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Chemometrics approach based on chromatographic behavior, *in silico* characterization and molecular docking study of steroid analogs with biomedical importance

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ABSTRACT

Physicochemical characterization of steroid analogs (triazole, tetrazole, toluenesulfonylhydrazide, nitrile, dinitrile and dione) is considered to be a very important step in further drug selection. This study applies to the determination of lipophilicity of previously synthesized steroid derivatives using reversed-phase high-performance liquid chromatography (RP HPLC). Chemometric aspect of chromatographic lipophilicity is given throughout multiple linear regression (MLR) quantitative structure-retention relationships (QSRR) approach. Minimal inhibitory concentration (MIC) is determined for two steroid derivatives possessing antimicrobial activity against *Staphylococcus aureus*. Molecular docking study was performed in order to identify the compound with the most promising potential as human cytochrome P450 CYP17A1inhibitor. Identified 3β-hydroxyandrost-5-eno[16,17-*d*]-1,2,3-triazole (**I.2.**) could be recommended for further trials for anticancer drugs and subjected to the absorption, distribution, metabolism, excretion and toxicity (ADMET) evaluation.

1. Introduction

Steroid molecules act as ligands or substrates for hormone receptors and enzymes in various biological processes, cell growth and proliferation (Gupta et al., 2013). A number of steroid derivatives have been synthesized to exert the antiproliferative activity against human cancers (Elmegeed et al., 2011; Abdelhalim et al., 2011). Numerous steroid analogs have been proved to have antiproliferative activity against human prostate cancer cell lines (Attard et al., 2005; Moreira et al., 2008; DeVore and Scott, 2012; Yu et al., 2013; Savić et al., 2013; Richmond et al., 2014). Abiraterone is a 17α-hydroxylase inhibitor, approved by U.S. FDA for the treatment of prostate cancer (Wölfling et al., 2004).

The first step in the evaluation of the possible application of synthesized steroid derivatives is the determination of their physicochemical parameters. One of the most important and most analyzed physicochemical parameter of biologically active compounds is lipophilicity, because the intermolecular interactions in biological systems can be compared with the interactions that occur in chromatographic systems (Sârbu et al., 2012; Kovačević et al., 2015). High-performance liquid chromatography (HPLC) has proved to be a method of choice for lipophilicity determination because of its high throughput ability, good accuracy and low sample consumption. Steroids in medicines and drugs (Lau et al., 2003; Klinsunthorn et al., 2011; Kovačević et al., 2016a), animal feed (Gonzalo-Lumbreras et al., 2007), cosmetics (Gagliardi et al., 2002) and urine (Andersen et al., 2008; Saito et al., 2010) have

Abbreviations: ACN, acetonitrile; ADMET, absorption, distribution, metabolism, excretion and toxicity; Caco2, *in vitro* Caco2 cell permeability (Human colorectal carcinoma); ANN, artificial neural networks; CADD, computer-aided drug design; DAD, diode array detector; ECCEN, eccentric connectivity index; EI, enzyme inhibitor; FMF, fraction of molecular framework; LR, linear regression; MBC, minimal bactericidal concentration; MDCK, *in vitro* MDCK cell permeability (Madin Darby Canine Kidney); MH agar/broth, Muller-Hinton agar/broth; MIC, minimal inhibitory concentration; MinPA, minimal projection area; MLR, multiple linear regression; MM2, molecular mechanics force field; NRL, nuclear receptor ligand; PC, principal component; PCA, principal component analysis; PC agar, plate count agar; PCR, principal component regression; PLS, partial least squares; PR, polynomial regression; QSAR, quantitative structure–activity relationship; QSPR, quantitative structure–property relationship; QSRR, quantitative structure-retention relationships; RMS, root mean square; RMSD, root mean square deviation; RMSE, root mean square error; RP HPLC, reversed-phase high-performance liquid chromatography; SD agar/broth, Sabouraud dextrose agar/broth; SP, skin permeability; SS, stepwise selection; VIF, variance inflation factor

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been studied using HPLC. For chromatographic lipophilicity assessment, logarithm of the retention factor (log*k*) could be used and it can be determined using different software packages (Kovačević et al., 2016a; Virtual Computational Chemistry Laboratory ALOGPS 2.1 Online Program, n.d.). Lipophilicity assessment studies explain more detailed the appearance of molecular lipophilicity phenomenon (Andrić and Héberger, 2015a, 2015b; Andrić et al., 2016).

Microbiological activity of various steroid molecules against bacterial and fungal strains has been published in few papers. Androgen derivatives were tested against *Escherichia coli* and *Staphylococcus aureus* and the absence of antibacterial activity against *S. aureus* was noticed (Figueroa-Valverde et al., 2015). Antimicrobial activity of oxysterols and novel steroidal chalcones was tested against *E. coli*, *S. aureus* and *Candida albicans* and these compounds expressed antimicrobial activity against these two microorganisms (Savage et al., 2002; Shingate et al., 2013; Kakati et al., 2013). Antimicrobial activity of steroids isolated from two *Chromolaena* species and cationic steroid compounds were tested against *E. coli* and *S. aureus* (Taleb-Contini et al., 2003; Epand et al., 2007). Tested steroid derivatives have antimicrobial activity against *E. coli* and *S. aureus*.

As one of the most commonly used techniques in the structure-based drug design, protein/ligand docking occurs. Molecular docking could be defined as a task to identify the ligand conformation with the most favorable binding energy (Tuccinardi et al., 2014). Structure-based drug design has a task to identify and optimize interactions between ligand and their host molecule, such as protein, given their three-dimensional structures (Bissantz et al., 2010). Docking a small molecule to a bioactive macromolecule in order to measure the interactions between them and visualize the optimal pose of ligand-protein complex is a widely used method in computer-aided drug design (CADD). Molecular docking is widely used for protein inhibitors binding models (Erić et al., 2012; Randjelović et al., 2013; Kozielewicz et al., 2014). Based on molecular docking results, a potential mechanism of action of a selected compound is proposed and further development of this steroid derivative as an anticancer agent is discussed.

The retention behavior of investigated steroid analogs such as triazole, tetrazole, toluenesulfonylhydrazide, nitrile, dinitrile and dione on reversed phase HPLC was investigated (Jevrić et al., 2017). As the investigated steroid analogs have biomedical importance, chromatographic behavior and *in silico* molecular modeling contribute to their characterization and better understanding of the interactions in given chromatographic system.

Therefore, the aim of this study was to assess the chromatographic lipophilicity and select the molecular characteristics that mostly contribute to the behavior of the analyzed steroid analogs in the applied chromatographic system using multiple linear regression (MLR) quantitative structure-retention relationships (QSRR) approach. As the studied steroid derivatives have biomedical importance, the evaluation of the binding affinity of the selected steroid derivatives with the best potential as human cytochrome P450 CYP17A1inhibitor was investigated.

2. Material and Methods

2.1. Investigated Steroid Analogs

The synthesis procedures of the investigated compounds and their antiproliferative activity (IC_{50}) toward androgen receptor negative prostate cancer cell line (PC-3) have been published earlier (Sakač et al., 2008; Sakač et al., 2009; Penov-Gaši et al., 2013; Nikolić et al., 2015). The IUPAC names and chemical structures of the analyzed analogs are presented in Table 1. The set of twenty-nine studied steroid analogs is divided into four groups according to their substituents. The substituents that occur within these four groups are: OH (hydroxyl), O (oxo), AcO (acetoxy) and BnO (benzyloxy) functional groups. The investigated molecules are grouped in the following way: triazole and tetrazole (**I.1 – I.7**), toluenesulfonylhydrazide (**II.8 – II.11**), nitrile and dinitrile (**III.12 – III.27**) and dione (**IV.28** and **IV.29**).

2.2. Chromatographic Instrumentation and Chemicals

Agilent Technologies 1200 Series HPLC system (Santa Clara, California, USA) with diode array detector (DAD) was used for chromatographic analysis. HPLC system contained binary pump, degasser and automatic injector connected to a computer for data processing with AgilentChemStation program. As the stationary phases, chromatographic column ZORBAX Eclipse Plus C8, Rapid Resolution HT (2.1 × 100 mm, 1.8 μm) (Santa Clara, California, USA) was used. Acetone used for dissolving and acetonitrile for mobile phase were both HPLC grade, purchased from J. T. Baker (Deventer, Netherlands). Ultrapure water was obtained using Millipore, Elix UV system and Simplicity Water Purification System (Molsheim, France).

2.3. Chromatographic Procedure

All investigated compounds were dissolved in acetone to obtain a concentration of 1 mg/mL and filtered throughout Captiva Econofilter (nylon membrane, 25 mm diameter, 0.45 μm pore size) (Santa Clara, California, USA). A binary mixture of acetonitrile and water was used as a mobile phase, with acetonitrile volume fractions in the range of 60–90 v/v. Isocratic elutions were performed at the flow rate and injection of 1.0 mL/min and 0.1 μL, respectively. The column temperature was kept at 30 °C. Detection of compounds on DAD was done at 210 nm. Retention data were expressed as the logarithm of retention factor (log*k*) values, defined by Eq. (1), and they were used for chromatographic lipophilicity modeling. All analyses were done in triplicate.

$$\log k = \log\left(\frac{t_r - t_0}{t_0}\right) \quad (1)$$

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

2.4. Microorganisms and Growth Conditions

Dichloromethane used for dissolving the investigated compounds was purchased from Lach-Ner (Neratovice, Czech Republic). Laminar-flow Biobase Biosafety BSC-1100IIA2-X chamber, Jinan Biobase Biotech Co., Ltd., (Jinan, China) was used for manipulation with microorganisms, as well as for dichloromethane evaporation. The bacterial strains used for this study were: *Escherichia coli* ATCC 25922 (gram-negative) and *Staphylococcus aureus* ATCC 25923 (gram-positive), while *Candida albicans* ATCC 10231 was chosen as test yeast strain. All microorganisms were maintained according to ISO11133:2014 standard. Muller-Hinton broth (MHB, HiMedia, Mumbai, India) for bacterial strains and Sabouraud dextrose broth (SDB, HiMedia, Mumbai, India) was inoculated with working cell suspension made from the stocked strains that are kept in deep freezer at − 80 °C (Snijders Labs, Tiliburg, Holland) and incubated at 37 °C.

2.5. Antimicrobial Assay

A modified microdilution method (standard broth micro-dilution technique as per CLSI guidelines) in a series of glass test tubes (7.5 × 80 mm) was used to determine the minimum inhibitory concentration (MIC) of the investigated steroid analogs (CLSI, 2002; CLSI, 2012). Investigated compounds were dissolved in cooled dichloromethane in eppendorf tubes in the initial concentration of 10 mg/mL. From the initial solution serial dilution (1:1) was made up to the final concentration 0.078 μg/mL by using the same solvent. For one test microorganism 50 μL of substance solution was used. After transferring