J. Chem. Sci. (2020) 132:98
https://doi.org/10.1007/s12039-020-01803-3

REGULAR ARTICLE

New oxygen-containing androstane derivatives: Synthesis and biological potential

MARINA P SAVIĆ[a,*], IVANA Z KUZMINAC[a], DUŠAN Đ ŠKORIĆ[a], DIMITAR S JAKIMOV[b], LUCIE RÁROVÁ[c], MARIJA N SAKAČ[a] and EVGENIJA A DJURENDIĆ[a]

[a]Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia
[b]Oncology Institute of Vojvodina, Faculty of Medicine, University of Novi Sad, Put Dr Goldmana 4, 21204 Sremska Kamenica, Serbia
[c]Laboratory of Growth Regulators, Faculty of Science and Institute of Experimental Botany, Palacký University and the Czech Academy of Sciences, Šlechtitelů 27, 78371 Olomouc, Czech Republic
E-mail: marina.savic@dh.uns.ac.rs

MS received 13 December 2019; revised 20 April 2020; accepted 21 April 2020

Abstract. New steroidal D-homo androstane derivatives with 5β,6β-epoxy-3,16-dicarbonyl, 6α- and 6β-hydroxy-3,16-dicarbonyl and 3β,5α-dihydroxy-6,16-dicarbonyl moieties were synthesized and confirmed by NMR spectroscopy. Novel and starting compounds were evaluated for their potential cytotoxicity *in vitro* against seven human cancer cell lines (MCF-7, MDA-MB-231, PC3, HeLa, HT-29, A549 and CEM) and one human noncancerous cell line (MRC-5). The most sensitive cell line was MDA-MB-231 derived from female reproductive tissue, wherein all compounds showed moderate to strong cytotoxic activity. Also, new compound with 5β,6β-epoxy-3,16-dicarbonyl moieties showed strong cytotoxic activity against colon adenocarcinoma (HT-29). In this work, *in silico* ADME properties of novel compounds were assessed by comparing calculated molecular properties with Lipinski, Veber, Egan, Ghose and Muegge criteria.

Keywords. D-homo androstane lactones; cytotoxicity; NMR analysis; *in silico* ADME studies.

1. Introduction

Steroids are important class of natural products with various biological, chemical and pharmaceutical applications.[1–3] Structural features, and their diverse native biological activities make these molecules an interesting starting material for the synthesis of novel compounds with potential biological activities[4,5] and consequently they have been raw materials for commercially important drugs for decades.[6–9] The hydrophobic steroid skeleton enables transport through biological membranes and, after binding to the compatible receptors, steroids can express their specific physiological function.[10] Efficient membrane permeation is necessary for bioavailability and therefore, rules that have been devised in medicinal chemistry to achieve favorable bioavailability are a convenient guide for the design of membrane-permeating molecules.[11–13]

Since chemical affinity for receptor binding was related to the distances between nucleophilic sites and electronic and hydrophobic interactions between the receptor and ligands, heteroatoms may be involved in the formation of additional hydrogen bonds with the receptors, which leads to changes in their biological activity.[14–16] The literature survey has discovered that the modification of the steroid skeleton by oxygen-containing functional groups leads to significant changes in the bioactivity of the parental molecules.[17–21] Thus, oxygenated derivatives of cholesterol, known as oxysterols, showed inhibitions of human cancer cells: HT-29 (from colorectal adenocarcinoma), HepG2 (from hepatocellular carcinoma), LAMA-84 (from myeloid leukemia), A549 (from lung adenocarcinoma

*For correspondence

Electronic supplementary material: The online version of this article (https://doi.org/10.1007/s12039-020-01803-3) contains supplementary material, which is available to authorized users.

Published online: 08 August 2020

epithelium), PC3 (from prostate metastasis), MCF-7 (from breast adenocarcinoma) and SH-SY5Y (human neuroblastoma).[18,19] The oxidation state on rings A and B of the steroidal nucleus and on the oxygenated groups is known to be essential for their cytotoxicity. Also, C-3, C-5 and/or C-6 oxygen-containing derivatives of androst-5-ene, dehydroepiandrosterone, pregnenolone and cholesterol proved to be selective inhibitors against the human aldo–keto reductase 1B10 (AKR1B10), which is highly expressed in several types of cancers, and has been regarded as a promising cancer therapeutic target.[20,21] On the other hand, epoxides are three-membered cyclic ethers that have highly polarized oxygen–carbon bonds in addition to a highly strained ring, which results in their specific reactivity.[22] Carvalho *et al.*[23] have studied epoxy steroids and found that their cytotoxicity is dependent on the position and stereochemistry of the epoxide and on the presence of additional hydroxyl substituents. Naz *et al.*[24] in their research suggested that the epoxide ring in steroidal skeleton is at least partly responsible for the observed activity against prostate (LnCap) and lung cancer (Calu-3) cell lines. Also, the literature describes natural withanolides, in which epoxide functionality in ring B steroidal core contributes to their biological activities.[25,26] For example, the recently identified potent inhibitor of MDA-MB-231 cell proliferation, tubocapsenolide A, poses the 3β-hydroxy-5β,6β-epoxy moiety.[27] Kasal *et al.*[28] reported the preparation of several steroidal A ring epoxides in pregnane serie and evaluation as neuronal modulators.

After the discovery of the clinical activity of testolactone in 1962,[29] a large effort has been invested in the synthesis of bioactive D-ring lactone-based compounds.[30] Presence of the lactone ring in the steroidal nucleus, may lead to forming of new 5α-reductase inhibitors[31] or aromatase inhibitors[4] or potential cytotoxic agents.[32]

Based on these and other studies, the aim of this research included the synthesis of oxygen-containing D-homo lactone androstane derivatives and their preliminary biological screening. In our previous work,[32–34] we have shown that the introduction of polar functional groups into ring A of androstane derivatives, such as an isoxazole or pyridine ring, hydroxymethylene or hydroxyimino groups, can altered cytotoxic activity of parental compounds. As a continuation of our research on D-modified androstane derivatives, in this study, we turned to an investigation of oxygen-containing moieties, such as epoxides, hydroxyl and carbonyl functional groups, their arrangement and number, and their influence on cytotoxic activity against a panel of human cancer cell lines versus normal fetal lung fibroblasts (MRC-5) control cells. In order to evaluate combined effects of D-homo lactonic ring and oxygen-containing functional groups on cytotoxic activity of the steroidal compounds, herein we report the synthesis, NMR characterization, cytotoxic activity and *in silico* ADME studies of new 5β,6β-epoxy-17-oxa-17a-homoandrostane-3,16-dione (**4**), 6α- and 6β-hydroxy-17-oxa-17a-homoandrost-4-ene-3,16-dione (**5a** and **5b**) and 3β,5α-dihydroxy-17-oxa-17a-homoandrostane-6,16-dione (**8**).

2. Experimental

2.1 *General*

Melting points were determined using an Electrothermal 9100 apparatus and are uncorrected. Infrared spectra (wave numbers in cm^{-1}) were recorded on PerkinElmer Spectrum Two. NMR spectra were recorded on a Bruker AV III HD spectrometer operating at 400 MHz (^{1}H), 100 MHz (^{13}C), and are reported in ppm downfield from the tetramethylsilane internal standard. Chemical shifts are given in ppm (δ-scale). High resolution mass spectra (HRMS) were recorded on a Thermo LTQ Orbitrap XL instrument in ESI+ mode. Chromatographic separations were performed on silica gel columns (Kieselgel 60, 0.063–0.20 mm, Merck). All reagents used were of analytical grade.

2.2 *Synthesis of new compounds **4**, **5a**, **5b** and **8***

Starting compounds **1**–**3a**, **3b**, **6a, 6b** and **7** were synthesized earlier from commercially available dehydroepiandrosterone and they are described in our previous papers.[35–37]

2.2a *5β,6β-Epoxy-17-oxa-17a-homoandrostane-3,16-dione (**4**) and 6α- and 6β-hydroxy-17-oxa-17a-homoandrost-4-ene-3,16-dione (**5a** and **5b**)* A mixture of 5α,6α- and 5β,6β-epoxy-3β-hydroxy-17-oxa-17a-homoandrostane-16-one (**3a** and **3b**)[35] (0.219 g, 0.6 mmol) was dissolved in dichloromethane (12 mL) and pyridinium chlorochromate (PCC) (0.197 g, 0.9 mmol) was added. Reaction mixture was stirred at room temperature for 48 h. After the reaction was completed, HCl (1:1) was added to pH 1. The resulting mixture was poured into water (15 mL) and extracted with dichloromethane (4 x 10 mL). The combined organic extract was dried (anh. Na_2SO_4) and solvent evaporated to yield crude product (0.213 g). The resulting product was purified by column chromatography (8 g silica gel, petroleum ether/ethyl-acetate, 1:1 and 1:3). After recrystallization from hexane/ethyl-acetate (4:1) pure 5β,6β-epoxy-17-oxa-17a-homoandrostane-3,16-dione (**4**) (0.025 g, 11.5%, mp 172–173 °C) in a form of white crystals and a mixture of 6α- and 6β-hydroxy-17-oxa-17a-homoandrost-4-ene-3,16-

dione (**5a** and **5b**) (0.092 g, 42.4%) in ratio 4 : 1, in a form of yellow powder were obtained. Compound **4**. IR (film, ν_{max}, cm^{-1}): 2950, 2909, 1739, 1700, 1467, 1432, 1384, 1238, 1039, 976. ^{1}H NMR ($CDCl_3$, δ, ppm): 1.07 (s, 3H, H-18); 1.17 (m, 1H, H-12α); 1.45 (s, 3H, H-19); 1.48 (m, 1H, H-11α); 1.54 (m, 1H, H-14); 1.56 (m, 1H, H-12β); 1.62 (m, 1H, H-9); 1.63 (m, 1H, H-11β); 1.71 (m, 2H, H-1); 1.75 (m, 1H, H-8); 1.90 (dt, 1H, J_1= 14 Hz, J_2=2.8 Hz, H-7α); 2.02 (m, 1H, H-7β); 2.16 (m, 1H, H-15β); 2.20 (m, 1H, H-4β); 2.39 (m, 2H, H-2); 2.72 (dd, 1H, J_1=6.1 Hz, J_2=18.6 Hz, H-15α); 3.36 (d, 1H, J=15.4 Hz, H-4α); 3.91 (m, 1H, H-6α); 3.96 (m, 2H, H-17a). ^{13}C NMR ($CDCl_3$, δ, ppm): 15.09 (CH_3,C-18); 17.90 (CH_3, C-19); 19.50 (CH_2, C-11); 30.96 (CH, C-8); 31.83 (CH_2, C-15); 32.35 (Cq, C-13); 33.66 (CH_2, C-7); 34.27 (CH_2, C-12); 34.50 (CH_2, C-1); 37.68 (CH_2, C-2); 39.56 (Cq, C-10); 43.42 (CH, C-14); 44.48 (CH, C-9); 50.41 (CH_2, C-4); 62.76 (CH, C-6); 78.12 (Cq, C-5); 80.99 (CH_2, C-17a); 170.62 (Cq, C-16); 210.96 (Cq, C-3). HRMS m/z: $C_{19}H_{26}O_4$ $[M+H]^+$ calculated: 319.19093; found: 319.19058. Compounds **5a** and **5b**. IR (KBr, ν_{max}, cm^{-1}): 3426, 2946, 2916, 1731, 1666, 1652, 1382, 1244, 1186, 1061, 735. ^{1}H NMR ($CDCl_3$, δ, ppm): 1.06 (s, 3H, H-18); 1.20 (s, 3H, H-19); 2.78 (dd, 1H, J_1=18.7 Hz, J_2=5.7 Hz, H-15α); 3.97 (m, 2H, H-17a); 4.34 (m, H6-β from 6α-hydroxy isomer); 4.40 (t, J= 3.1 Hz, H6-α from 6β-hydroxy isomer); 5.84 (s, H-4 from 6β-hydroxy isomer); 6.20 (d, J=1.4 Hz, H-4 from 6α-hydroxy isomer). ^{13}C NMR ($CDCl_3$, δ, ppm): 14.97 (C-18); 18.39 (C-19); 19.38; 31.81; 32.26; 33.69; 34.15; 34.87; 36.03; 38.90; 39.24; 43.73; 52.55; 67.80 (CH, C-6, α-isomer); 72.41(CH, C-6, β-isomer); 80.73 (CH_2, C-17a); 120.10 (CH, C-4); 169.88 (Cq, C-5); 170.16 (Cq, C-16); 199.03 (Cq, C-3). HRMS m/z: $C_{19}H_{26}O_4$ [M+K]+ calculated: 357.14627; found: 357.14638.

2.2b *3β,5α-Dihydroxy-17-oxa-17a-homoandrostane-6,16-dione (**8**).* 5α-Hydroxy-17-oxa-17a-homoandrostane-6,16-dion-3β-yl acetate (**7**) (0.151 g, 0.4 mmol)[32,37] was dissolved in absolute methanol (9 mL) and KOH (0.1 g, 1.7 mmol) was added. The reaction mixture was stirred under reflux for 80 min. When the reaction was completed, methanol was evaporated and water (10 mL) was added. Reaction mixture was acidified to pH 1 with HCl (1:1) and extracted with dichloromethane (4 x 10 mL). The combined organic extract was dried (anh. Na_2SO_4) and solvent evaporated to yield crude product (0.112 g). After recrystallization from hexane/ethyl-acetate (4:1), pure 3β,5α-dihydroxy-17-oxa-17a-homoandrostane-6,16-dione (**8**) (0.092 g, 70%, mp >250 °C) in a form of white powder was obtained. IR (film, ν_{max}, cm^{-1}): 3469, 3392, 2948, 1709, 1379, 1245, 1030. ^{1}H NMR (acetone-d_6, δ, ppm): 0.80 (s, 3H, H-19); 1.02 (s, 3H, H-18); 1.27 (m, 1H, H-12α); 1.39 (m, 1H, H-11β); 1.43 (m, 1H, H-2β); 1.53 (m, 1H, H-1α); 1.58 (m, 1H, H-12β); 1.63 (m, 1H, H-11α); 1.67 (m, 1H, H-8); 1.68 (m, 1H, H-4β); 1.78 (m, 1H, H-1β); 1.79 (m, 1H, H-12β); 1.81 (m, 1H, H-14); 1.85 (m, 1H, H-4α); 2.08 (m, 1H, H-7α); 2.11 (m, 1H, H-9); 2.15 (m, 1H, H-15β); 2.57 (dd, 1H, J_1=18.4 Hz, J_2=5.9 Hz, H-15α); 2.73 (t, 1H, H-7β, J=12.4 Hz); 3.52 (bs, 1H, 3β-OH); 3.94 (m, 1H, H-3); 3.96 (m, 2H, H-17a); 4.48 (bs, 1H, 5α-OH). ^{13}C NMR (acetone-d_6, δ, ppm): 13.32 (CH_3, C-19); 14.30 (CH_3, C-18); 19.76 (CH_2, C-11); 29.61 (CH_2, C-1); 30.57 (CH_2, C-2); 31.16 (CH_2, C-15); 32.62 (Cq, C-13); 34.11 (CH_2, C-12); 36.05 (CH_2, C-4); 37.47 (CH, C-8); 39.44 (CH_2, C-7); 41.87 (Cq, C-10); 43.42 (CH, C-9); 44.45 (CH, C-14); 65.96 (CH, C-3); 79.52 (Cq, C-5); 80.24 (CH_2, C-17a); 169.07 (Cq, C-16); 210.61 (Cq, C-6). HRMS m/z: $C_{19}H_{28}O_5$ $[M+Na]^+$ calculated: 359.1834; found: 359.18371.

2.3 *In silico ADME prediction*

The drug likeliness profile of the compounds was predicted through the analysis of pharmacokinetic properties of the compounds by using SwissADME[38] online prediction tool. Pharmaceutically important properties of compounds **1–3a, 4, 5a**, **5b**, **7** and **8** (Table 1) were compared to five sets of criteria:

1. Lipinski (MW ≤ 500, HBD ≤ 5, HBA ≤ 10, LogP ≤ 5),[13]
2. Veber (nrotb ≤ 10, TPSA ≤ 140 Å^2),[12]
3. Egan (LogP ≤ 5.88, TPSA ≤ 131.6 Å^2),[39]
4. Ghose (160 ≤ MW ≤ 480, -0.4 ≤ LogP ≤ 5.6, 40 ≤ MR ≤ 130, 20 ≤ No. atoms ≤ 70)[40] and
5. Muegge (200 ≤ MW ≤ 600, -2 ≤ LogP ≤ 5, TPSA ≤ 150 Å^2, No. rings ≤ 7, No. carbons > 4, No. heteroatoms > 1, nrotb ≤ 15, HBD ≤ 5, HBA ≤ 10).[41]

In addition, possibility of the gastrointestinal absorption and brain penetration was analyzed using the BOILED-Egg model.

2.4 *Cell lines and cell culture*

Seven human tumor cell lines: estrogen receptor positive (MCF-7) and estrogen receptor negative (MDA-MB-231) breast adenocarcinoma, androgen receptor negative prostate cancer (PC3), cervical carcinoma (HeLa), colon adenocarcinoma (HT-29), lung adenocarcinoma (A549) and human T-lymphoblastic leukemia cell line (CEM) and one human noncancerous cell line (normal fetal lung fibroblasts MRC-5), were used in the present study.

2.4a *Cytotoxicity testing* Compounds were evaluated for cytotoxic activity toward MCF-7, MDA-MB-231, HeLa, HT-29 and A549 cell lines using the tetrazolium colorimetric MTT assay,[42] after exposure to test compounds, in concentrations ranging from 10^{-8} to 10^{-4} M, for 72 h, as described in our previous work.[34] Doxorubicin and cisplatin as a nonselective anti-proliferative agents were used as reference compounds. Two independent experiments were conducted in quadruplicate for each concentration of tested compound. Mean values and standard deviations (SD) were calculated for each concentration. The IC_{50} value is defined

Table 1. *In silico* physicochemical properties of compounds **1–3a**, **4**, **5a**, **5b**, **7** and **8**.

Comp.	Formula	MW	HBD	HBA	LogP	nrotb	TPSA	MR	No. rings
1	$C_{19}H_{28}O_3$	304.42	1	3	3.25	0	46.53	86.44	4
2	$C_{21}H_{30}O_4$	346.46	0	4	3.63	2	52.60	96.18	4
3a	$C_{19}H_{28}O_4$	320.42	1	4	2.75	0	59.06	85.93	5
4	$C_{18}H_{23}O_4$	303.37	0	4	1.95	0	55.90	79.91	5
5a	$C_{19}H_{26}O_4$	318.41	1	4	2.46	0	63.60	86.64	4
5b	$C_{19}H_{26}O_4$	318.41	1	4	2.46	0	63.60	86.64	4
7	$C_{21}H_{30}O_6$	378.46	1	6	2.46	2	89.90	98.05	4
8	$C_{19}H_{28}O_5$	336.42	2	5	1.94	0	83.83	88.32	4

MW: molecular weight expressed in Daltons; HBD: number of hydrogen bond donors; HBA: number of hydrogen bond acceptors; LogP: Average of partition coefficient five predictions (iLOGP, XLOGP3, WLOGP, MLOGP and Silicos-IT LogP); nrotb: number of rotatable bonds; TPSA: topological polar surface area in $Å^2$; MR: molar refractivity.

as the dose of compound that inhibits cell growth by 50%. The IC_{50} of each tested compound was determined by median effect analysis.

Anti-cancer activity of new and standard compounds towards human T-lymphoblastic leukemia cell line CEM was determined after 72 h of incubation by Alamar blue as described earlier.[43] The data were obtained from two independent experiments performed in triplicates.

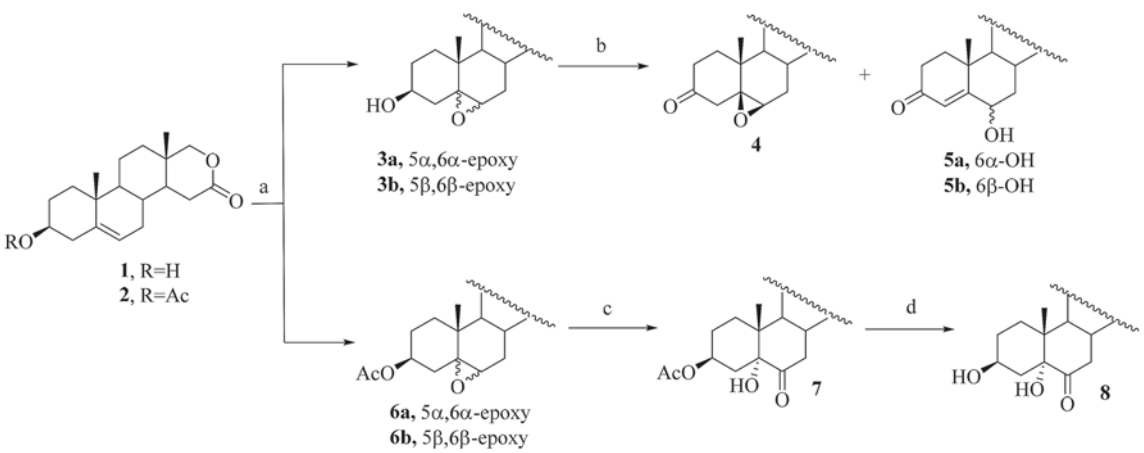
3. Results and Discussion

3.1 *Synthesis and structure discussion*

We report here synthesis of some new oxygen-containing 17-oxa-17a-homoandrostane derivatives (**4**, **5a**, **5b** and **8**; Scheme 1), based on convenient synthesis from parental compounds (**1**–**3a**, **3b**, **6a** and **6b**, **7**), which were prepared earlier.[32,35,37]

In order to further modification, a mixture of 5α,6α- and 5β,6β-epoxy-derivatives **3a** and **3b** was used for the synthesis of new compounds **4**, **5a** and **5b**. The reaction was carried out with pyridinium chlorochromate in dichloromethane for 48 h at room temperature. After chromatographic purification 5β,6β-epoxy-17-oxa-17a-homoandrostane-3,16-dione (**4**) and a mixture of 6α- and 6β-hydroxy-17-oxa-17a-homoandrost-4-ene-3,16-dione (**5a** and **5b**) were isolated. Structure of compound **4** (Figure 1) was confirmed by NMR spectroscopic analysis and total assignation of all proton and carbon resonances was performed (see Experimental).

2D NOESY experiment was of particular use in determination of stereochemistry of 5,6-epoxy function. It can be seen that signal at 3.91 ppm, which is assigned to H-6 proton, shows NOE interactions with H-4α and H-7α protons. Further, NOE interaction with angular methyl group protons H-19 was not detected.



Scheme 1. a. *m*-CPBA, CH_2Cl_2, $NaHCO_3$, 0 °C, 1.5 h (R=H) or 1 h (R=Ac); b. PCC, CH_2Cl_2, 48 h, rt; c. CrO_3, acetone/water (10:1), 0 °C, 30 min → rt, 40 min; d. KOH, abs. methanol, reflux, 80 min.

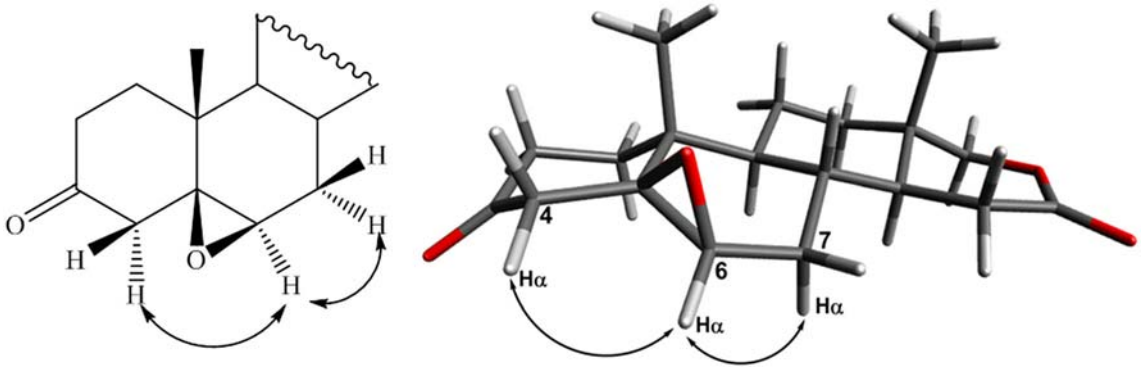


Figure 1. 2D and 3D NOE interactions of proton H-6 in compound **4.**

All of this indicates alpha orientation of H-6 proton which, in turn, means that 5,6-epoxide is beta oriented.

Mixture of compounds **5a** and **5b** was characterized by NMR spectroscopy. Integration of two signals from H6-β proton at 4.34 and H6-α proton at 4.40 ppm showed that 6α-hydroxy (**5a**) and 6β-hydroxy (**5b**) isomers were obtained in 4:1 ratio. Stereochemistry of 6α-hydroxy isomer (Figure 2) in the mixture was confirmed by 1D NOESY experiment (Supplementary informations). In ^{13}C NMR of the mixture signal at 199.03 ppm indicates the presence of α,β-unsaturated ketone at C-3.

In the last step, by saponification of 5α-hydroxy-17-oxa-17a-homoandrostane-6,16-dion-3β-yl acetate (**7**) with potassium hydroxide in absolute methanol under the reflux for 80 min, 3β,5α-dihydroxy-17-oxa-17a-homoandrostane-6,16-dione (**8**) was obtained. Total NMR assignation of all proton and carbon resonances was performed (see Experimental).

3.2 *In silico ADME prediction*

In order to identify newly synthesized compounds, as well as some precursors, as drug like molecules, physicochemical properties were calculated using SwissADME[38] online prediction tool and compared with Lipinski, Veber, Egan, Ghose and Muegge criteria. As shown in Table 1, compounds **1–3a, 4, 5a**, **5b**, **7** and **8** are well in accordance with the five sets of rules, indicating their potential for use as drug like molecules. As expected, stereoisomers **5a** and **5b** have identical physicochemical parameters. Drug-likeness of these compounds can be easier and faster to examine using bioavailability radars (Figure 3). For compounds **3a** and **3b**, also for mixture of **5a** and **5b**, only radars for α-isomers are presented since physicochemical properties for both α and β-isomers are the same. From Figure 3 it can be clearly observed that all tested compounds are well in compliance with all criteria.

In addition, the BOILED-Egg model was analyzed for selected compounds in order to get insight into possibility of the gastrointestinal absorption and brain penetration (Figure 4).[44] All four tested compounds are predicted to be absorbed by intestine, but only molecules **4**, **5a** and **5b** can penetrate the brain. Since compounds **7** and **8** are not predicted to penetrate the blood–brain barrier, it means that they are probably inactive in central nervous system. For newly synthesized compounds **8**, **5a** and **5b**, as well as compound **7** is predicted possibility of elimination from

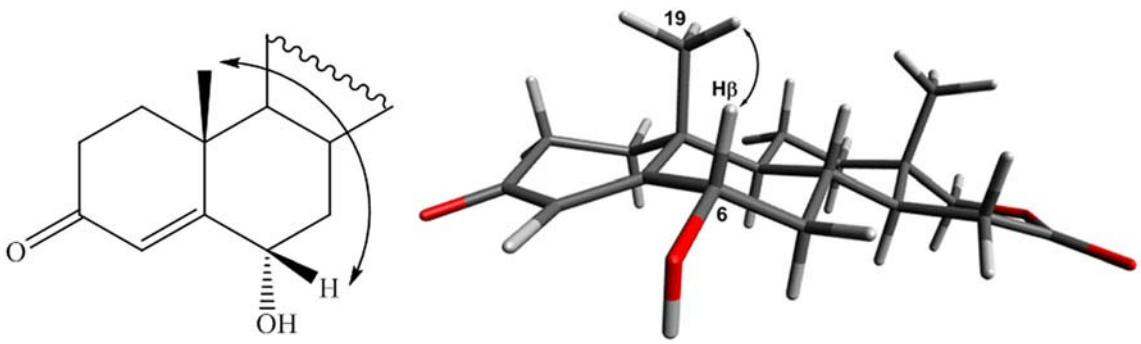


Figure 2. 2D and 3D NOE interaction of H-6 proton with angular methyl group H-19 protons in compound **5a**

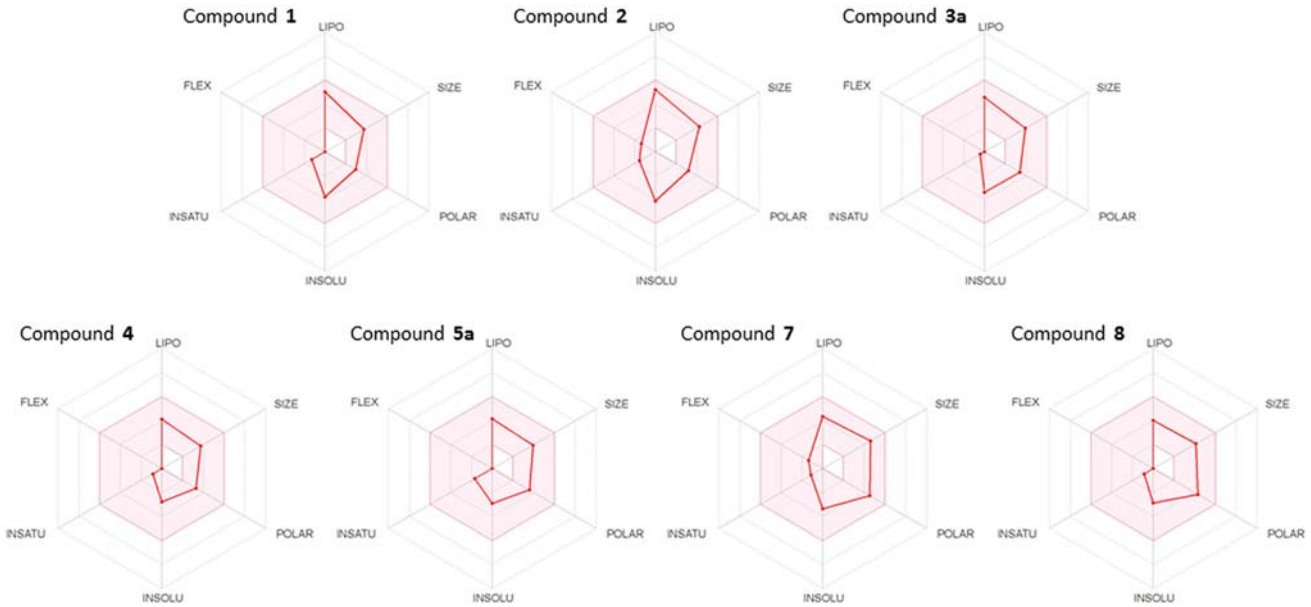


Figure 3. The bioavailability radars of compounds **1, 2, 3a, 4, 5a**, **7** and **8** enable faster insight into the drug-likeness of compounds. The pink area represents the optimal range for each properties (lipophilicity: XLOGP3 between −0.7 and +5.0, size: MW between 150 and 500 g/mol, polarity: TPSA between 20 and 130 $Å^2$, solubility: log *S* not higher than 6, saturation: fraction of carbons in the sp^3 hybridization not less than 0.25, and flexibility: no more than 9 rotatable bonds).

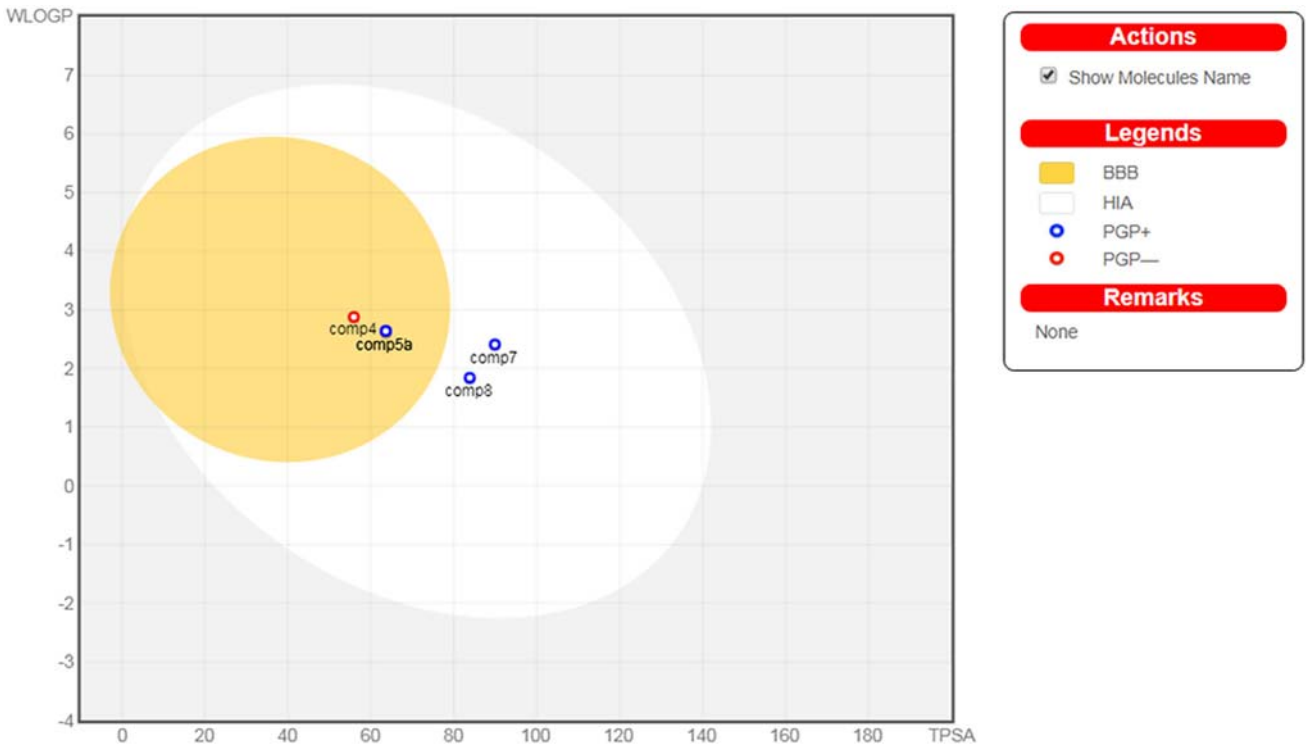


Figure 4. Graphical distribution of compounds **4**, **5a** and **5b**, **7** and **8** using the BOILED-Egg predictive model for intestine and brain permeation. The grey region is the physicochemical space predicted to exhibit high intestinal absorption and the yellow region is the physicochemical space predicted to permeate the brain. Blue dots are for molecules predicted to be effluated from central nervous system by P-glycoprotein (PGP+), while red dots are for those predicted not to be effluated by PGP (PGP-).

Table 2. *In vitro* cytotoxic activity of the tested compounds, doxorubicin and cisplatin.

	IC_{50} (μM), 72 h						
Compound	MCF-7	MDA-MB-231	PC3	HeLa	HT-29	A549	MRC-5
1	>100	10.15	22.39	40.23	36.03	>100	>100
2	>100	2.09	41.29	69.44	54.84	>100	>100
3a	>100	18.32	28.21	32.73	>100	>100	>100
4	>100	85.82	41.33	>100	11.04	92.17	>100
5a and **5b**	>100	25.02	>100	>100	>100	>100	>100
6a	>100	27.62	>100	99.15	>100	>100	>100
7	81.77	23.73	>100	>100	>100	>100	>100
8	>100	6.16	>100	33.65	>100	>100	>100
Doxorubicin	0.20	0.09	84.23	0.07	0.15	>100	0.10
Cisplatin	1.60	2.64	4.56	2.10	4.10	3.20	0.24

central nervous system by P-glycoprotein, unlike compound **4**. This indicates that compounds **7** and **8** have less probability of inducing side effects than compounds **5a** and **5b**, especially **4**.

3.3 *Cytotoxic activity*

The oxygen-containing derivatives **1**, **2**, **3a, 4**, mixture of **5a** and **5b**, **6a, 7** and **8** (Table 2) were evaluated for their *in vitro* cytotoxicity against six types of solid human cancer cell lines. Cytotoxic activity against MCF-7, MDA-MB-231, PC3, HeLa, HT-29, A549 and MRC-5 was evaluated *in vitro* using the MTT assay, following 72 h treatment with tested compounds. Results were compared with the nonselective anticancer agents, doxorubicin and cisplatin. As can be seen from Table 2, all the tested compounds were nontoxic on normal MRC-5 cells, whereas the doxorubicin and cisplatin were highly toxic to healthy cells. The most sensitive cell line was MDA-MB-231 estrogen receptor negative carcinoma derived from female reproductive tissue. Strong cytotoxic activity against MDA-MB-231 cells was observed for compound **1**, **2** and **8**, with IC_{50} values 10.15 μM, 2.09 μM and 6.16 μM, respectively, while compounds **3a**, mixture of **5a** and **5b**, **6a** and **7** showed moderate cytotoxic activity (IC_{50}=18.32 μM, IC_{50}=25.02 μM, IC_{50}=27.62 μM and IC_{50}=23.73 μM, respectively). Most of the tested compounds were practically inactive against estrogen receptor positive breast cancer cell line MCF-7, except compound **7**, with weak cytotoxic activity (IC_{50}=81.77 μM). Design and synthesis of potential drugs with selectivity against estrogen receptor negative MDA-MB-231 cells, over estrogen receptor positive cells MCF-7, may contribute to the development of more effective breast cancer chemotherapies. Moderate cytotoxic activity against androgen receptor negative prostate cancer (PC3) showed compounds **2**, **3a** and **4** (IC_{50}=41.29 μM, IC_{50}=28.21 μM and IC_{50}=41.33, respectively), while compound **1** showed cytotoxicity of 22.39 μM. Moderate cytotoxicity against cervical carcinoma (HeLa) showed compounds **1**, **3a** and **8** (IC_{50}=40.23 μM, IC_{50}=32.73 μM and IC_{50}=33.65 μM, respectively), while compound **2** had weak cytotoxicity (IC_{50}=69.44 μM). Only compound **4** showed strong cytotoxic activity (IC_{50}=11.04 μM) against colon adenocarcinoma (HT-29), while compounds **1** and **2** showed moderate (IC_{50}=36.03 μM) and weak cytotoxicity (IC_{50}=54.84 μM), respectively. All the tested compounds were almost inactive to the lung adenocarcinoma (A549) cells. Comparing these results with cytotoxic activity of doxorubicin and cisplatin, it can be concluded that compound **2** showed stronger cytotoxic activity than cisplatin against MDA-MB-231 cells, while other compounds were more active compared to doxorubicin against PC3 cells.

All tested compounds were evaluated on cytotoxicity in acute T-lymphoblastic leukemia cells CEM *in vitro*. There was no significant activity detected after 72 h of treatment with 50 μM of androstane derivatives.

4. Conclusions

We report here convenient synthesis of novel oxygen-containing 17-oxa-17a-homoandrostane derivatives. Also, we have investigated the effects of chemical transformations of the synthesized compounds on their cytotoxic activity. The cytotoxic activity was tested on

compounds **1**, **2**, **3a**, **4**, mixture of **5a** and **5b**, **6a**, **7** and **8**, and compared with cisplatin and doxorubicin. Additon of epoxy- or hydroxy-functions to the parental compounds resulted in significant changes in cytotoxic activity against MDA-MBA-231 and HT-29 cancer cell lines. Having in mind the high toxicity of doxorubicin and cisplatin against healthy cells MRC-5, the investigated compounds with strong cytotoxic activity and nontoxicity to healthy cells, deserved further studies. *In silico* calculated physicochemical properties of all tested compounds were in accordance with five sets of rules for oral bioavailability. This is especially important for new compound **8**, that showed excellent cytotoxic properties against MDA-MB-231 cells, also it is not predicted to permeate the brain and therefore has less probability to induce side effects.

Supplementary Information (SI)

Supplementary information features copies of ^{1}H and ^{13}C NMR spectra of newly synthesized compounds is available at www.ias.ac.in/chemsci.

Acknowledgements

The authors thank the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 172021). This work was also supported by the European Regional Development Fund – Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868).

References

1. Shahidi N T 2001 A review of the chemistry, biological action, and clinical applications of anabolic-androgenic steroids *Clin. Ther.* **23** 1355 https://doi.org/10.1016/S0149-2918(01)80114-4
2. Abdel-Razek A S, Hamed A, Frese M, Sewald N and Shaaban M 2018 Penicisteroid C: New polyoxygenated steroid produced by co-culturing of Streptomyces piomogenus with Aspergillus niger *Steroids* **138** 21 https://doi.org/10.1016/j.steroids.2018.06.005
3. Jabeen M, Choudhry M I, Miana G A, Rahman K M, Rashid U, Khan H, Arshia and Sadiq A 2018 Synthesis, pharmacological evaluation and docking studies of progesterone and testosterone derivatives as anticancer agents *Steroids* **136** 22 https://doi.org/10.1016/j.steroids.2018.05.008
4. Cepa M M D S, Tavares da Silva E J, Correia-da-Silva G, Roleira F M F and Teixeira N A A 2005 Structure-activity relationships of new A,D-ring modified steroids as aromatase inhibitors: design, synthesis, and biological activity evaluation *J. Med. Chem.* **48** 6379 https://doi.org/10.1021/jm050129p
5. Larik F A, Saeed A, Shahzad D, Faisal M, El-Seedi H, Mehfooz H and Channar P A 2017 Synthetic approaches towards the multi target drug spironolactone and its potent analogues/derivatives *Steroids* **118** 76 https://doi.org/10.1016/j.steroids.2016.12.010
6. David K, Dingemanse E, Freud J and Laqueur E 1935 Über kristallinisches männliches Hormon aus Hoden (Testosteron), wirksamer als aus Harn oder aus Cholesterin bereitetes Androsteron *Hoppe-Seyler's Z. Physiol. Chem.* **233** 281
7. Manson A J, Stonner F W, Neumann H C, Christiansen R G, Clarke R L, Ackerman J H, Page D F, Dean J W, Philips D K, Potts G O, Arnold A, Beyler A L and Clinton R O 1963 Steroidal heterocycles. VII.[1] Androstano[2,3-d]isoxazoles and related compounds *J. Med. Chem.* **6** 1 https://doi.org/10.1021/jm00337a001
8. Potter G A, Barrie S E, Jarman M and Rowlands M G 1995 Novel steroidal inhibitors of human cytochrome $P450_{17\alpha}$ (17a-Hydroxylase-$C_{17,20}$-lyase): Potential agents for the treatment of prostatic cancer *J. Med. Chem.* **38** 2463 https://doi.org/10.1021/jm00013a022
9. Nathan M R and Schmid P 2017 A review of fulvestrant in breast cancer *Oncol. Ther.* **5** 17 https://doi.org/10.1007/s40487-017-0046-2
10. Beato M and Klug J 2000 Steroid hormone receptors: an update *Hum. Reprod. Update* **6(3)** 225 https://doi.org/10.1093/humupd/6.3.225
11. Yang N J and Hinner M J 2015 In *Site-Specific Protein Labeling. Methods in Molecular Biology* Gautier A and Hinner M (Eds.) Vol 1266 (New York: Humana Press) p.29 https://doi.org/10.1007/978-1-4939-2272-7_3
12. Veber D F, Johnson S R, Cheng H-Y, Smith B R, Ward K W and Kopple K D 2002 Molecular properties that influence the oral bioavailability of drug candidates *J. Med. Chem.* **45** 2615 https://doi.org/10.1021/jm020017n
13. Lipinski C A, Lombardo F, Dominy B W and Feeney P J 1997 Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings *Adv. Drug Deliv. Rev.* **23** 3 https://doi.org/10.1016/S0169-409X(96)00423-1
14. Todorov M, Mombelli E, Aït-Aïssa S and Mekenyan O 2011 Androgen receptor binding affinity: a QSAR evaluation *SAR QSAR Environ. Res.* **22** 265 https://doi.org/10.1080/1062936X.2011.569508
15. Popadyuk I I, Markov A V, Morozova E A, Babich V O, Salomatina O V, Logashenko E B, Zenkova M A, Tolstikova T G and Salakhutdinov N F 2017 Synthesis and evaluation of antitumor, anti-inflammatory and analgesic activity of novel deoxycholic acid derivatives bearing aryl- or hetarylsulfanyl moieties at the C-3 position *Steroids* **127** 1 https://doi.org/10.1016/j.steroids.2017.08.016
16. Scherbakov A M, Komkov A V, Komendantova A S, Yastrebova M A, Andreeva O E, Shirinian V Z, Hajra A, Zavarzin I V and Volkova Y A 2018 Steroidal pyrimidines and dihydrotriazines as novel classes of anticancer agents against hormone-dependent breast cancer cells *Cells. Front. Pharmacol.* **8** Article No. 979 https://doi.org/10.3389/fphar.2017.00979
17. Ghosh P, Ghosh A, Mandal A, Sultana S S, Dey S and Pal C 2016 Oxysterols: Synthesis and anti-leishmanial activities *Steroids* 107 65 https://doi.org/10.1016/j.steroids.2015.12.020

18. Carvalho J F S, Cruz Silva M M, Moreira J N, Simões S and Sá e Melo M L 2010 Sterols as anticancer agents: Synthesis of Ring-B oxygenated steroids, cytotoxic profile, and comprehensive SAR analysis *J. Med. Chem.* **53** 7632 https://doi.org/10.1021/jm1007769
19. Carvalho J F S, Cruz Silva M M, Moreira J N, Simões S and Sá e Melo M L 2011 Selective cytotoxicity of oxysterols through structural modulation on Rings A and B. Synthesis, in vitro evaluation, and SAR *J. Med. Chem.* **54** 6375 https://doi.org/10.1021/jm200803d
20. Zhang W, Wang L, Zhang L, Chen W, Chen X, Xie M, Yan G, Hu X, Xu J and Zhang J 2014 Synthesis and biological evaluation of steroidal derivatives as selective inhibitors of AKR1B10. *Steroids* **86** 39 https://doi.org/10.1016/j.steroids.2014.04.010
21. Chen W, Chen X, Zhou S, Zhang H, Wang L, Xu J, Hu X, Yin W, Yan G and Zhang J 2016 Design and synthesis of polyhydroxy steroids as selective inhibitors against AKR1B10 and molecular docking *Steroids* **110** 1 https://doi.org/10.1016/j.steroids.2016.03.004
22. Parker R E and Isaacs N S 1959 Mechanisms of epoxide reactions *Chem. Rev.* **59(4)** 737 https://doi.org/10.1021/cr50028a006
23. Carvalho J F S, Cruz Silva M M, Moreira J N, Simões S and Sá e Melo M L 2009 Efficient chemoenzymatic synthesis, cytotoxic evaluation, and SAR of epoxysterols *J. Med. Chem.* **52** 4007 https://doi.org/10.1021/jm9003973
24. Naz S, Kerra R G and Narayanan R 2000 New antiproliferative epoxysecosterols from Pseudopterogorgia americana *Tetrahedron Lett.* **41** 6035 https://doi.org/10.1016/S0040-4039(00)01015-7
25. Joshi P, Misra L, Siddique A A, Srivastava M, Kumar S and Darokar M P 2014 Epoxide group relationship with cytotoxicity in withanolide derivatives from Withania somnifera *Steroids* **79** 19 https://doi.org/10.1016/j.steroids.2013.10.008
26. Misra L, Lal P, Chaurasia N D, Sangwan R S, Sinha S and Tuli R 2008 Selective reactivity of 2-mercaptoethanol with 5β,6β-epoxide in steroids from Withania somnifera *Steroids* **73** 245 https://doi.org/10.1016/j.steroids.2007.10.006
27. Chen W-Y, Chang F-R, Huang Z-Y, Chen J-H, Wu Y-C and Wu C-C 2008 Tubocapsenolide A, a Novel Withanolide, Inhibits Proliferation and Induces Apoptosis in MDA-MB-231 Cells by Thiol Oxidation of Heat Shock Proteins *J. Biol. Chem.* **283** 17184 https://doi.org/10.1074/jbc.M709447200.
28. Kasal A, Buděšínský M, Mareš P, Krištofíková Z, Leitão A J, Sá e Melo M L and Silva M M C 2016 Neurosteroids: Can a 2alpha,3alpha-epoxy ring make up for the 3alpha-hydroxyl group? *Steroids* **105** 12 https://doi.org/10.1016/j.steroids.2015.11.007
29. Segaloff A, Weeth J B, Meyer K K, Rongone E L and Cunningham M E 1962 Hormonal therapy in cancer of the breast. XIX. Effect of oral administration of Δ^1-testololactone on clinical course and hormonal excretion *Cancer* **15** 633 https://doi.org/10.1002/1097-0142(196205/06)15:3%3c633::AID-CNCR2820150327%3e3.0.CO;2-L
30. Blickenstaff R T 1992 *Antitumor steroids* (San Diego: Academic Press Inc.) p. 68 https://doi.org/10.1016/C2012-0-01447-0
31. Garrido M, Bratoeff E, Bonilla D, Soriano J, Heuze Y and Cabeza M 2011 New steroidal lactones as 5α-reductase inhibitors and antagonists for the androgen receptor *J. Steroid. Biochem.* 127 367 https://doi.org/10.1016/j.jsbmb.2011.07.001
32. Savić M P, Djurendić E A, Petri E T, Ćelić A, Klisurić O R, Sakač M N, Jakimov D S, Kojić V V and Penov Gaši K M 2013 Synthesis, structural analysis and antiproliferative activity of some novel D-homo lactone androstane derivatives *RSC Adv.* **3** 10385 https://doi.org/10.1039/C3RA41336E
33. Savić M P, Klisurić O R, Penov Gaši K M, Jakimov D S, Sakač M N and Djurendić E A 2016 Synthesis, structural analysis and cytotoxic activity of novel A- and B-modified D-homo lactone androstane derivative *J. Chem. Crystallogr.* **46** 84 https://doi.org/10.1007/s10870-016-0631-5
34. Savić M P, Ajduković J J, Plavša J J, Bekić S S, Ćelić A S, Klisurić O R, Jakimov D S, Petri E T and Djurendić E A 2018 Evaluation of A-ring fused pyridine D-modified androstane derivatives for antiproliferative and aldo–keto reductase 1C3 inhibitory activity *MedChemComm* **9** 969 https://doi.org/10.1039/c8md00077h
35. Djurendić E, Sakač M, Zaviš M, Gaković A, Čanadi J, Andrić S, Klisurić O, Kojić V, Bogdanović G and Penov Gaši K 2008 Synthesis and biological evaluation of some new A,B-ring modified steroidal D-lactones *Steroids* **73** 681 https://doi.org/10.1016/j.steroids.2008.02.006
36. Djurendić E A, Zaviš M P, Sakač M N, Čanadi J J, Kojić V V, Bogdanović G M and Penov Gaši K M 2009 Synthesis and antitumor activity of new D-seco and D-homo androstane derivatives *Steroids* **74** 983 https://doi.org/10.1016/j.steroids.2009.07.007
37. Djurendić E A, Savić M P, Klisurić O R, Sakač M N, Bogdanović G M, Jakimov D S and Penov Gaši K M 2012 Synthesis, X-ray, structural analysis, and cytotoxic activity of some new androstane D-homo lactone derivatives *Struct. Chem.* **23** 1761 https://doi.org/10.1007/s11224-012-9986-1
38. Daina A, Michielin O and Zoete V 2017 SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules *Sci. Rep-UK* **7** No. 42717 https://doi.org/10.1038/srep42717
39. Egan W J, Merz K M and Baldwin J J 2000 Prediction of drug absorption using multivariate statistics *J. Med. Chem.* **43** 3867 https://doi.org/10.1021/jm000292e
40. Ghose A K, Viswanadhan V N and Wendoloski J J 1999 A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases *J. Comb. Chem.* 1 55 https://doi.org/10.1021/cc9800071
41. Muegge I, Heald S L and Brittelli D 2001 Simple selection criteria for drug-like chemical matter *J. Med. Chem.* **44** 1841 https://doi.org/10.1021/jm015507e

42. Mosmann T 1983 Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays *J. Immunol. Methods* **65** 55 https://doi.org/10.1016/0022-1759(83)90303-4
43. Rárová L, Steigerová J, Kvasnica M, Bartůněk P, Křížová K, Chodounská H, Kolář Z, Sedlák D, Oklestková J and Strnad M 2016 Structure activity relationship studies on cytotoxicity and the effects on steroid receptors of AB-functionalized cholestanes *J. Steroid Biochem.* **159** 154 https://doi.org/10.1016/j.jsbmb.2016.03.017
44. Daina A and Zoete V 2016 A BOILED-Egg to predict gastrointestinal absorption and brain penetration of small molecules *ChemMedChem* **11** 1117 https://doi.org/10.1002/cmdc.201600182