C18-UHPLC ANALYSIS OF RETENTION BEHAVIOR OF NEWLY DESIGNED *O*-ALKYLATED ANDROSTANE DERIVATIVES IN TERNARY MIXTURE METHANOL/ACETONITRILE/WATER

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Abstract
Different new groups of steroid compounds are the center of attention in a grate number of scientific publications since the majority of compounds are being researched as anticancer drugs. One of the most important feature of potential drug is its lipophilicity. Nowadays, ultra high-performance liquid chromatography (UHPLC) is used as one of the most sophisticated techniques for determination of the retention behavior determination of different biologically active compounds. The series of 18 newly designed *O*-alkylated androstane derivatives was investigated in reversed phase (RP)-UHPLC system using ternary mixture methanol/acetonitrile/water. A good agreement between experimentally observed and *in silico* lipophilicity was noticed given that coefficient of determination of 0.8406 was achieved.

Introduction
Improved anticancer activity of different steroid compounds originates from heterocyclic systems with a nitrogen atom in the steroid nucleus [1,2]. Nowadays, different steroids are taken as a relevant scaffold for new anticancer drugs development since they possess suitable physicochemical properties, high selectivity and reduced side effects when applied as drugs. Different steroid derivatives are proven to achieve results in the treatment of hormone independent tumors, reproductive tissues and a variety of tumor cell lines [3-5]. Scientist are constantly working on design and development of safer, effective and target-specific steroid drugs.
Novel alkylaminoethyl derivatives of androstane 3-oximes were synthesized and their cytotoxic effects were evaluated [6]. *In vitro* cytotoxic activity was investigated against several common human tumor cell lines, as well as on normal fetal lung cells (MRC-5) and human foreskin fibroblasts (BJ). Additionally, *in vitro* screening against cytochrome P450 enzymes were conducted. Also, quantitative structure-activity relationship (QSAR) modeling of cytotoxic activity of these compounds towards malignant melanoma cells was conducted [7]. Univariate linear regression (ULR) and multivariate linear regression (MLR) models were presented as well as several support vector machines regression (SVM)-based quantitative structure-activity relationship (QSAR) models. Previously determined cytotoxic activity of the studied androstane 3-oximes against melanoma G-361 cells was used for molecular docking analysis and molecular dynamics of the most promising compounds.
This study represent the extension of research of this group of 18 newly synthesized *O*-alkylated androstane derivatives in terms of characterization regarding their retention behavior and lipophilicity. Using ternary mixture methanol/acetonitrile/water in RP-UHPLC system the retention behavior of investigated compounds was tested and correlated with their *in silico* lipophilicity.

Experimental

The series of the analyzed compounds was synthesized at the Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad [6]. The IUPAC names of the compounds are presented in Table 1. The studied *O*-alkylated androstane derivatives are generally divided into two series: the series I of 17β-hydroxy-17α-(pyridin-2-yl)methylandrost-4-en-(3*E*,*Z*)-one oximes (the compounds **1**-**9**) and the series II of (17*E*,*Z*)-(pyridin-2-yl)methylideneandrost-4-en-(3*E*)-one oximes (the compounds **10**-**18**).

Table 1. The IUPAC names of the analyzed compounds

No.	The IUPAC name
1	17β-Hydroxy-17α-(pyridin-2-yl)methylandrost-4-en-(3*E*)-one oxime
2	17β-Hydroxy-17α-(pyridin-2-yl)methylandrost-4-en-(3*Z*)-one oxime
3	17β-Hydroxy-17α-(pyridin-2-yl)methylandrost-4-en-(3*E*)-one-*O*-[2-(pyrrolidin-1-yl)ethyl] oxime
4	17β-Hydroxy-17α-(pyridin-2-yl)methylandrost-4-en-(3*E*)-one-*O*-[3-(*N*,*N*-dimethylamino)propyl] oxime
5	17β-Hydroxy-17α-(pyridin-2-yl)methylandrost-4-en-(3*E*)-one-*O*-[2-(*N*-methylpyrrolidin-2-yl)ethyl] oxime
6	17β-Hydroxy-17α-(pyridin-2-yl)methylandrost-4-en-(3*E*)-one-*O*-[2-(piperidin-1-yl)ethyl] oxime
7	17β-Hydroxy-17α-(pyridin-2-yl)methylandrost-4-en-(3*E*)-one-*O*-[2-(morpholin-4-yl)ethyl] oxime
8	17β-Hydroxy-17α-(pyridin-2-yl)methylandrost-4-en-(3*E*)-one-*O*-[2-(*N*,*N*-diethylamino)ethyl] oxime
9	17β-Hydroxy-17α-(pyridin-2-yl)methylandrost-4-en-(3*E*)-one-*O*-[2-(*N*,*N*-dimethylamino)ethyl] oxime
10	(17*E*)-(Pyridin-2-yl)methylideneandrost-4-en-(3*E*)-one oxime
11	(17*E*)-(Pyridin-2-yl)methylideneandrost-4-en-(3*Z*)-one oxime
12	(17*E*)-(Pyridin-2-yl)methylideneandrost-4-en-(3*E*)-one-*O*-[2-(pyrrolidin-1-yl)ethyl] oxime
13	(17*E*)-(Pyridin-2-yl)methylideneandrost-4-en-(3*E*)-one-*O*-[3-(*N*,*N*-dimethylamino)propyl] oxime
14	(17*E*)-(Pyridin-2-yl)methylideneandrost-4-en-(3*E*)-one-*O*-[2-(*N*-methylpyrrolidin-2-yl)ethyl] oxime
15	(17*E*)-(Pyridin-2-yl)methylideneandrost-4-en-(3*E*)-one-*O*-[2-(piperidin-1-yl)ethyl] oxime
16	(17*E*)-(Pyridin-2-yl)methylideneandrost-4-en-(3*E*)-one-*O*-[2-(morpholin-4-yl)ethyl] oxime
17	(17*E*)-(Pyridin-2-yl)methylideneandrost-4-en-(3*E*)-one-*O*-[2-(*N*,*N*-diethylamino)ethyl] oxime
18	(17*E*)-(Pyridin-2-yl)methylideneandrost-4-en-(3*E*)-one-*O*-[2-(*N*,*N*-dimethylamino)ethyl] oxime

The chromatographic analysis was carried out by RP-UHPLC system on UHPLC Agilent 1290 Infinity LC System with Diode Array Detector under isocratic conditions with the ZORBAX column Eclipse C18, 95Å, 2.1 × 50 mm, 1.8 μm (1200 bar pressure limit, LC Platform, Low Dispersion UHPLC). Prior to analysis, the compounds were dissolved in methanol (concentration 0.5 mg/mL). Afterwards, the solutions were submerged into ultrasonic bath and filtered by Captiva Econofilter with nylon membrane (25 mm diameter, 0.45 μm pore size, 1000/pk). Temperature in the column was maintained at 25 °C. The injection volume was 10 μL. The flow was set at 0.2 mL/min. The mobile phase used was ternary mixture of methanol, acetonitrile and water (45/45/10 v/v, respectively). The peaks were measured at 210 nm. The chemicals used in analysis are methanol (HPLC gradient grade, Baker), acetonitrile (for HPLC analysis, Acros Organics) and ultrapure water. The retention parameters of the analyzed molecules were expressed as capacity factor (log*k*):

$$\log k = \log((t_r - t_0)/t_0)$$

where t_r is the retention time of a compound and t_0 the dead time (the time of the first disturbance on the chromatogram).

The lipophilicity parameters (log*P*) of the compounds were calculated by ChemBioDraw 13.0 program [8] based on 2D molecular structures.

Results and discussion

One of the representative chromatograms of the analysis of the compound **16** ((17*E*)-(Pyridin-2-yl)methylideneandrost-4-en-(3*E*)-one-*O*-[2-(morpholin-4-yl)ethyl] oxime) is presented in Figure 1, together with its structural formula. The peak detected as the compound of interest is clearly and unambiguously separated from other peaks on the observed chromatogram.

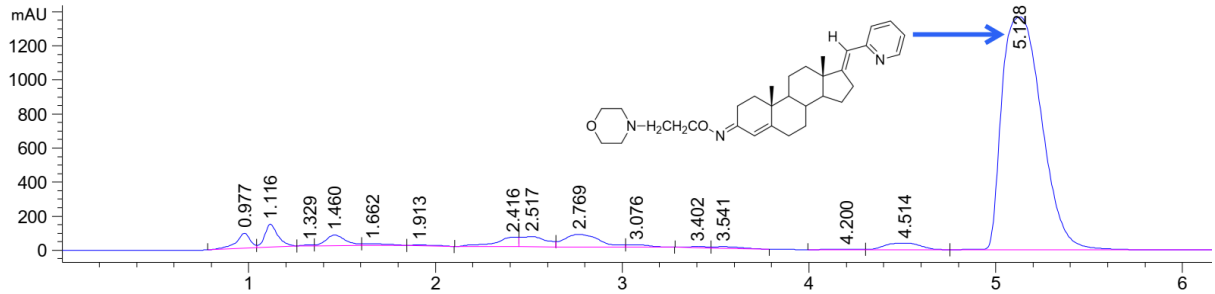

Figure 1. The representative chromatogram of the analysis of the compound **16** ((17*E*)-(Pyridin-2-yl)methylideneandrost-4-en-(3*E*)-one-*O*-[2-(morpholin-4-yl)ethyl] oxime)

Retention and lipophilicity parameters obtained experimentally and *in silico* are presented in Table 2. For all eighteen compounds, dead time, retention time of a compound, capacity factor and lipophilicity parameter are given. It can be noticed that compounds from the series I of 17β-hydroxy-17α-(pyridin-2-yl)methylandrost-4-en-(3*E,Z*)-one oximes (the compounds **1**-**9**) have lower values of log*k* and log*P* parameters, while compounds from series II of (17*E,Z*)-(pyridin-2-yl)methylideneandrost-4-en-(3*E*)-one oximes (the compounds **10**-**18**) have higher values of log*k* and log*P* parameters. According to log*P* parameter values, compound **7** occurs as the one with the lowest lipophilicity (4.07), whilst compound **17** stands out as the one with the highest lipophilicity (5.98).

Table 2. Retention parameters and lipophilicity data of the analyzed androstane compounds

Compounds	t_0	t_r	**log*k***	**log*P***
1	0.974	1.701	-0.127	4.210
2	0.978	1.679	-0.145	4.210
3	1.108	3.257	0.288	4.790
4	0.960	4.296	0.541	4.580
5	1.020	4.746	0.563	4.870
6	1.101	5.328	0.584	5.200
7	1.024	1.717	-0.170	4.070
8	1.099	4.561	0.498	5.150
9	1.054	3.377	0.343	4.470
10	0.965	5.086	0.630	5.040
11	0.963	5.037	0.626	5.040
12	1.001	7.513	0.813	5.620
13	1.000	9.059	0.906	5.410
14	1.100	7.585	0.771	5.710
15	1.100	11.113	0.959	6.040
16	0.977	5.128	0.628	4.910
17	1.009	10.857	0.989	5.980
18	1.034	5.823	0.666	5.310

In Figure 2 the relationship between the lipophilicity (log*P*) and retention parameter (log*k*) of the analyzed series of androstane derivatives is graphically presented. An overview of the distribution of the points around the regression line as well as slope (0.5603) and intercept (-2.3001) are presented.. Very high coefficient of determination (0.8406) indicates the presence of good concurrence between experimentally observed and *in silico* lipophilicity.

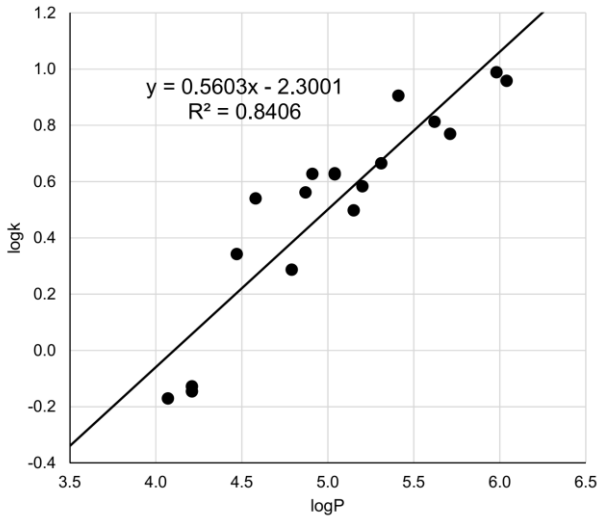

Figure 2. The relationship between the lipophilicity (log*P*) and retention parameter (log*k*) of the analyzed series of androstane derivatives

Conclusion

In the present study, C18-UHPLC analysis of retention behavior of 18 newly designed *O*-alkylated androstane derivatives in ternary mobile phases with methanol and acetonitrile as modifiers was conducted. This study was conducted in order to investigate chromatographic lipophilicity as one of the most important feature of the potential anticancer drug. Results of this study indicate that chromatographic lipophilicity of investigated compounds as future drug candidates of biomedical importance can be successfully correlated with *in silico* lipophilicity descriptor. The lipophilicity parameter can be presented as function of retention value and in that way it reflects the lipophilicity of investigated steroid derivatives.

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