RETENTION BEHAVIOR OF NOVEL *O*-ALKYLATED ANDROSTANE 3-OXIMES IN RP(C18)-UHPLC SYSTEM WITH METHANOL/WATER MOBILE PHASE

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Abstract
The retention behavior of newly synthesized *O*-alkylated androstane 3-oximes has been investigated applying reversed-phase ultra-high performance liquid chromatography (RP-UHPLC) with octadecyl stationary phase and methanol/water mobile phase. The set of the analyzed compounds takes into account two groups of steroidal derivatives: 17α-(pyridin-2-yl)methyl and (17*E*)-(pyridin-2-yl)methylidene derivatives. The retention behavior of the compounds in the applied chromatographic system was determined as logarithm of the capacity factor (*logk*). Afterwards, the *logk* values were correlated with *in silico* lipophilicity (logP) in order to establish the quantitative structure-retention relationship (QSRR) model and to confirm that the obtained retention parameters can be considered chromatographic (anisotropic) lipophilicity parameters. The obtained QSRR model successfully correlates the *logk* and logP parameters (the strong correlation has been determined). Considering the lipophilicity parameters, the analyzed compounds can be considered from moderately to highly lipophilic.

Introduction
Generally, steroid compounds are considered to be an excellent basis for development of new anticancer and anti-inflammatory drugs. Androstane derivatives are biologically active compounds whose biological role can be different depending on substituents present in their structure. The novel series of alkylaminoethyl derivatives of androstane 3-oximes possess significant anticancer activity towards various types of cancer (malignant melanoma - G-361, lung adenocarcinoma - A549 and colon adenocarcinoma - HT-29) [1]. Considering their biological potential, the characterization of physicochemical properties of these compounds, particularly the estimation of their lipophilicity, is of a great importance. Also, the set of these compounds was the subject of quantitative structure-activity relationship (QSAR) analysis and molecular docking and molecular dynamics studies that contributed to the better understanding of their anticancer potential [2].
Chromatography is one of the most used analytical techniques in estimation of anisotropic lipophilicity of biologically active molecules. The most applied techniques used for this purpose are high performance liquid chromatography (HPLC) and thin-layer chromatography (TLC). The retention parameters determined by HPLC and TLC are a good basis for the QSRR analysis in which their correlations with different molecular descriptors are investigated. Experimental determination of the chromatographic lipophilicity by RP-HPLC of the series of *O*-alkylated androstane 3-oximes provides the data about their retention behavior and reveals the retention mechanisms in the applied chromatographic system. The chromatographic system with C18 stationary phase and methanol/water mobile phase is the most common system for determination of the chromatographic lipophilicity.

Lipophilicity is one of the most important molecular features of biologically active compounds. It determines the pharmacokinetic/pharmacodynamic properties and absorption, distribution, metabolism and excretion (ADME). Besides, it plays a significant role in toxic behavior of compounds.

Experimental

The general structures of the analyzed compounds are presented in Fig. 1. The series is divided into two groups of compounds: (1) the group I contains nine 17β-hydroxy-17α-(pyridin-2-yl)methylandrost-4-en-(3*E*,*Z*)-one oximes (compounds **1**-**9**); (2) the group II contains nine (17*E*,*Z*)-(pyridin-2-yl)methylideneandrost-4-en-(3*E*)-one oximes (compounds **10**-**18**). Prior to the RP-UHPLC analysis, the compounds were dissolved in methanol (0.5 mg/ml).

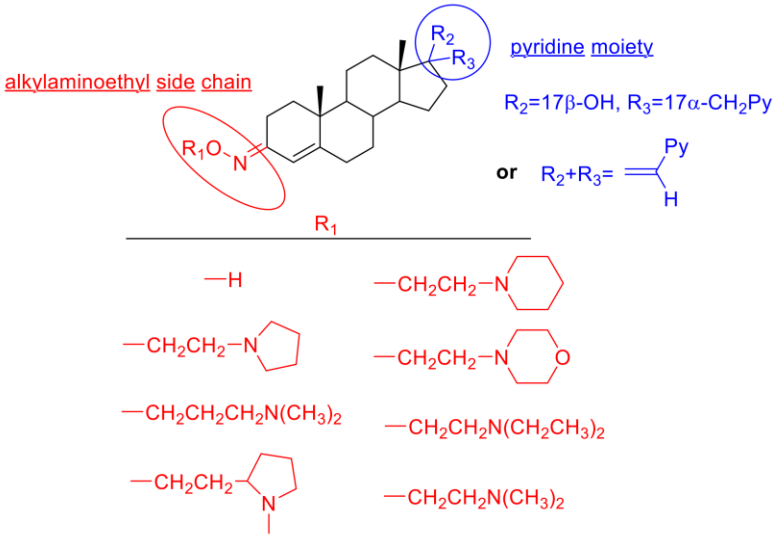

Figure 1. The general molecular structures of the analyzed *O*-alkylated androstane 3-oximes in 17α-(pyridin-2-yl)methyl and (17*E*)-(pyridin-2-yl)methylidene series

The chromatographic analysis was performed on UHPLC Agilent 1290 Infinity LC System with Diode Array Detector under isocratic conditions, by using the column ZORBAX Eclipse C18, 95Å, 2.1 × 50 mm, 1.8 µm (1200 bar pressure limit, LC Platform, Low Dispersion UHPLC) at 25 °C. The flow was set at 0.2 ml/min and the injection volume was 10 µL and. The analysis was carried out by using methanol/water (90/10 v/v) mobile phase. The peaks were recorded at λ_1 = 210 nm. The capacity factor (*logk*) was calculated as follows:

$$logk = \log((t - t_m)/t_m) \quad (1)$$

where t is the retention time of a compound and t_m is the dead time.

The *in silico* lipophilicity parameter (logP) was calculated by using ChemBioDraw 13.0 program.

Results and discussion

The obtained retention parameters indicate that (17*E*,*Z*)-(pyridin-2-yl)methylideneandrost-4-en-(3*E*)-one oximes (group I) have higher retention in the applied chromatographic system than 17β-hydroxy-17α-(pyridin-2-yl)methylandrost-4-en-(3*E*,*Z*)-one oximes (group II). The reason for that might be the fact that the group II possesses higher lipophilicity. In Fig. 4 there are two chromatograms of representative compounds from group I. The compounds **1** and **2** have the

lowest retention since they do not possess highly non-polar substituents. The addition of more non-polar substituents induce the significant increase in the retention time, as it can be seen from Fig. 1. The compound **1** have relatively small retention time comparing to the compound **4** that possesses *N*,*N*-dimethylaminopropyl substituent. The compounds **15** and **17** have the highest retention and the highest lipophilicity.

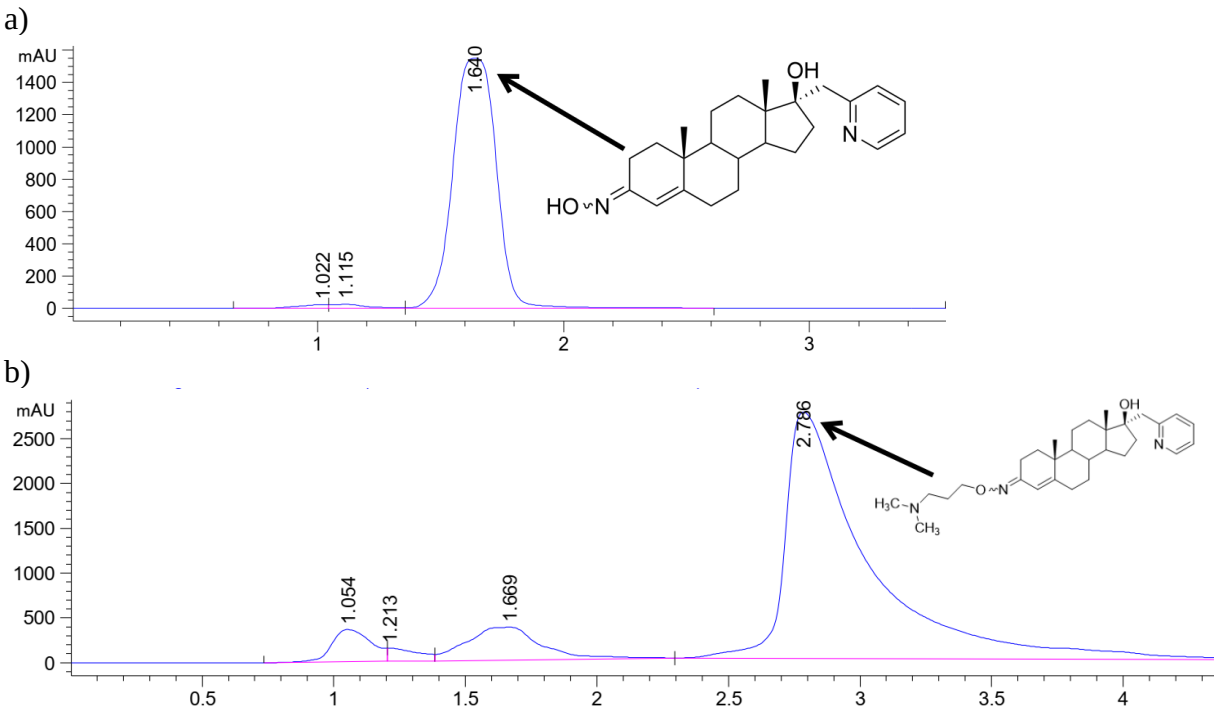

Figure 2. The representative chromatograms of a) the compound **1** (17β-Hydroxy-17α-(pyridin-2-yl)methylandrost-4-en-(3*E*)-one oxime and b) compound **4** (17β-Hydroxy-17α-(pyridin-2-yl)methylandrost-4-en-(3*E*)-one-*O*-[3-(*N*,*N*-dimethylamino)propyl] oxime)

The QSRR model that correlates *in silico* lip ophilicity parameters (logP) with capacity factor of the studied compounds is presented in Fig. 3.

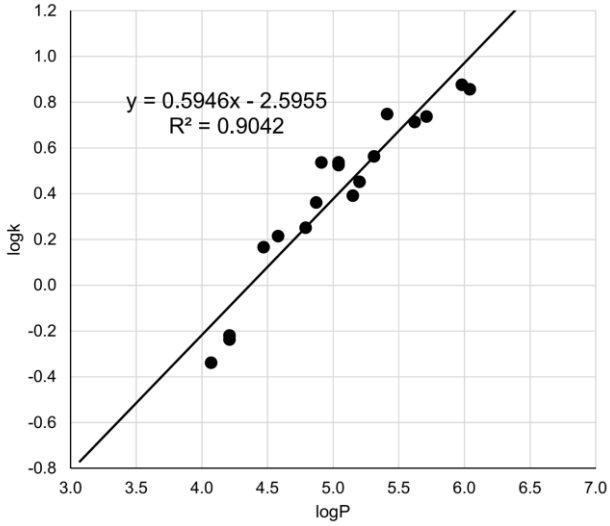

Figure 3. The relationship between the lipophilicity parameter (logP) and retention parameter (*logk*) determined in the applied chromatographic system

The obtained QSRR model indicate the strong linear relationship between logP and *logk* parameters. The determination coefficient is significantly high (R^2 = 0.9042) and no outlier has been detected in the set of the compounds. The obtained model implies that *logk* values, determined in the applied chromatographic system, can be considered the lipophilicity measures of the studies series of novel *O*-alkylated androstane 3-oximes.

Conclusion

The retention behavior of the analyzed series of newly synthesized *O*-alkylated androstane 3-oximes, determined in C18-UHPLC system with methanol/water mobile phase, is well correlated with *in silico* lipophilicity parameters. The experimental retention parameters, expressed as *logk* values, can be considered to be anisotropic lipophilicity of the studied androstane derivatives.

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