strong antiproliferative activity (IC_{50} 9.30 μM) against the MDA-MB-231 cells. A weak antiproliferative activity against the ER+ human breast adenocarcinoma cell line (MCF-7) showed only compound **14** (IC_{50} 44.99 μM), with a B-seco system, compared with the starting compound **9** (IC_{50} >100 μM). All tested compounds were non-toxic on the healthy MRC-5 cells, whereas Doxorubicin was very toxic on these cells.

It was found that, in addition to the lactone function, the 3-keto-4-ene system and 5α-hydroxy-6-keto system also increased the antiproliferative activity, but only against the MDA-MB-231 cells.

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СИНТЕЗА И АНТИПРОЛИФЕРАТИВНА АКТИВНОСТ НОВИХ А- И Б-МОДИФИКОВАНИХ Д-ХОМО ЛАКТОНСКИХ АНДРОСТАНСКИХ ДЕРИВАТА

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У овом раду је описана синтеза А- и Б-модификованих Д-хомо лактонских деривата из 3β-хидрокси-17-окса-Д-хомоандрост-5-ен-16-она (**1**). 17-Окса-Д-хомоан-

дрост-4-ен-3,16-дион (**2**), добијен *Oppenauer*-овом оксидацијом једињења **1**, је преко нестабилног интермедијера 3,16-диоксо-4,17-диокса-Д-хомоандростан-5α-карбалдехида (**3**) преведен у 17-окса-Д-хомо-3,5-секо-4-норандростан-5-он-3-карбоксилну киселину (**4**), која је добијена и директно оксидацијом једињења **2**. Ацетиловањем једињења **1** је добијен 17-окса-Д-хомоандрост-5-ен-16-он-3β-ил ацетат (**5**), који је са хром(VI)-оксидом у 50%-тној сирћетној киселини дао смешу једињења **6**-**8**, а са *meta*-хлорпербензоевом киселином и хром(VI)-оксидом је дао 5α-хидрокси-17-окса-Д-хомоандростан-6,16-дион-3β-ил ацетат (**9**). Оксиминовањем једињења **9** добијена је смеша 6(*E*)- и 6(*Z*)-хидроксимино-5α-хидрокси-17-окса-Д-хомоандростан-16-он-3β-ил ацетата (**10**, **11**), који су хидролизом дали смешу 6(*E*)- и 6(*Z*)-хидроксимино-3β,5α-дихидрокси-17-окса-Д-хомоандростан-16-она (**12**, **13**), односно *Beckmann*-овом фрагментацијом је добијен 6-нитрил-17-окса-5,6-секо-Д-хомоандростан-5,16-дион-3β-ил ацетат (**14**). Чиста и стабилна једињења **1**, **2**, **4**, **5**, **9** и **14** су тестирана *in vutro* на шест малигних ћелијских линија (MCF-7, MDA-MB-231, PC-3, HeLa, HT-29, K562) као и на једну здраву ћелијску линију (MRC-5). Резултати антипролиферативне активности су показали да полазно једињење **1** са 3β-хидроксилном функцијом има задовољавајућу антипролиферативну активност (IC_{50} 20,20 μM), а једињење **2** са 3-кето-4-енским системом јаку антипролиферативну активност (IC_{50} 9,30 μM), према MDA-MB-231 ћелијама. Једињење **5** са 3β-ацетокси функцијом је показало нешто мању антипролиферативну активност (IC_{50} 23,54 μM) у поређењу са једињењем **1**, док је једињење **4** (IC_{50} >100) са А-секо системом нетоксично према MDA-MB-231 ћелијама. Такође, једињење **9** са 5α-хидрокси-6-кето системом је показало задовољавајућу антипролиферативну активност (IC_{50} 15,67 μM), већу од активности једињења **1** и **5**. Према MCF-7 ћелијама слабу антипролиферативну активност је показало само једињење **14** (IC_{50} 44,99 μM) са Б-секо системом, која је ипак већа од антипролиферативне активности полазног једињења **9** (IC_{50} >100 μM). Тестирана једињења (**1**, **2**, **4**, **5**, **9** и **14**) нису била токсична према здравим ћелијама (MRC-5), док је Доксорубицин показао јаку токсичност и према здравим ћелијама.

Кључне речи: деривати андростана, Д-хомо лактони, А- и Б-секо деривати, антипролиферативна активност

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