European Journal of Pharmaceutical Sciences 92 (2016) 194–202

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Retention data of bile acids and their oxo derivatives in characterization of pharmacokinetic properties and *in silico* ADME modeling

Jovana Trifunović [a,*], Vladan Borčić [a], Saša Vukmirović [a], Svetlana Goločorbin Kon [b], Momir Mikov [a]

[a] Department of Pharmacology, Toxicology and Clinical Pharmacology, Medical Faculty, University of Novi Sad, Hajduk Veljkova 3, 21000 Novi Sad, Serbia
[b] Department of Pharmacy, Medical Faculty, University of Novi Sad, Hajduk Veljkova 3, 21000 Novi Sad, Serbia

ARTICLE INFO

Article history:
Received 10 February 2016
Received in revised form 11 July 2016
Accepted 12 July 2016
Available online 14 July 2016

Keywords:
Bile acids
Oxo derivatives of bile acids
ADME
In silico correlation
Multiple linear regression

ABSTRACT

Purpose: Information on ADME properties of examined bile acids and their oxo derivatives are scarce, although the interest for bile acids and their use in nanochemistry and macromolecular chemistry is increasing. The purpose of this research was to evaluate the lipophilicity, a crucial physicochemical parameter for describing ADME properties of selected bile acids and their oxo derivatives, and to compare two approaches: experimentally determined hydrophobicity parameters and calculated log*P* values.

Methods: Commercially available bile acids - deoxycholic, chenodeoxycholic, hyodeoxycholic and ursodeoxycholic acid were used to synthesize oxo derivatives. Lipophilicity was evaluated in two solvent systems: toluene/ethanol and toluene/butanol. Retention parameters were acquired by normal-phase TLC. The correlations between calculated log*P* values obtained using five different software and experimentally determined hydrophobicity parameters ($R_M{}^0{}_{(tol/eth)}$, $R_M{}^0{}_{(tol/but)}$, $b_{(tol/eth)}$ and $b_{(tol/but)}$) were examined.

Results: Correlation analysis confirmed significant dependence between experimental R_M^0 values and software calculated parameters. Results suggest satisfactory intestinal absorption after oral administration for all of the examined compounds as well as low volumes of distribution, and high affinity for binding with plasma proteins. Penetration through blood-brain barrier and skin is not satisfactory. All of the examined compounds show high affinity for binding with G-protein coupled receptors and consequently inhibition of ionic channels. Results also suggest possible binding with nuclear receptors.

Conclusions: Established lipophilicity testing model of studied compounds showed excellent predictive ability and might represent significant tool in development of relations between chromatographic behavior and ADME properties. Compounds 3α-hydroxy-7,12-dioxo-5β-cholanoic and 12α-hydroxy-3,7-dioxo-5β-cholanoic acid might be the most suitable candidates for further development studies (satisfactory pharmacokinetic properties and lowest haemolytic potential) followed by 3α-hydroxy-12-oxo-5β-cholanoic acid and 3α-hydroxy-7-oxo-5β-cholanoic acid (slightly higher haemolytic potential, but better ligand properties).

1. Introduction

Bile acids are amphiphilic compounds, composed of a steroid structure with four rings, and they represent surface active substances (Calabresi et al., 2012). Surface activity of bile acids is enabled due to differences between two sides of the basic steroid ring α-side, which is perceived as concave side and β-side, which is defined as convex side (Armstrong and Carey, 1982). Bile acids synthesis is one of the most important routes of cholesterol metabolism in mammals. Two pathways are significant in the synthesis of bile acids: classical and acidic pathway. Both of these pathways contribute in forming of primary bile acids, cholic acid (CA) and chenodeoxycholic (CDCA). Primary bile acids further undergo modifications of their chemical structure *via* conjugation with amino acids which leads to increasing hydrophobicity of the molecule. After secretion into the lumen of intestine, conjugated bile acids become exposed to bacterial degradation and removal of glycine and taurine part, which allows production of secondary bile acids, deoxycholic acid (DCA) and lithocholic acid (LCA). All of synthesized bile acids can be returned to liver and re-secreted *via* enterohepatic circulation (Chiang, 2009; Heusser and Wurthier, 1947; Hirofuji, 1965).

Abbreviation: log*P*, logarithm of partition coefficient; R_M^0, intercept; *b*, slope of the linear plot; C, volume fraction of the organic solvent in the mobile phase; TPSA, total polar surface area; natoms, number of atoms; M_w, molecular weight; nOH, number of hydrogen bond donors; nOHNH, number of hydrogen bond acceptors; nviol, number of violations; nrotb, number of rotatable bonds; V, molecular volume; logBB, logarithm of the blood-brain barrier partition coefficient; HIA, human intestinal absorption in percent; MDCK, *in vitro* Mandin Darby Canine Kidney cell permeability; PPB, plasma protein bound in percent; PE, jejunum pH = 6, 5 (cm/s); V_d, human volume of distribution (l/kg); SP, skin permeability; GPCR, G-protein coupled receptor ligand; ICM, Ion channel modulator; NRL, Nuclear receptor inhibitor; PI, protease inhibitor.

* Corresponding author.
E-mail addresses: jovanatrifunovic@gmx.com, jovanatrifunovic@uns.ac.rs (J. Trifunović).

http://dx.doi.org/10.1016/j.ejps.2016.07.011
0928-0987/

Presence of rigid steroid skeleton and amphiphilic properties has made them very attractive as building blocks in pharmaceutical chemistry and supramolecular chemistry (Sepe et al., 2014; Vasovic et al., 2014). Hydroxyl groups localized on α side of the molecule are easily accessible for further modifications what implies their use for attaching different binding moieties. There are many examples of synthesized conjugates of bile acids with various drugs, which become substances with improved properties such as low molecular heparin-bile acids conjugates, bile acids cisplatin complex and polyaminocarboxylate chelator attached to bile acid residue (Kim et al., 2011; Paschke et al., 2000; Chong et al., 2009; Anjul et al., 2010; Glanzer et al., 2015).

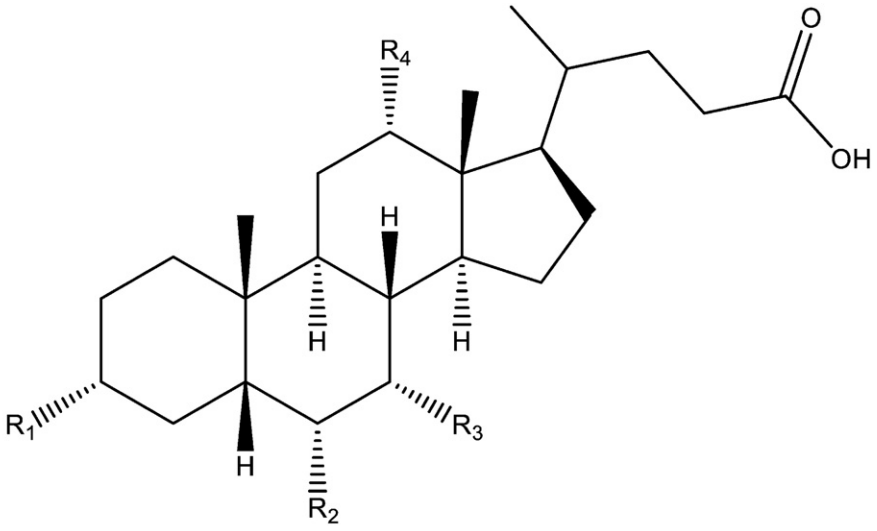
Rise of interest concerning the role and mechanism of action of bile acids has been noticed in the past few decades. In the past, bile acids were mostly considered to be acting in the intestine where they play a role in digestion of fats and mediate absorption of fat-soluble vitamins. Recent studies confirm that bile acids do not only facilitate solubilization of fats but also behave as signal molecules that interact with various receptors including nuclear receptors and G protein-coupled receptors (Coplea and Tiangang, 2016; Sepe et al., 2014).

As amphipathic molecules they are able to interact with phospholipids of cells membranes and enhance drugs permeation. Thus, bile acids are considered as drug promoters in buccal, ocular, nasal, and transdermal dosage forms (Eskandar et al., 2015). In latest pharmaceutical studies their dual nature has been exploited to improve delivery of physiologically active compounds which are practically water insoluble (such as amphotericin B, resveratrol, and oxaprozin) (Elnaggar, 2015). Different types of nanocarriers are investigated as nanoemulsions, nanoparticles, nanogels and liposomes to overcome major obstacles of drug delivery.

Beside classical bile acids, their derivatives, called oxo bile acids, have recently been increasingly investigated. Oxo bile acids possess one, two or three keto groups as a part of the molecule and show less toxic effects in cells that regular bile acids. They are especially significant for their hemolytic potential, which is significantly lower compared to deoxycholic and chenodeoxycholic acid (Poša and Kuhajda, 2010). The chemical structures of thirteen investigated bile acids and their oxo derivatives used in this study are presented in Fig. 1.

Lipophilicity (hydrophobicity) is an essential intrisic physicochemical factor for predicting specific behavior of a compound in passive diffusion through biological membranes. This molecular parameter is connected with biological activity of components and it is also crucial for determining other important physicochemical properties. Therefore, lipophilicity is key descriptor in investigating correlation between biological activity and structure in modern QSAR and QSPR studies. Lipophilicity is usually related with polarity, hydrogen bonding of the molecule and molecular weight. Widely accepted reference system for the quantification of lipophilicity is the logarithm of partition coefficient (log*P*) in an octanol/water two-liquid phase system (Nasal et al., 2003).

While the conventional method for calculating log*P* is prone to flaws and lacks the necessary reproducibility, there are specifically developed



Name of bile acid	Groups
Deoxycholic acid (1)	R_1=OH ,R_2=H, R_3=H, R_4=OH
Chenodeoxycholic acid (2)	R_1=OH, R_2=H, R_3=OH, R_4=H
3α-hydroxy-12-oxo-5β-cholanoic acid(3)	R_1=OH, R_2=H, R_3=H, R_4=O
3α-hydroxy-7-oxo-5β-cholanoic acid(4)	R_1=OH, R_2=H, R_3=O, R_4=H
3α-hydroxy-7,12-dioxo-5β-cholanoic(5)	R_1=OH, R_2=H, R_3=O, R_4=O
12α-hydroxy-3,7-dioxo-5β-cholanoic acid(6)	R_1=O, R_2=H, R_3=O, R_4=OH
3,7,12-Trioxo-5β-cholanoic acid(7)	R_1=O, R_2=H, R_3=O, R_4=O
Hyodeoxycholic acid(8)	R_1=OH, R_2=OH, R_3=H, R_4=H
Ursodeoxycholic acid(9)	R_1=OH ,R_2=H, R_3=OH(β), R_4=H
3,12-dioxo-5β-cholanoic acid(10)	R_1=0, R_2=H, R_3=H, R_4=O
3,7-dioxo-5β-cholanoic acid (11)	R_1=0, R_2=H, R_3=0, R_4=H
3α , 7α-dihdroxy-12-oxo- 5β-cholanoic acid(12)	R_1=0H, R_2=H, R_3=OH, R_4=O
3α , 12α-dihdroxy-7-oxo- 5β-cholanoic acid (13)	R_1=0H, R_2=H, R_3=O, R_4=OH

Fig. 1. Structure of the investigated bile acids and their oxo derivatives.