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Synthesis of Androstane Derivatives with a Heteroatom

ISIDORA NOGO[1,*], MAJA SREMACKI[2] and EVGENIJA ĐURENDIC[3]

[1]Faculty of Environmental Engineering, University Educons, Vojvode Putnika 87, Sremska Kamenica, Serbia
[2]Faculty of Technical Sciences, University of Novi Sad, Dositeja Obradovica 6, Serbia
[3]Faculty of Sciences, University of Novi Sad, Dositeja Obradovica 6, Serbia

*Corresponding author: E-mail: isidoranogo@gmail.com

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New information, data and findings of mechanism of steroid hormones, as well as the influence of specific structure on hormones activity, give excellant possibilities for designing new potentially biologically active androgen agonists and antagonists. The goal of the research shown in the work is synthesis of new androstan derivatives as potentially biologically active antiandrogen substances. Present study is particularly focused on synthesis of new androstan derivatives with a heteroatom obtained by multiphase synthesis from the initial dehydroepiandrosteron. The work describes and shows methods and processes of obtaining known and newly synthesized compounds, the structures of which were tested with spectroscopic data. Also, the assignment of spectroscopic data is given in the work (IR, ^{1}H NMR and ^{13}C NMR spectrum), characterizes the synthetized compounds.

Keywords: Synthesis, Steroids androst-5-ene derivatives, Aromatase inhibitors.

INTRODUCTION

Steroids are compounds of big therapy importance and it is estimated that completely one third of pharmacological compositions contains certain steroid compounds [1].

There are many findings on mechanism of action of steroid hormones. For example, by conversion of one keto group of steroid compounds into a ketal group, at least one of series of enzymatic reactions that normally take place in the environment of a keto group of corresponding steroid compound is inhibited [2].

Many steroid compounds are also inhibitors of aromataze enzymes. Steroid compounds with heteroatom in molecule are biologically active compounds and steroids with heterocycles connected with A- or D-ring of steroid nucleus are of particular pharmaceutical importance [3-9]. They show antitumour, anti-inflammatory activity and stand for chemotherapeutic anti-cancer agents [10].

Cholesterol (7) (C 27 steroid) is transported to mitochondria where key reaction in the process of steroid geneses takes place, so a part of side cholesterol chain is being riven. During this process a gradual hydroxylation in positions C20 and C22 is being done, followed by disconnection between these two atoms. In this way pregnenolon is created which is being transported to cytoplasm where its further conversion into progesterone takes place (9) (C21 steroid). Further on progesterone takes part into adrenal cortex hormone synthesis [mineral and glucocorticoids, cortisol (17), aldosterone (13) as well as androgen], ovarian hormone [estrogens: estradiol (20), estrone (18)] and testicular hormones [androgens: testosterone (1) androstendione (3), dihydrotestosterone (6) and dehydroepiandrosterone (5)]. Converting progesterones into androgens (C19 steroids) includes hydroxylation on C-17, then riving C20 and C22 chains. In further steroid genesis androgens are transformed into estrogens with the participation of aromatise enzymes.

The most important androgen hormones are: testosterone, epitestosterone, androstendione, androsterone, dehydroepiandrosterone and dihydrotestosterone. On the bases of great number of androgen researches, many authors [11-13] found that 17β-hydroxyl-group and 3-oxo group are essential for biological activity of androgens in a body. Testosterone is biologically active due to C-17 hydroxyl group, which is in a β position, while with epitestosterone hydroxyl group is in α position, which is manifested by its reduced biological activity. On C-17 atom, instead of a hydroxyl group androstendione, androsterone and dehydroepiandrosterone have keto function and therefore reduced activity. Dehydroepiandrosterone shows mild androgenic effects but acting anabolic [14].

It is also shown that characteristics of A ring are of great importance for biological activity, as well as the position of double bonds and substituent on it [15].

Androgen hormones have basic part in the processes of growth and development, differentiation and function of the male sex glands as well as in regulating the process of reproduction and the whole metabolism [16]. Which of the hormones will bind to a receptor and cause its activation as well as the series of causally related reactions depends on the kind of target cell and tissue and on the level of ontogenetic development [17]. It is determined that androgen hormones have strong impact on metabolism of carbohydrates and fats, cholesterol balance and triglycerides and insulin level [18].

Scientific researches show that androgens inhibit the ability of some fat cells for lipid storage by blocking the signal of transduction pathway, which normally support adipocyte function [19]. Dehydroepiandrosterone, one of the members of androgen hormones, was called ''neurosteroid'' after the proof of its synthesis in human brain had been found [20]. Former research has shown that 7α-hidroxylation of dehydroepiandrosterone takes place both in liver and in brain and the given derivative shows neuroprotective effect in central nervous system [21-24]. The presence of dehydroepiandrosterone in CSF detected in patients with neuro degenerative diseases shows the possibility of pharmacological use of this representative and/or its derivatives for therapeutic use [25,26]. Pyridine derivatives have an important part in synthesis of natural products and are used in the treatment of atherosclerosis because they are effective to inhibit HMG-CoA reductase and thus cholesterol transport. Although there are many methods for synthesis of steroid derivatives to pyridine they are rarely applied due to the lack of selectivity and poor yields. Abbiati *et al.* [27] successfully synthesized steroidal derivatives which have pyridine nucleus in their structure. The reaction is run in the presence of N-propargylamine and gold ions as catalysts, at certain temperature, with the mechanism of regioselective 6-endo-dig-cyclization where different products were obtained at a given yield. When the pyridine ring is introduced in the 3,4-position on A ring, testosterone (17β-hydroxyandrost-4-en-3-one, 17α-methyl-testosterone (17β-hydroxy-17α-methyl-androsta-4-en-3-one) and 19-nortestosterone (17β-hydroxy-19-norandrost-4-en-3-one), we obtain a new compound which has been shown to possess powerful biological activities such as antimicrobial, antiestrogens, anti-inflammatory, hypotensive, cardiovascular and anabolic.

In terms of therapeutic importance, the heterocyclic steroid compounds are of the utmost importance and their synthesis was performed by Yan *et al.* [28].

The reaction of the starting compounds is performed in the presence N-propargylamine, copper(II) nitrate trihydrate in ethanol, with stirring and reflux for 12 h under a controlled atmosphere (atm. Ar, 4Pa). By purification of the resulting product by flash column chromatography, we obtained colourless crystals of 17β-hydroxy-pyridino[2',3':3,4]androst-5-ene and colourless crystals of 17β-hydroxy-17α-methyl-pyridino-[2',3':3,4]androst-5-ene, white crystals of 17β-hydroxy-pyridino-[2',3':3,4]-19-norandrost-5-ene, 17β-hydroxy-pyridino[2',3':3,4]-androstan, 17β-hydroxy-17α-methyl-pyridino [2',3':3,4]androstan and 17β-hydroxy-pyridino[2',3':3,4]-19-norandrostan whose structures were confirmed by spectral methods.

Steroidal compounds condenses with N-propargylamine, with the help of copper(II) nitrate trihydrate in ethanol (about catalysts), to give the imine derivatives. Imine derivatives, further isomerization to give a compound subject to regioselective 6-endo-dig intramolecular nucleophilic attack of the carbon of the enamino moiety and to give an organometallic intermediate.

Further protonation of intermediates form dihydropyridine. It is assumed that the resulting compound is subject to an oxidation reaction in the presence of copper, wherein the copper is reduced from Cu^{2+} to Cu^{+} and radical intermediate. In the presence of oxygen in air, Cu^{+} is transformed into Cu^{2+} and a radical intermediate is protonated. Deprotonation of the pyridine derivatives gives the final product of this synthesis. The structure of the newly synthesized compound, 17β-hydroxy-pyridino[2',3':3,4]androst-5-ene, is confirmed by X-ray diffraction analysis [27].

In our work we have carried out the chemical transformation of dehydroepiandrosterone to provide new modified derivative there of as a potentially biologically active substances, for example. Inhibitors of the enzyme aromatase, the antiproliferative activity of which was tested. The study included the synthesis of new derivatives of androst-5-ene with a D-lactone function of the new derivatives containing a heteroatom in the molecule. As the lactone function proved to be a structural feature that contributes to the biological activity of steroids, as described earlier a synthesis in which in addition to lactone function a pyridine ring was introduced in positions 3 and 4 of A androstene ring, was done, all in order to increase biological activity.

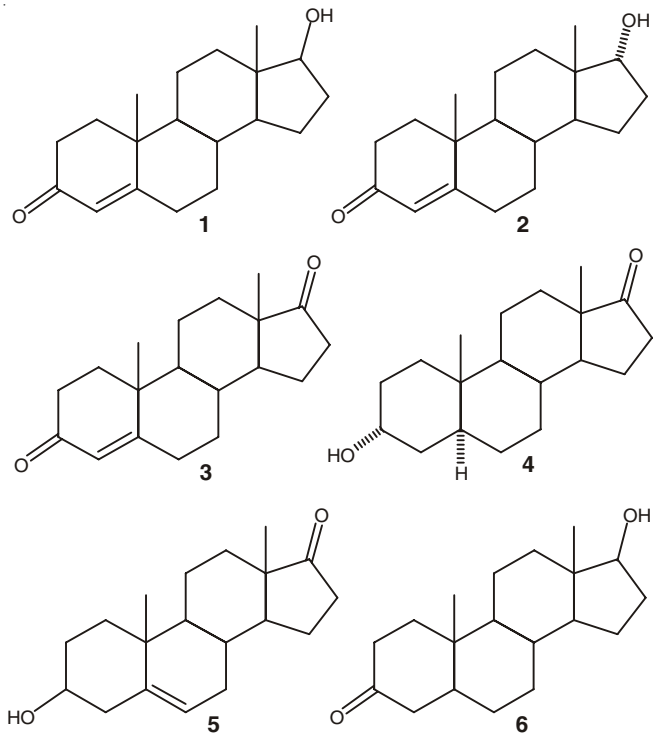

Fig. 1. Structure of androgen hormones

EXPERIMENTAL

IR spectra were recorded on a Nexus 670 SP-IR spectrophotometer and readings are expressed in cm^{-1}. All ^{1}H and ^{13}C NMR spectra were recorded on a Brucker AC 250 E device

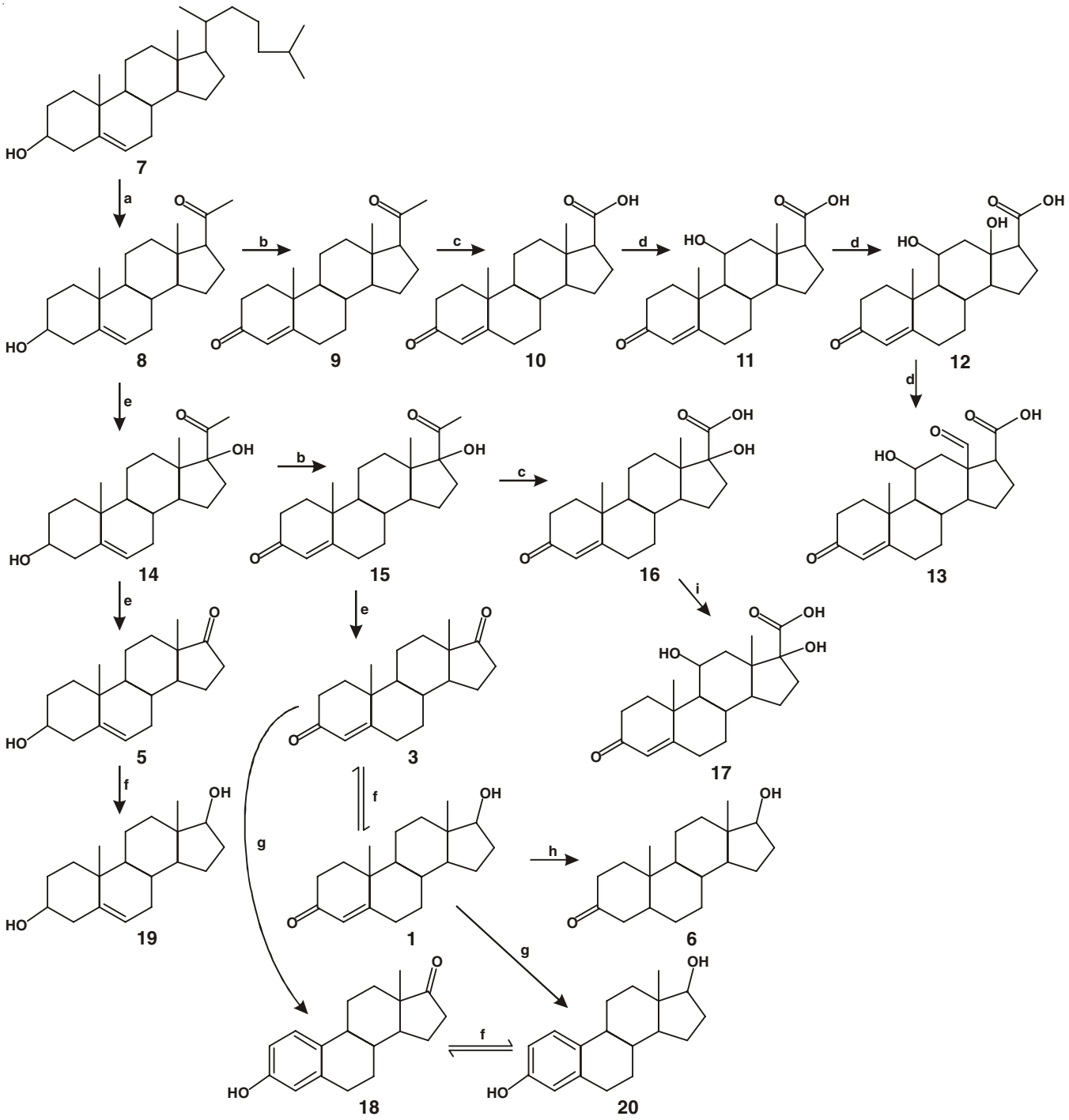

Fig. 2. Biosynthesis of androgen **a** (P450scc); **b** (3βHSD); **c** (21-hydroxylase); **d** (11β-hydroxylase, 18-hydroxylase, 18-oxidase); **e** (P450c17); **f** (17βHSD); **g** (P450arom); **h** (5α-reductase); **i** (11β-hydroxylase)

with the use of the standard Bruker software, anticipating the presence of tetramethylsilane (TMS, δ 0.00) as an internal standard at a frequency of 250 MHz for ^{1}H NMR spectra, respectively at 62.9 MHz for ^{13}C NMR spectra, in which the centre line of the carbon out of the chloroform-d is in a 77 ppm. Chemical shift values (δ-scale) are expressed in ppm values. Melting points were determined on Kofler apparatus and are not corrected. For chromatography silica gel Kieselgel 60 (0.063-0.2 mm, Merck) was used. The extracts were dried with anhydrous sodium sulfate (Na_2SO_4) and the solvents were evaporated on a rotary evaporator (Büchi). Dehydroepiandrosterone (6.00 g, 20.80 mmol) and isoamyl nitrite (6.5 mL) were dissolved in a solution of potassium *tert*-butoxide in *tert*-butanol, with vigorous stirring. The reaction mixture was left for 24 h at room temperature. After completion of the reaction, the reaction mixture was poured into water (600 mL) and acidified with hydrochloric acid (1:1) maintain to pH = 6, whereby the crude product separates as a white precipitate. The resulting precipitate was separated by filtration and washed with water. After drying, the pure compound **2** as a white crystalline in a yield of 84.76 % (5.60 g, m.p. = 223 °C, [22] m.p. = 223 °C) is obtained. Potassium *tert*-butoxide is prepared as follows: dry *tert*-butanol (180 mL) was added potassium metal, previously cut into small pieces (3.00 g, 76.70 mmol) with

vigorous stirring and the reaction is completed when all of the potassium disappear.

3β-Hydroxy-16-oximino-androst-5-en-17-one: IR (KBr, ν_{max}, cm^{-1}): 3392, 2936, 2896 1732, 1635, 1458, 1377, 1047, 1003, 946. ^1H NMR ($CDCl_3$, δ, ppm): 0.99 (s, 3H, H-18); 1.06 (s, 3H, H-19); 3.5 (m, 1H, H-3); 5:39 (d, 1H, H-6, *J* = 5.21 Hz). ^{13}C NMR ($CDCl_3$, δ, ppm): 205.18 (C-17); 156.56 (C=NOH); 141.07 (C-5); 120.61 (C-6); 71.55 (C-3); 50.04; 48.63; 46.74; 42.07; 37.03; 36.67; 31.44; 31.17; 31.01; 30.68; 25.58; 20:18; 19:42 (C-19); 13.90 (C-18).

3β-Hydroxy-16-oximino-androst-5-ene-17-one (4.80 g, 15 mmol) was dissolved in 95 % ethanol (280 mL) and add sodium borohydride (4.03 g, 106 mmol). The reaction mixture was stirred at room temperature for 1.5 h. Upon expiry of the reaction time, the reaction mixture was poured into water (600 mL). A precipitate of white solid is obtained, which was separated by filtration. After drying, the compound **3** as a crude product (4.70 g) is obtained, which without purification can be used in later stages of the synthesis. The pure compound **3** was obtained by recrystallization from absolute methanol in a yield of 96.42 % (4.65 g, m.p. = 255-257 °C, [22] m.p. = 255-257 °C).

3β,17β-Dihydroxy-16-oximinoandrost-5-ene: IR (KBr, ν_{max}, cm^{-1}): 3395, 2934, 2891, 2853, 1636, 1437, 1381, 1081, 1052, 979, 950. ^1H NMR (DMSO-d_6, δ, ppm): 0.62 (s, 3H, H-18); 0.96 (s, 3H, H-19); 3.26 (m, 1H, H-3); 3.85 (d, 1H, H-17, *J* = 5.8 Hz); 4.63 (d, 1H, 3β-OH, *J* = 5.0 Hz); 4.94 (d, 1H, 17β-OH, *J* = 5.8 Hz); 5.28 g (d, 1H, H-6, *J* = 5.0 Hz); 10.39 (s, 1H, =NOH). ^{13}C NMR (DMSO-d_6, δ, ppm): 162.34 (C-16); 141.38 (C-5); 120.22 (C-6); 80.62 (C-17); 70.02 (C-3); 19:20 (C-19); 11:38 (C-18). 3β,17β-Dihydroxy-16-oximinoandrost-5-ene (0.249 g, 0.78 mmol) was dissolved in ethylene glycol (8.00 mL) and added potassium hydroxide (1.675 g, 29.85 mmol).

The reaction mixture is stirred at the boiling point for 3 h. After completion of the reaction, the mixture was poured into water (50 mL), acidified with hydrochloric acid (1:1) to pH = 1 and extracted with methylene chloride (5 × 10 mL). The extracts were combined, dried with anhydrous sodium sulfate, remove drying agent and the solvent is evaporated. A yellow oil is obtained, which is purified by chromatography on a silica gel column chromatography (benzene/ethyl acetate, 9:1), to give the pure compound **4** (0.178 g, 73.94 %, [23] m.p. = 207 °C) in the form of crystals.

3β-Hydroxy-17-oxa-D-homoandrost-5-en-16-one: IR (KBr, ν_{max}, cm^{-1}): 3481, 3348 2962, 2933 2887 1714 1471 1263, 1207, 1038, 812; ^1H NMR ($CDCl_3$, δ, ppm): 1.02 (s, 3H, H-18); 1.03 (s, 3H, H-19); 2.72 (dd, 1H, H-15-b, $J_{15A, 15b}$ = 18.6 Hz, $J_{14a, 15b}$ = 5.6 Hz); 3.64 (m, 1H, H-3); 3.89 (q, 1H, $J_{17a, 17b}$ = 10.7 Hz, H-17a); 3.99 (q, 1H, H-17b); 5.35 (d, 1H, *J* = 5.0 Hz, H-6); ^{13}C NMR ($CDCl_3$, δ, ppm): 170.80 (C-16, C=O); 140.61 (C-5); 120.49 (C-6); 81.11 (C-17); 19:33 (C-19); 14.88 (C-18).

3β-Hydroxy-17-oxa-D-homoandrost-5-en-16-one (0.173 g, 0.57 mmol) was dissolved in cyclohexanone (3 mL) and was added aluminum(III) *t*-butoxide (0.245 g, 0.995 mmol). The reaction mixture is heated to the boiling point with vigorous stirring for 12 h. After completion of the reaction, the reaction mixture was soured with hydrochloric acid (1:1) to pH = 1, the excess of cyclohexanone was removed by distillation with steam. Thereafter, extraction was carried out with methylene chloride (5 × 5 mL). The organic extracts were combined and dried with anhydrous sodium sulfate, the dryer was removed and the solvent removed by evaporation. A light yellow oil is obtained, which was purified by flash chromatography on a silica gel column (benzene/ethyl acetate, 9: 1), to afford the pure compound (0.0876 g, 50.69 %, m.p. = 189 °C, [23] m.p. = 189 °C) in the form of white crystals.

17-Oxa-D-homoandrost-4-ene-3,16-dione: IR (KBr, ν_{max}, cm^{-1}): 2941, 2857, 1731, 1670, 1617 1449, 1381, 1272, 1 242, 1039, 875; ^1H NMR ($CDCl_3$, δ, ppm): 1.05 (s, 3H, H-18); 1.20 (s, 3H, H-19); 2.79 (dd, 1H, H-15b); 3.89 (d, 1H, H-17b); 3.99 (d, 1H, $J_{17a, 17b}$ = 10.8 Hz, H-17a); 5.76 (d, *J* = 1.5 Hz, H-4); ^{13}C NMR ($CDCl_3$, δ, ppm): 199.02 (C-3, C=O); 170.32 (C-16, C=O); 169.38 (C-5); 124.13 (C-4); 80.84 (C-17); 17:47 (C-19); 14.92 (C-18).

17-Oxa-D-homoandrost-4-ene-3,16-dione (**5**) (0.161 g, 0.533 mmol) was dissolved in 95 % ethanol (4 mL) N-propargylamine (0.061 g, 1.1 mmol) and $Cu(NO_3)_2{\cdot}3H_2O$ (0.0143 g, 5.919 mmol × 10^{-8}) were added. The reaction mixture was heated at the boiling point under an argon atmosphere with vigorous stirring for 6 h. After the expiry of the reaction time, water (50 mL) was added to the reaction mixture, after which it was extracted with methylene chloride (6 × 5 mL). The organic extracts were combined, dried with anhydrous sodium sulfate, drying agent was removed and the solvent is removed by evaporation, after which the crude reaction product in the form of dark brown crystals (0.1655 g). By purification of the crude product on a silica gel column (hexane/ethyl acetate, 1:1, 1: 2, 1: 3, 1: 4) a mixture of compound 17-oxa-pyridino[2',3':3,4]-D-homoandrostane-16-one (**6**) and 17-oxa-pyridino[2',3':3,4]-D-homoandrosta-1,5-dien-16-one (**7**) is obtained, from which purely compound **7** as a yellow oil (0.0538 g, 29.7 %) is isolated.

17-Oxa-pyridino[2',3':3,4]-D-homoandrostane-16-one: IR (film, ν_{max}, cm^{-1}): 2934, 1731, 1573, 1440, 1423, 1382, 1241, 1188, 1042, 752; ^1H NMR ($CDCl_3$, δ, ppm): 0.69 (s, 3H, H-18); 1.02 (s, 3H, H-19); 2.77 (dd, 1H, J_1 = 5.6 Hz, J_2 = 18.7 Hz, H-15); 3.90 (d, 1H, *J* = 10.8 Hz, H-17b); 3.98 (d, 1H, *J* = 10.7 Hz, H-17a); 7.54 (d, 1H, *J* = 7.8 Hz, N=CH-CH=CH-); 7.65 (d, 1H, *J* = 8.1 Hz, N=CH-CH=CH-); 8.38 (bs, 1H, -N=CH-CH=CH-); ^{13}C NMR ($CDCl_3$, δ, ppm) 12.13 (C-18); 19:57 (C-19); 22.97; 23:24; 28.67; 29.42; 31.67; 31.80; 34.27; 34.46; 35.45; 36.16; 40.55; 44.36; 46.07; 52.10; 81.10 (C-17); 120.89 (N=CH-CH=CH-); 133.39 (N=CH-CH=CH-); 145.75 (N=CH-CH=CH-); 156.13 (C-3); 170.83 (C16 = O).

17-Oxa-pyridino-[2',3':3,4]-D-homoandrosta-1,5-dien-16-one: IR (film, ν_{max}, cm^{-1}): 2934, 1731, 1573, 1440, 1423, 1382, 1241, 1188, 1042, 752; ^1H NMR ($CDCl_3$, δ, ppm): 0.79 and 1.00 (2s, 6H, 2H-18); = 1.03 i 1.07 (2s, 6H, 2H-19); 2.79 (dd, 1H, J_1 = 5.7 Hz, J_2 = 18.5 Hz, H-15); 3.93 (d, 1H, *J* = 10.7 Hz, H-17b); 4.00 (d, 1H, *J* = 10.7 Hz, H-17a); 15.06 (t, 1H, *J* = 2.7 Hz, H-6); 7.16 (m, 1H); 7.52 (d, 1H, *J* = 7.9 Hz, N=CH-CH=CH-); 7.78 (d, 1H, *J* = 8.0 Hz, N=CH-CH=CH-); 8.35 (s, 1H, -N=CH-CH=CH-); ^{13}C NMR ($CDCl_3$, δ, ppm): 14.91 and 15.12 (2C-18); 18:39 (C-19); 23:38; 29.15; 29.48;

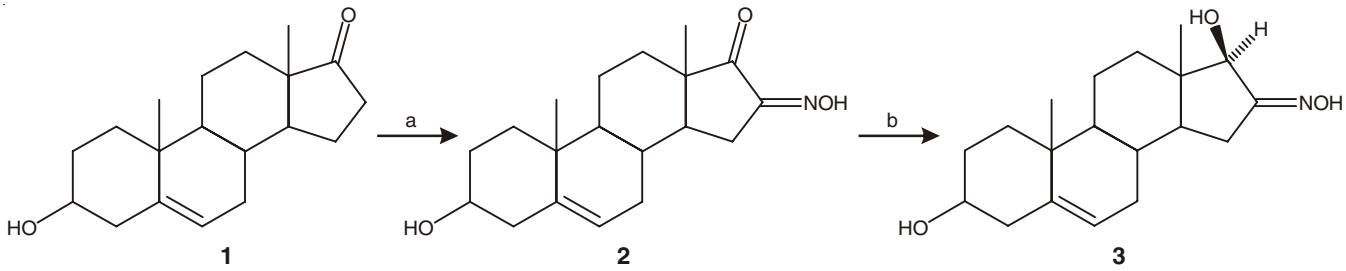

Fig. 3. a. Isoamyl nitrite, *t*-BuOK, *t*-BuOH, 25 °C, 24 h; b. $NaBH_4$, C_2H_5OH, 25 °C, 1.5 h

31.71; 31.84; 34.27; 36.18; 35.55; 44.41; 44.92; 46.15; 47.88; 52.08; 81.00; 81.06; 120.64; 120.97; 121.42; 132.37; 132.95; 134.08; 134.50; 139.35; 146.44 (N=CH-CH=CH-); 147.81; 156.42; 170.81 and 170.83 (2C16 = O).

RESULTS AND DISCUSSION

The starting compound in these synthesis is dehydroepiandrosterone (**1**), which is converted to 3β-hydroxy-16-oxyiminoandrost-5-en-17-one (**2**) of the known reactions of oximination [29] from the isoamyl nitrite in the presence of potassium *tert*-butoxide in potassium *tert*-butanol (Fig. 3), wherein the compound (**2**) with a yield of 84.76 %, m.p. = 223 °C [30] was obtained.

Compound **2** was reduced with sodium borohydride in ethanol at room temperature during 1.5 h, to give a 3β,17β-dihydroxy-16-oxyiminoandrost-5-one (**3**) with a yield of 96.42 %, m.p. = 255 °C (m.p. = 255-257 °C). In the next phase, shown in Fig. 4, the compound **3** is heated in ethylene glycol with potassium hydroxide at the boiling point for 3 h and then the reaction mixture was acidified with hydrochloric acid (pH = 2), to give a 3β-hydroxy-17-oxa-D-homoandrost-5-en-16-one (**4**) with a yield of 73.94 %, m.p. = 207 °C) [29].

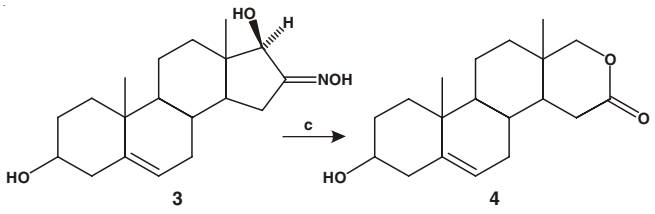

Fig. 4. c. Ethylene glycol, KOH, reflux, 3 h, HCl (1:1)

In the study new D-homo derivates of a lactone androst-5-ene were synthesized and D-homo lactone **4** is used as a starting compound. Lactone 17-oxa-D-homoandrost-4-ene-3,16-dione (**5**) with a yield of 50.69 % (m.p. = 189 °C) was obtained with Oppenauer oxidation with aluminum-*tert*-butoxide in cyclohexanone at reflux for 12 h (Fig. 5) [30]. The structure of compound **5** was confirmed on the basis of spectroscopic data of IR, ^{1}H NMR and ^{13}C NMR spectra and these data were consistent with literature data [29,30].

The obtained compound 17-oxa-D-homoandrost-4-ene-3,16-dione (**5**), has served as a precursor in the synthesis of new androstrane derivatives with a heteroatom. Compound **5** is reacted with N-propargylamine in the presence of a catalyst [$Cu(NO_3)_2{\cdot}3H_2O/C_2H_5OH$], in special conditions (atm., Ar, 4Pa) at reflux for 6 h, modification of the procedure, whereby the mixture of a compound 17-oxa-pyridino[2',3':3,4]-D-

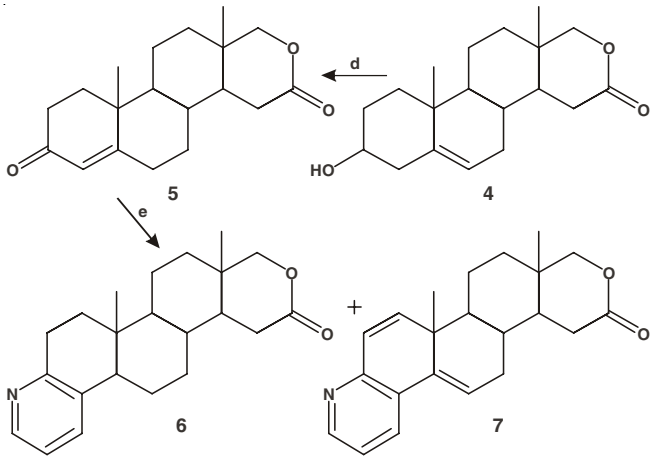

Fig. 5. d. Cyclohexanone, Al(*t*-OBu)$_3$, reflux, 12 h, HCl (1:1); e. N-propargyl amine, $Cu(NO_3)_2{\cdot}3H_2O/C_2H_5OH$, Ar, 4 Pa, reflux 6 h

homoandrostane-16-one (**6**) and 17-oxa-pyridino[2',3':3,4]-D-homoandrosta-1,5-dien-16-one (**7**) was obtained. The whole synthesis process is shown in Fig. 6.

The structure of obtained compounds **6** and **7** was confirmed by spectroscopic data and the respective spectra (IR, ^{1}H NMR and ^{13}C NMR). An intense band 1731 cm^{-1}, corresponds to the vibration of C=O function at C-3 and C-16 atoms, can be seen in the IR spectrum of the compound **6**. In ^{1}H NMR spectrum a multiplet at 7.10 ppm, doublet at 7.52 ppm and a broad singlet at 8.38 pm were observed, which correspond to a hydrogen atom of the pyridine nucleus. In the ^{13}C NMR spectrum new signals at 121.01 ppm, 132.95 ppm and 146.54 ppm were registered, which correspond to the carbon atoms of the pyridine nucleus.

^{1}H NMR spectrum of compound **7** indicates the presence of C_5=C_6 double bond and a fused pyridine ring in the compound. However, the emergence of multiple signals in the ^{13}C NMR spectrum at 132.37-134.49 ppm, indicates that besides the compound **6**, there is a compound **7** in the mixture as well.

Conclusion

A multi-stage synthesis of the new androstane derivatives with a heteroatom, as potentially biologically active substances, was carried out successfully. Dehydroepiandrosterone served as a starting compound in the multiphase synthesis. With synthesized compounds of 3β-hydroxy-17-oxa-D-homo-androst-5-en-16-one and 17-oxa-D-homoandrost-4-ene-3,16-dione antiproliferative activity according to the selected tumor cell lines was tested : towards cells of human breast adenocarcinoma ER + MCF-7, cells in ER- breast adenocarcinoma, MDA-MB-231, the prostate cancer cells PC3 as well as on

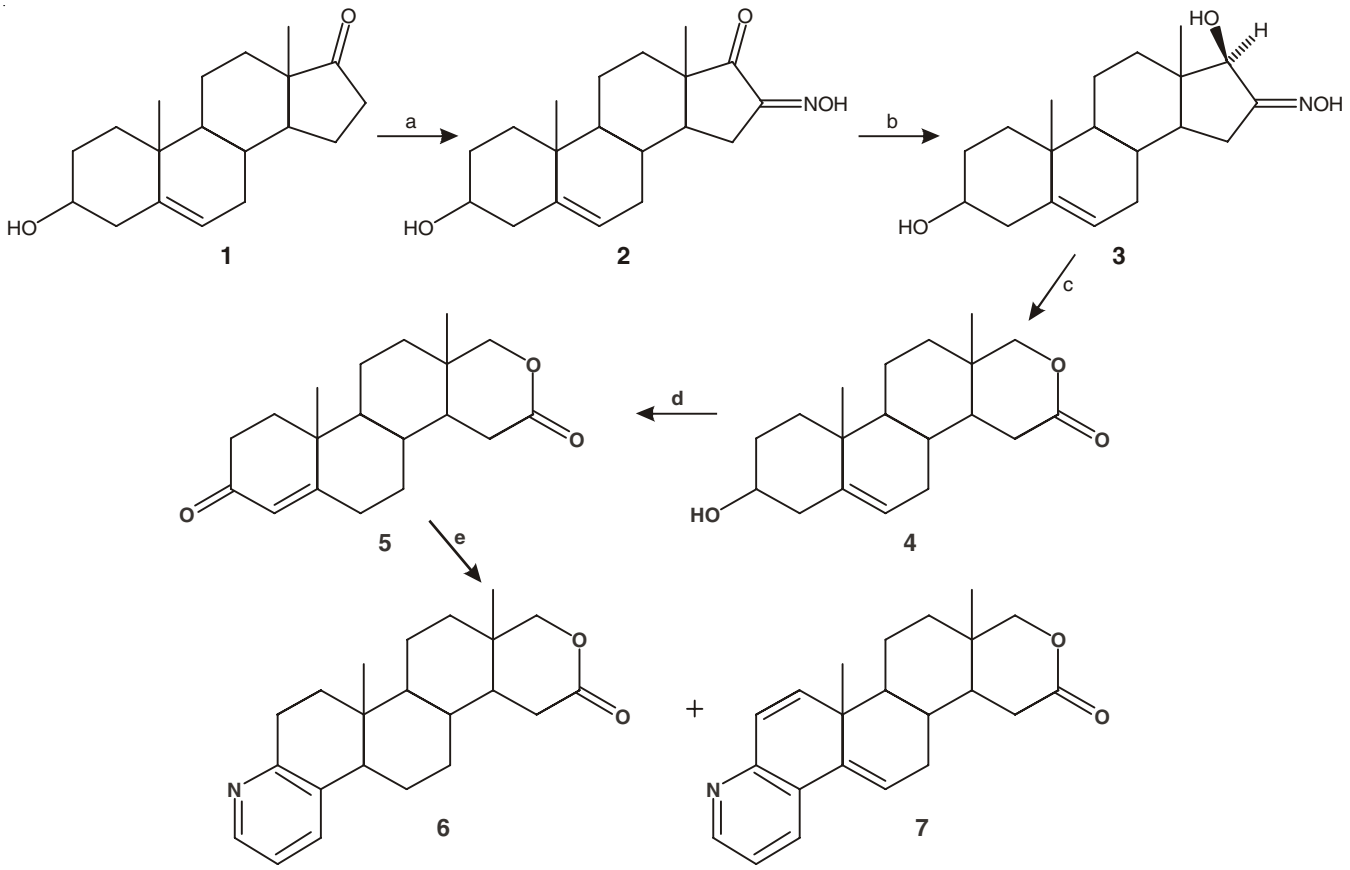

Fig. 6. The whole process of synthesis - a. isoamyl nitrite, *t*-BuOK, *t*-BuOH, 25 °C, 24 h; b. $NaBH_4$, C_2H_5OH, 25 °C, 1.5 h; c. Ethylene glycol, KOH, reflux, 3 h, HCl (1:1); d. Cikloheksanone, Al(*t*-$OBu)_3$, reflux, 12 h, HCl (1:1); e.N-Propargylamine, $Cu(NO_3)_2 \cdot 3H_2O$/ C_2H_5OH, Ar, 4 Pa, reflux, 6 h

healthy cells of fetal lung fibroblast MRC-5. The results indicate that the greater cytotoxicity was achieved in the presence of 4-en-3-one system, or in the case of compound 17-oxa-D-homoandrost-4-ene-3,16-dione. Thus, the introduction of 4-en-3-one system in a steroid molecule, results in new androstane derivatives which exhibit antiproliferative effects by cells of breast adenocarcinoma, ER-(MDA-MB-231).

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