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Docking-based analysis and modeling of the activity of bile acids and their synthetic analogues on large conductance Ca^{2+} activated K channels in smooth muscle cells

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Abstract. – OBJECTIVE: The objective of this study was to perform docking-based analysis of bile acid binding on the protein complex of channels and to derive neural network that predicts the influence of bile acids and their synthetic analogues on the activity of BK(Ca) channels in smooth muscle cells based on descriptors for bile acids and their synthetic analogues and on their already published activities using patch-clamp techniques.

MATERIALS AND METHODS: Ligands for molecular docking were optimized using computer routine for minimization of energy by using the force field MMFF94 *via* Chem3D 15.0 and ligands and protein channel complex were prepared in AutoDockTools 1.5.6. AutoDock Vina 4.0 software was used for blind docking; processing and verification of the obtained results was performed *via* Discovery Studio 4.0. Neural network was derived using descriptors for bile acids and their synthetic analogues and their already published activities on calcium-activated K^+ channels in smooth muscle cells (*ChemDraw Professional 15.0*, Dragon 6 software).

RESULTS: Molecular docking was performed for: lithocholic acid, deoxycholic acid, 5β-cholanoic acid, 3β-hydroxi-5β-cholanoic acid, henodeoxycholic acid, ursocholic acid and α-muricholic acid. Neural network model Multiple layer perceptron is derived, having 0.9259 training performances and 0.3673 test performances, training error 0.0073 and test error 0,1607. Model was tested for henodeoxycholic, ursocholic and α-muricholic acid, and internal validation of the model is performed.

CONCLUSIONS: Molecular docking suggested that the pharmacophore for maximizing the activity of BK(Ca) channels in the steroid skeleton of bile acids is the C3 quasi-axial α-OH group and the C24 carboxyl function. Derived neural network model successfully predicted activities of tested bile acids on Ca^{2+} activated K^+ channels in smooth muscle cells.

Introduction

Bile acids have been primarily associated to cholesterol metabolism in the liver, stimulation of cholesterol, fat-soluble vitamins and lipid absorption from the intestines[1] but lately, bile acids have gained increasing recognition as signaling molecules in homeostasis of triglycerides, cholesterol, glucose and energy[2-6].

Lipophilicity of bile acids and their salts, expressed with the partition coefficient, depends on the number, position, and orientation of hydroxyl groups[7,8] and determines their interaction with the receptors, enzymes, ionic channels, cell membranes, etc.[9]. Chemical modifications of the functional groups yield bile acid derivatives with different lipophilicity, oxidation of hydroxyl groups to oxo groups leads to derivatives with less tendency for self-aggregation, less membranolytic activity[10], and a decrease in the solubilization power of cholesterol[11].

Calcium-activated K^+ channels BK(Ca) can be synergistically activated by both voltage and intracellular Ca^{2+}, with a large carboxy-terminal intracellular portion responsible for Ca^{2+} sensitivity[12]. Activity of these channels is proved to be increased in vascular smooth muscle due to fatty acid acids[13]. Also, natural bile acids and their synthetic analogues reversibly increase BK(Ca) channel activity in rabbit mesenteric artery smooth muscle cells[14], probably as the result of a direct interaction of bile acid with the duct complex itself or with a closely related membrane component[15]. It has also been reported[16] that higher is the possibility of direct activation of BKCa channels *via* bile acid in rabbit smooth muscle cells, which suggests that increasing BKCa channel activity is

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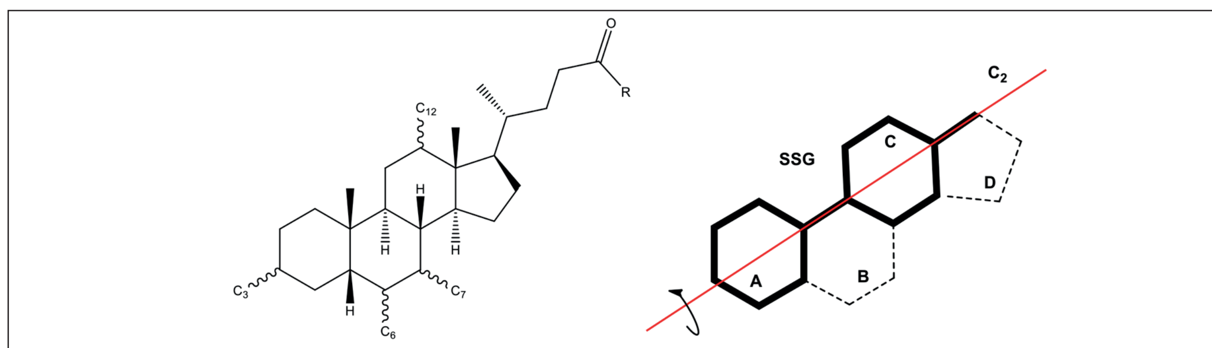

Figure 1. Steroid ring system and the largest planar symmetric steroid subgraph (SSG).

at least one of the mechanisms by which bile acids can affect peripheral vascular resistance.

Given that bile acids and their synthetic analogues affect the activity of BK(Ca) channels in smooth muscle cells[14], the objective of this study was to perform docking-based analysis of bile acid binding on the protein complex of channels and to derive neural network that predicts influence of bile acids and their synthetic analogues on the activity of BK(Ca) channels in smooth muscle cells based on descriptors for bile acids and their synthetic analogues and on their already published activities using patch-clamp techniques.

Materials and Methods

Bile Acids and Their Derivatives

For analysis of the influence of bile acids and other analogues on the increase in BK(Ca) channel activities in smooth muscle following molecules are selected (Figure 1, Table I).

Molecular Docking Studies

The molecular docking material was taken from electronic databases: .sdf files of ligands (lithocholic, 5-β-holanic and 3-β-hydroxy-5-β-cholanic acid and α-muricholic acid, deoxycholic acid, henodeoxycholic acid, ursocholic acid (PubChem

Table I. The list of studied bile acids and their synthetic analogues.

	Trivial names	Substituents				
		C3	C6	C7	C12	R
(1)	Lithocholic acid	α-OH	/	/	/	OH
(2)	Deoxycholic acid	α-OH	/	/	α-OH	OH
(3)	Cholic acid	α-OH	/	α-OH	α-OH	OH
(4)	Taurine conjugate of lithocholic acid	α-OH	/	/	/	NH (CH2) 2SO3H
(5)	Methyl ester of cholic acid	α-OH	/	α-OH	α-OH	OCH3
(6)	3,7,12,24-tetrahydroxi-5β-cholane	α-OH	/	α-OH	α-OH	/
(7)	7α,12α - dihydroxi-5β-cholanoic acid	/	/	α-OH	α-OH	OH
(8)	3-hemisuccinat of lithocholic acid	-OCO(CH_2)$_2$ COOH	/	/	/	OH
(9)	Methyl ester of ursodeoxicholic acid	α-OH	/	β-OH	/	OCH3
(10)	3-Epideoxycholic acid	β-OH	/	/	α-OH	OH
(11)	5β-cholanoic acid	/	/	/	/	OH
(12)	3β-hydroxi-5β-cholanoic acid	β-OH	/	/	/	OH
(13)	3β,7β,12β-trihydroxi-5β-cholanoic acid	β-OH	/	β-OH	β-OH	OH
(14)	Henodeoxycholic acid	α-OH	/	α-OH	/	OH
(15)	Ursocholic acid	α-OH	/	β-OH	α-OH	OH
(16)	α-muricholic acid	α-OH	β-OH	α-OH	/	OH

ID: 9903, 92803, 164853, 5283852, 222528, 10133, 122340) were downloaded from PubChem, the three-dimensional crystal structure of the Ca^{2+} activated K^+ channel was taken from the Protein Data Bank (PDB ID: 3NAF) in the form of .pdb files.

Ligands were optimized using computer routine for minimization of energy by using the force field MMFF94 *via* Chem3D 15.0 (PerkinElmer, 2011.) and ligands and protein channel complex were prepared in *AutoDockTools* 1.5.6 (Scripps Research, 2007).

AutoDock Vina 4.0 software (Scripps Research, 2009) was used for blind docking and determination of binding affinity for protein channel complex and processing and verification of the obtained results was performed *via Discovery Studio* 4.0 (BIOVIA Discovery Studio 2016) and through which images of binding of the given ligands and BKCa channels were obtained. Docking protocol was validated using redocking studies with known ligands of Ca^{2+} activated K^+ channel.

Neural Network Analysis

Derived descriptors for bile acids and their analogues (*ChemDraw Professional 15.0* (PerkinElmer, 2011), Dragon 6 software (Talete, 2010.) are standardized, and descriptive statistic is performed in order to find correlations between descriptors and activities of examined molecules on BK(Ca) channels. Due to low level of correlation, 53 decriptors are chosen with the coefficient of correlation with molecule activity higher than 0.4. Selected descriptors are from following groups: Walk and path counts (20), Connectivity indices (4), Information indices (4), 2D matrix-based descriptors (1), 2D autocorrelations (18), Burden eigenvalues (5), Molecular properties (1).

As predictors in neural network are used published data: mean values of the increase in BK(-Ca) activity for molecules examined bile acids and synthetic analogues recorded by the imposed patch clamp method in three sub-methods: Cell-attached patch (CA), Inside-out patch (IO) and Outside-out patch (OO [14]): molecules (1), (2), (3), (4), (5), (8), (10), (11), (13) for training, molecules (6), (9) for test, molecules (1), (7) for validation (Table I), (STATISTICA 8 (StatSoft, 200.).

Results

Docking Results

The molecular docking results for following ligands: lithocholic acid (1), deoxycholic acid (2), 5β- cholanoic acid (11), 3β-hydroxi-5β-cholanoic acid (12), henodeoxycholic acid (14), and α-muricholic acid (16) and published [or predicted result for molecule (16)] in increase in BK(Ca) channel activity are presented in Table II and Figure 2.

Based on the results of molecular docking amino acids od proteins mainly involved in interactions with bile acids and their analogues are TYR332, GLU388, GLY333.

Neural Network Results

Neural network model Multiple layer perceptron is derived, having 0.9259 training performances and 0.3673 test performances, training error 0.0073 and test error 0,1607. Model is then used to predict bile-acid induced increase in BK(-Ca) channel activity for henodeoxycholic acid (99.60%), for ursocholic acid (83.65%) and for α-muricholic acid (84.64%).

Internal validation of the neural network model is performed by the method leave one out. Results of the validation show that molecules (13) and (6) have the least influence for the construction of the neural network model. If molecule (13) 3β,7β,12β-trihydroxi-5β-cholanoic acid is left out, neural network modell whose training performance id 0.9799, test performance -0.4927, training error 0.0036 and test error is 0.1489. Also, if molecule (6) 3,7,12,24- tetrahydroxi-5β-cholan is left out model with similar performances is derived: training performance 0.9582, test performance +0.3946, training error 0.0069 and test error 0.188. By leaving out other molecules of the training group in the process of internal validation, models with poorer performance than the initial one were obtained.

Discussion

Previously published results[16] concerning activity of bile acids and their derivatives of the increase in activity of BK(Ca) channels suggests that all examined bile acids and their synthetic analogues except 3β-hydroxi-5β-cholanoic acid (12) increase the activity of BK(Ca) channels. 3β-hydoxi-5β-cholanoic acid has β-oriented hydroxyl group on the position 3, and if we compare it with derivatives (10) and (13) with the same structural element (C3 β-OH group), and show examined activity, we can conclude that the presence of C3 β-OH group is not reason for inactivity if there are some more hydroxyl groups present in the molecule [for molecule (10) that is C12 α-OH group

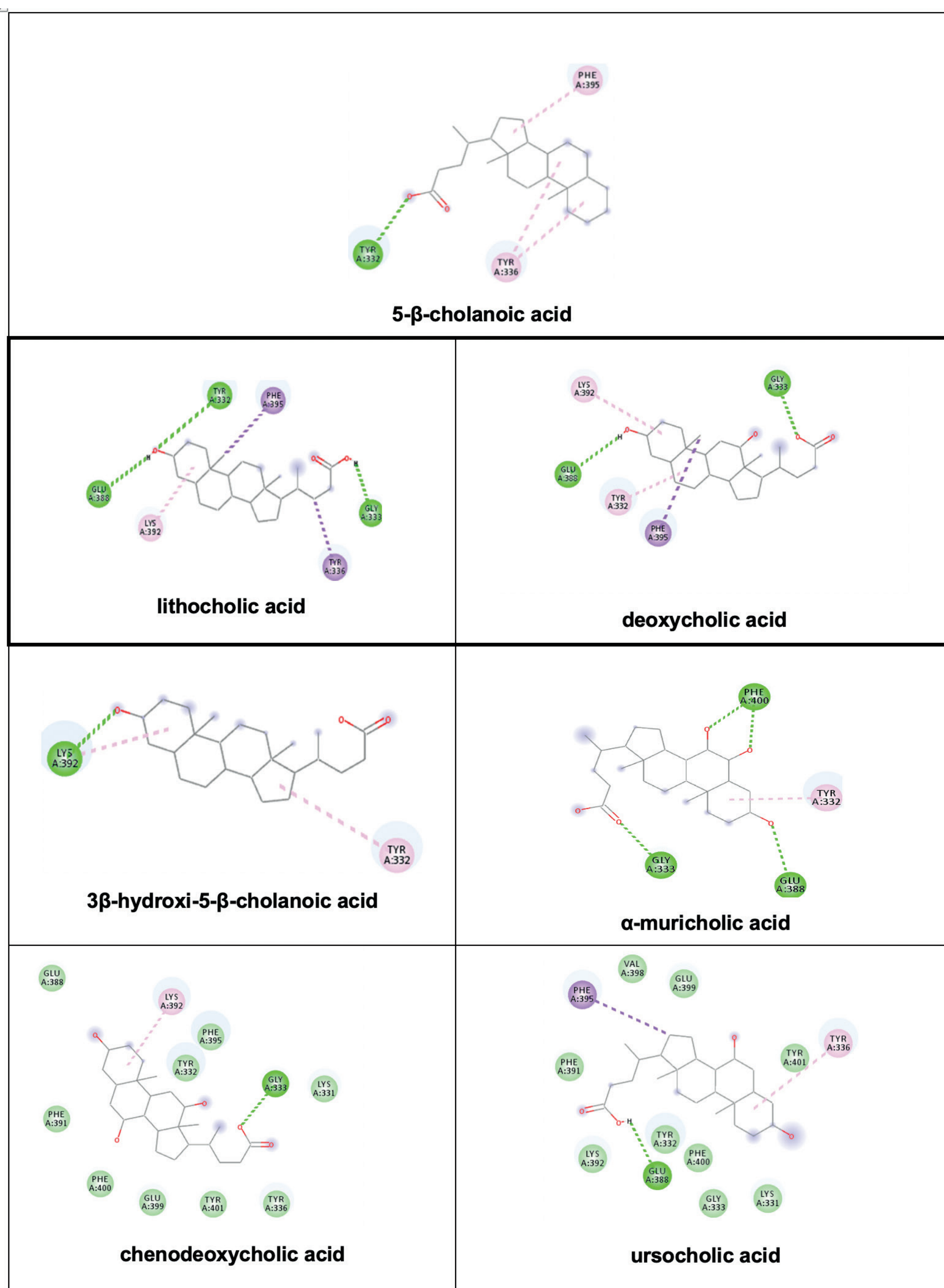

Figure 2. Ligands and protein binding interaction.

and in molecule (13) are present C7 β-OH and C12 β-OH]. Very low influence on the increase of activity of BK(Ca) channels shows molecule (11), 5β-cholanoic acid, that has no hydroxyl group, and it is the most hydrophobic molecule. However, we cannot conclude that hydrophobicity of examined molecules is associated with the increase in activity of BK(Ca) channels since among examined structures are molecules of different polarity and similar effect [specifically for cholic acid (3) it has

Table II. Molecular docking results and results in increase in BK(Ca) channel activity.

Molecule	Binding energy [kcal/mol]	Funcional part of ligand	Interactions Protein complex	Type of interaction	Increase in BK(Ca) channel activity (%)
(1)	-8,2	C3- α-OH	TYR332,GLU388	hydrogen bond	
		C24-OH	GLY333	hydrogen bond	
		C19-CH3	PHE395	pi-sigma	100
		C22-CH3	TYR336	pi-sigma	
		A ring	LYS392	pi-alkyl	
(2)	-8,1	C3- α-OH	GLU388	hydrogen bond	
		C24-OH	GLY333	hydrogen bond	100
		C19-CH3	PHE395	pi-sigma	
		A ring	LYS392	pi-alkyl	
		B ring	TYR332	pi alkyl	
(11)	-8,6	C24-OH	TYR332	hydrogen bond	
		A-ring	TYR336	pi-sigma	24.6
		B-ring	TYR336	pi-sigma	
		D-ring	PHE395	pi-sigma	
(12)	-7,9	C3-β_OH	LYS392	hydrogen bond	0
		D-ring	TYR336	pi-sigma	
(14)	-7,8	C24-OH	GLY333	hydrogen bond	99.6
		A-ring	LYS392	alkyl	
(15)	-8,5	C24-OH	GLU388	hydrogen bond	
		A-ring	TYR336	pi-alkyl	84.65
		C16	PHE395	pi-sigma	
(16)	-8,7	C3-α-OH	GLU388	hydrogen bond	
		C6-β-OH	PHE400	hydrogen bond	84.64
		C7-α-OH	PHE400	hydrogen bond	
		C24-OH	GLY333	hydrogen bond	
		A-ring	TYR332	pi-alkyl	

been shown to show slightly lower activity than deoxycholic (2) and lithocholic (1), and the most polar of them].

Molecular docking (MD) is performed for molecules from Table II. Lithocholic acid (1) and deoxycholic acid (2) are secondary bile acids synthetized in the gut microbiota from primary bile acids (chenodeoxycholic and cholic acid). According to MD, the following common amino acid units of the tested ion channel participate in the binding to BK(Ca) channels in these bile acids: GLU388, GLY333 (forms hydrogen bond with the bile acids' C3 α-OH group and the OH group of the bile acids' C24 carboxyl group) and PHE395 (it has been shown to have pi-sigma interaction with bile acids) (Table II). In terms of the energy of individual interactions, hydrogen bonds are certainly the most significant. These interactions make an energetic contribution to the initiation of the cascade of conformational changes of the ion channel which leads to an increase in its activity – bile acids (salts) as allosteric modulators of the activity of BK(Ca) channels. Therefore, lithocholic acid and deoxycholic acid increase the activity of BK(Ca) channels to the greatest extent.

Primary bile acid chenodeoxycholic acid (14) binds to the examined ionic channel over hydrogen bonds between C24-OH steroid group and amino acid residue GLY333 of BK(Ca) ionic channel [with the same OH group also bind (1) and (2) for the same amino acid residue of the ionic channel] whereby the increase in the activity of BK(Ca) channels is almost the same as with the secondary bile acids (1) and (2).

Geometry of binding of chenodeoxycholic acid for BK(Ca) ionic channel is identical to the geometry of binding of deoxycholic and lithocholic acid. The main axis of symmetry (C_2) of the largest planar symmetric steroid subgraph (SSG)[17] of chenodeoxycholic acid related to amino acid residue GLY333 and LYS392 of the examined ionic channel has the same orientation as C_2 axis od SSG of deoxycholic acid (Figure 1 and Table II), while C_2 axis of deoxycholic SSG and C_2 axis of SSG of lithocholic acid have the same orientation related to the amino acid residue GLU388,

GLY333. Ursocholic acid (15) and α-muricholic acid (16) in humans are present as metabolites of primary bile acids (intestinal flora) but in much smaller amounts than deoxycholic and lithocholic acid[18] [(16) is the primary bile acid in mice]. These two bile acids also bind for amino acid residue that participate in the binding of (1) and (2) (Figure 2 and Table II). However, the lower value of the increase in BK(Ca) channel activation in derivatives (15) and (16) can be explained by the deviation of the binding geometry of these bile acids in relation to the binding geometry of deoxycholic and lithocholic acid to the examined ion channel.

The axis of symmetry C_2 of SSG ursocholic acid is rotated by 180° in relation to the same axis of deoxycholic and lithocholic acid SSG, while α-muricholic acid [same binding geometry as (1) and (2)] when binding to BK(Ca) channels forms new hydrogen bonds that are not present in the binding of deoxycholic and lithocholic acid.

These new hydrogen bonds act antagonistically on the conformational changes in the examined ion channel induced by hydrogen bonds which are realized through the amino acid residues GLU388, GLY333. Epilitocholic acid (11), i.e., the C3 epimer of lithocholic acid (trace present in human feces), in BK(Ca) channels, does not bind by hydrogen bonding to any of the key amino acid residues GLU388, GLY333.

Therefore, there is no increase in the activity of the examined ion channel, therefore it probably acts as a partial antagonist for other bile acids. 5β-cholanoic acid (11) (also trace present in human feces) has the opposite binding geometry to the BK(Ca) channel with respect to deoxycholic and lithocholic acid, somewhat increasing the activity of the tested ion channel. Namely, it binds over hydrogen bond to TYR332 as well as lithocholic acid.

The largest increase in the activity of BK(Ca) channels occurs with deoxycholic acid and lithocholic acid, i.e., BK(Ca) channels were most adapted to the conformation (spatial distribution of OH and COOH groups) of secondary bile acids in humans. The biological role of increased activation of the examined ion channels by secondary bile acids is probably reflected (hypothetically) in the fact that, during digestion of primary bile salts secreted into the small intestine (i.e., into the duodenum), after some time, due to bacterial intestinal form, are transformed into (1) and (2), which are included in the enterohepatic circulation certainly when the resorption of nutrients is nearing completion or complete, i.e., when the concentration of nutrients in the blood decreases. However, then the secondary bile salts (most effectively) increase the activity of vascular BK(Ca) channels in smooth muscle cells.

This relaxes the vascular smooth muscle and reduces vascular resistance. This causes an increase in blood flow in the arterioles, so the cells of certain tissues in a unit of time are exposed to a larger volume of blood, i.e., higher amount of nutrients at their already low concentration (in the blood). Therefore, the role of secondary bile acids (1) and (2) through increasing the activity of BK(Ca) channels may be in increasing the efficiency in the supply of tissues with nutrients after the completion of the process of digestion-resorption.

The pharmacophore necessary for the maximum increase in the activity of BK(Ca) channels is the C3 quasi-axial α-OH group and the C24 carboxyl function. Other OH groups in the steroid skeleton of some bile acid (bound to different C atoms in different spatial orientations) may not alter the action of this pharmacophore or be weak.

Model of artificial neural network is derived for predicting increase in activity of BK(Ca) channels influenced by bile acids and their derivatives and tested for chenodeoxycholic acid, ursocholic acid, and α-muricholic acid in order to estimate how the number and position of hydroxyl groups present in a molecule influence their activity. It is estimated that chenodeoxycholic acid shows high increase of the activity of BK(Ca) channels similar as for example deoxycholic acid (2) that also has two α-oriented OH groups. Based on the model ursocholic acid (15) shows a little bit lower activity than cholic acid (3), only difference in their structures is that ursocholic acid in the position 7 has β-oriented OH group, and cholic acid possess C7 α-OH group. However, ursocholic acid (12) has a bit lower activity than the methyl ester of ursodeoxycholic acid (9) that has, besides C3 α-OH group also C7 β-hydroxyl group, so we cannot conclude that the presence of β-OH group in the position 7 responsible for the decrease of activity of ursocholic acid. For α-muricholic acid is predicted to possess similar activity as ursocholic acid, which is expected.

Conclusions

Molecular docking suggested that, for bile acids and their analogues, common binding places on protein are amino acids TYR332, GLU388, GLY333 and the pharmacophore for maximiz-

ing the activity of BK(Ca) channels in the steroid skeleton of bile acids is the C3 quasi-axial α-OH group and the C24 carboxyl function. Derived neural network model successfully predicted activities of teste bile acids on Ca^{2+} activated K^{+} channels in smooth muscle cells.

Conflicts of Interest

The authors declare no conflicts of interest.

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