

# *In silico* screening of natural products targeting chorismate synthase

Detección *in silico* de productos naturales dirigidos a la corismato sintasa

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## SCIENTIFIC RESEARCH

### How to cite this paper:

Al-Khayyat M, *In Silico* screening of natural products targeting chorismate synthase. *Innovaciencia*. 2019; 7 (1): 1-13. <http://dx.doi.org/10.15649/2346075X.505>

### Reception date:

Received: 20 January 2019  
Accepted: 16 May 2019  
Published: 25 October 2019

### Keywords:

Amentoflavone; Docking; Homology modeling; Pharmacokinetics.

## ABSTRACT

**Introduction:** Chorismate synthase catalyzes the final step in shikimate acid pathway involved in synthesis of aromatic compounds in bacteria. This enzyme can be a possible molecular target for design of antibiotics. **Materials and Methods:** Homology modeling and molecular docking were performed to screen about one hundred natural compounds in order to find inhibitors of enzymes as a possible new target. A model was built by SWISS-MODEL and its quality was assessed by ERRAT, ProSA, Rampage and MolProbity servers. Docking experiments were performed and pharmacokinetics and toxicities were studied by admetSAR. **Results:** The predicted model was reliable to be used in docking experiments. Amentoflavone had the highest binding affinity of -10.0 Kcal/mol. Probabilities indicated that rotenone may inhibit P-glycoprotein I, hinokiflavone and silybin may inhibit P-glycoprotein II, while taspine acts on both types of P-glycoproteins. Amentoflavone, hinokiflavone, rotenone and silybin have a probability of inhibiting cytochromes that are involved in oxidation stage of metabolism. **Conclusions:** These compounds had binding affinities towards FMN binding site of the enzyme model and may be considered in the research for new antibacterial agents but only when their drug interactions are fully investigated.

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## INTRODUCTION

*Staphylococcus aureus* is a Gram positive bacterium capable of causing infections in human ranging from superficial lesions such as cutaneous and subcutaneous abscesses to deep infections involving bones, lung as well as toxic shock syndrome (1,2). This is due to its ability to invade and colonize tissues through expression of numerous virulence factors like exfoliative toxins, haemolysins, leukocidins, protein A and clumping factor (1,3).

In 1961, *S. aureus* developed resistance to the antibiotic methicillin and later on, to other members of penicillin group, the  $\beta$ -lactams. This methicillin resistant *S. aureus* (MRSA) strains acquired a *mecA* gene encoding to an altered penicillin binding protein, PBP2a which is less susceptible to  $\beta$ -lactams antibiotics including methicillin (4). In Iraq, a study carried out by Al-Dahbi and Al-Mathkhury (5) on nasal isolates of *S. aureus* in a hospital found that MRSA constitutes about 94.3%. All the one hundred and six isolates were resistant to penicillin G and 94.3% of them were resistant to cefoxitin. These isolates had also 29.2-50% of resistance to ciprofloxacin, gentamicin, tetracycline, erythromycin and co-trimoxazole.

Due to antibiotic resistance, alternative targets are being considered upon which new antibiotics could be designed. Dias et al., (6) suggested that chorismate synthase may be a target. This enzyme is a component of the shikimic acid pathway which is responsible for the biosynthesis of aromatic amino acids in bacteria but not in human. Chorismate synthase catalyzes the formation of chorismate from 5-enolpyruvyl shikimate-3-phosphate using flavin mononucleotide (FMN) as a cofactor (7,8). In this study, homology modeling and molecular docking are carried out on chorismate synthase of *S. aureus* subsp. *aureus* MRSA252 (9) in order to identify ligands that are capable of binding the active site of the enzyme where FMN binds as a preliminary step in screening for enzyme inhibitors.

## MATERIALS AND METHODS

### Sequence retrieval

Amino acid sequence of chorismate synthase of *S. aureus* subsp. *aureus* MRSA252 (9) was obtained from

GeneBank database having ID: CAG40475.1, which can be accessed at <http://www.ncbi.nlm.nih.gov/protein/cag40475.1>.

### Binding site prediction

Binding site was predicted by (a) RaptorX (10) maintained by Xu Group at University of Chicago available at: ([raptorx.uchicago.edu](http://raptorx.uchicago.edu)). (b) GalaxyWEB web server for protein structure prediction, refinement and related methods at Computational Biology Lab., Department of Chemistry, Seoul University. The server uses LIGPLOT (11). This binding prediction tool can be accessed at: (<http://galaxy.seoklab.org/cgi-bin/submit.cgi?type=SITE>).

### Homology modeling

The 3 dimensional structure of the enzyme, chain A was built by SWISS-MODEL (12) maintained by Protein Structure Bioinformatics Group at Swiss Institute of Bioinformatics and Biozentrum, University of Basel, Basel, Switzerland. It is available at (<http://swissmodel.expasy.org>).

### Quality assessment of the model

The accuracy of the model was assessed by four online tools; (a) ERRAT (13), this tool analyzes interaction of non-bound heavy atoms and comparing it with refined structures, available at (<http://servicesn.mbi.ucla.edu/ERRAT/>) at Molecular Biology Institute, University of California, Los Angeles. (b) PROSA (14), to determine if the protein fold lies within the range of experimentally determined protein structures accessed at (<https://prosa.services.came.sbg.ac.at/prosa.php>) The site is maintained by Center of Applied Molecular Engineering, Department of Biosciences, University of Salzburg. (c) Ramachandran plot analysis by RAMPAGE (15). The site is maintained by Crystallography and Bioinformatics Group at Department of Biochemistry, School of Biological Sciences, University of Cambridge, available at: (<http://mordred.bioc.cam.ac.uk/~rapper/rampage.php>). (d) MolProbity structure validation (16) from Department of Biochemistry, School of Medicine, Duke University. The software can be accessed at: (<http://molprobity.biochem.duke.edu/>).

### Ligand selection

The chemical structures of the control, Flavin mononucleotide (FMN) and one hundred natural products were obtained from ZINC database (17), at (<http://>

zinc.docking.org/). This database also provides the molecular properties; mass, H-bond donors, H-bond acceptors and polar surface area.

### Molecular docking

Molecular docking was performed by AutoDock Vina (18) designed by Oleg Trott from Molecular graphics Laboratory at the Scripps Research Institute, La Jolla, California. The Autogrid tool was employed to pre-calculate a grid. This grid has a size of 66×66×66 and a box center of -8.118, 1.819 and 10.049 for x, y and z, respectively. Rigid protein-ligand docking was performed by Hex 8.0.0 using settings: grid dimension = 0.6, docking solutions = 100, an initial steric scan at N = 16, a final search at N = 26, receptor and ligand range 180 degrees (Ritchie and Venkatraman, 2010). Interactions between ligand and the enzyme model were visualized by LIGPLOT+ (11).

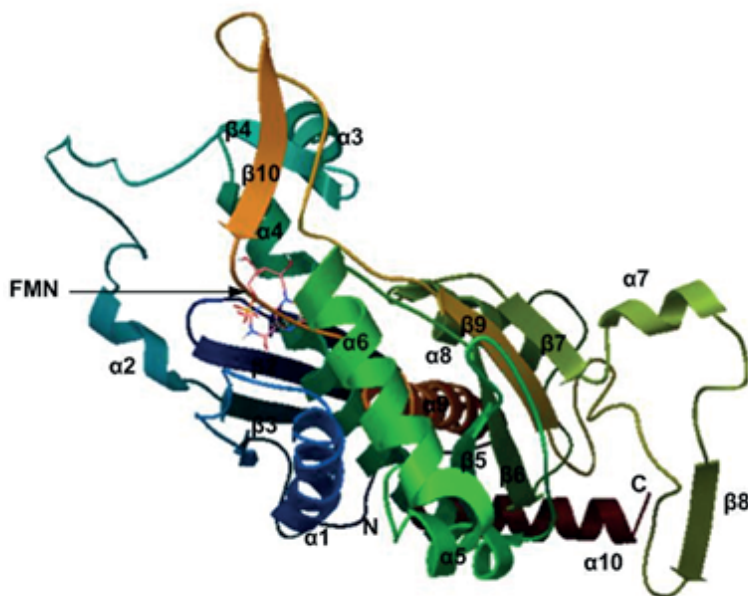
### Pharmacologic properties of the compounds

The pharmacokinetics and toxicity profiles (ADMET) of the compounds were predicted by admetSAR (19) at (<http://lmmd.ecust.edu.cn/admetSar1>). The computed parameters were: (1) human intestinal absorption (HIA), (2) blood-brain barrier (BBB) penetration, (3) human colon adenocarcinoma (Caco-2) permeability, (4) plasma glycoprotein (P-gp) substrate and inhibition probabilities, (5) renal organic cationic transporter protein (OCT2) inhibition, (6)

Cytochromes (CYP P450), substrate and inhibition probabilities. Toxicities were predicted by (1) carcinogenic activity and (2) acute rat toxicity (ART).

## RESULTS AND DISCUSSION

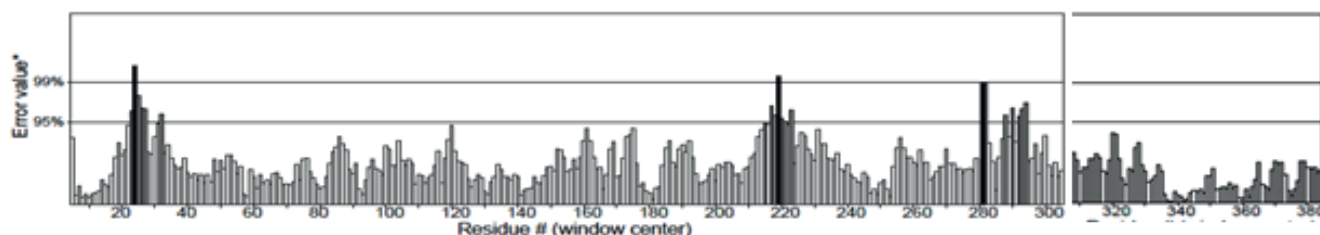
The three dimensional structure of chorismate synthase, chain A was built by SWISS-MODEL based on the template of *Streptococcus pneumoniae* serotype 4, PDB ID: 1xqo.1.A which had a resolution of 2.00 Å with a sequence similarity of 0.45. Figure 1 shows the model. Maclean and Ali (20) solved the X-ray structure of chorismate synthase of *S. pneumoniae* and found that (a) it is a tetramer of 2 dimer subunits, (b) Thr<sup>315</sup> or sometimes Ser is a conserved amino acid in the enzyme structure, (c) The amino acid His<sup>110</sup> forms Hydrogen bonding with O<sub>2</sub> of FMN, (d) the hydrophobic interactions of Ala<sup>342</sup>, Ala<sup>345</sup>, Ala<sup>346</sup>, Ile<sup>313</sup>, Met<sup>310</sup> mediates flexibility required to accommodate FMN molecule, and (e) O<sub>2</sub> of FMN also forms coordinate bonding with the side chain of Asn<sup>251</sup>. Binding site prediction by RaptorX server for FMN revealed the following amino acid residues of the model: Arg<sup>45</sup>, Gly<sup>111</sup>, His<sup>112</sup>, Ala<sup>135</sup>, Ile<sup>250</sup>, Asn<sup>251</sup>, Ala<sup>252</sup>, Met<sup>310</sup>, Lys<sup>311</sup>, Ile<sup>313</sup>, Pro<sup>314</sup>, Thr<sup>315</sup>, Ala<sup>342</sup>, Ala<sup>345</sup> and Ala<sup>346</sup>, while GalaxyWEB server predicted that FMN forms H bond with His122 and hydrophobic interactions with Arg<sup>39</sup>, Ala<sup>135</sup>, Ile<sup>250</sup>, Asn<sup>251</sup>, Met<sup>310</sup>, Ile<sup>313</sup>, Pro<sup>314</sup>, Ala<sup>342</sup> and Ala<sup>345</sup>.



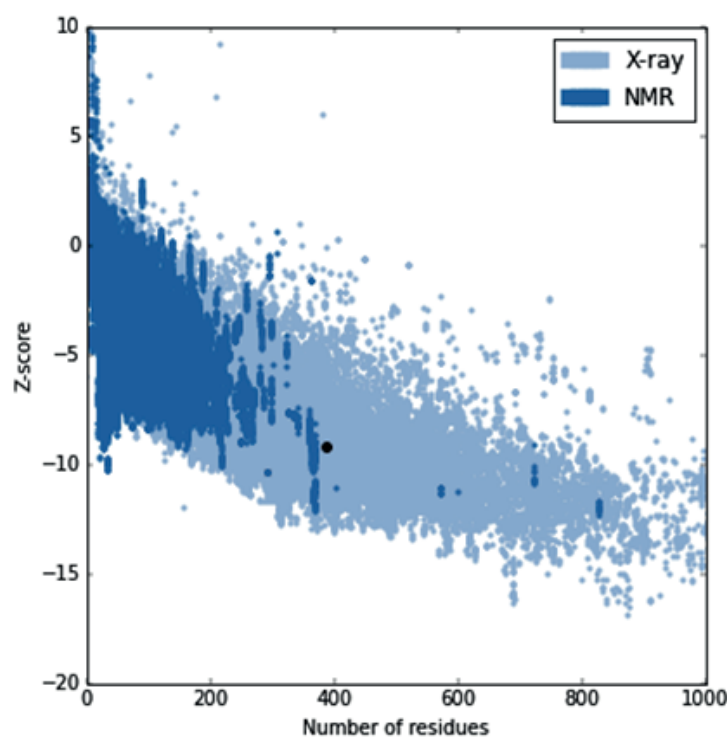
**Figure 1.** The Three dimensional structure of Chorismate synthase as predicted by SWISS-MODEL. Flavin mononucleotide is shown in its binding site. Visualized by Python Molecular Viewer, Sanner (21).

The model was evaluated by four on line methods. It has an overall quality of 94.7% in ERRAT (Figure 2). In ProSA, the model has a Z-score of -9.19; hence, it lies within the range of X-ray experimentally solved structures (Figure 3). In Ramachandran plot analysis (Figure 4), the number of residues in favored region was 367 (95.1%) and the number of the residues in allowed region was 16 (4.1%), but

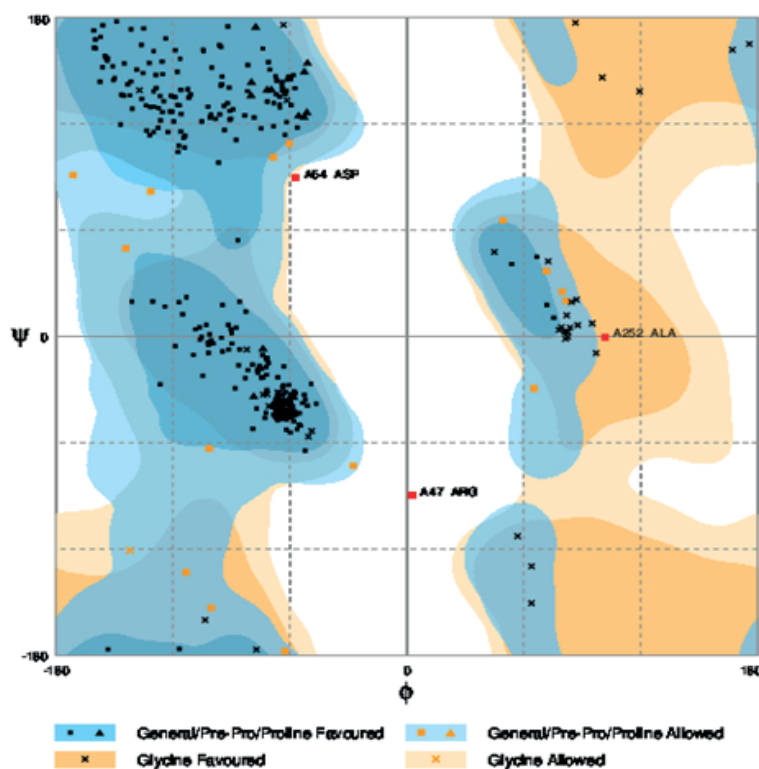
3(0.8%) of residues were in the outlier region. Normally, it is expected that 98% of the residues should lie in the favored region and about 2.0% should be in the allowed region. However, MolProbity server indicates that 364 (94.3%) of the residues are in favored regions and only 4 are outliers (Table 1). The model has good quality since more than 90% of the residues lie in the favored region <sup>(22)</sup>.



**Figure 2.** ERRAT analysis of the model. Black bars represent misfolded regions. On the error axis the two lines indicate the confidence in which it is possible to reject regions.



**Figure 3.** ProSA prediction of the model (black dot) which lies within the normal range of X-ray determined experimental structures.



**Figure 4.** Ramachandran plot of the model using Rampage. Amino acid residues of the model that lie in the disallowed regions (outliers) are marked by red squares.

**Table 1.** Structure validation by Molprobit server

Protein Geometry	Poor rotamers	9	2.76%	Goal: <0.3%
	Favored rotamers	299	91.72%	Goal: >98%
	Ramachandran outliers	4	1.04%	Goal: <0.05%
	Ramachandran favored	364	94.30%	Goal: >98%
	C $\beta$ deviations >0.25Å	2	0.57%	Goal: 0
	Bad bonds:	0 / 3061	0.00%	Goal: 0%
Bad angles:	27 / 4126	0.65%	Goal: <0.1%	
Peptide Omegas	Cis Prolines:	0 / 13	0.00%	Expected: $\leq 1$ per chain, or $\leq 5\%$
	Cis nonProlines:	1 / 374	0.27%	Goal: <0.05%

Molecular docking was performed using AutoDock Vina for one hundred natural products and the highest ten that had binding affinities more than FMN are presented in Table 2 with their interactions. Figure 5 shows the interactions with FMN, while Figure 6 shows the Hydrogen bonding and hydrophobic interactions between amentoflavone and residues of the predicted model. Amentoflavone forms interactions with Arg<sup>39</sup>, Ala<sup>135</sup>, Asn<sup>251</sup>, Ile<sup>313</sup>, Thr<sup>315</sup>, Ala<sup>342</sup> and Ala<sup>345</sup> that are also predicted as components of the binding residues by RaptorX and GalaxyWEB.

However, when redocked by Hex 8.0.0 which uses another method of estimating docking scores, only amentoflavone, hinokiflavone, apocynin A and vicenin had docking scores higher than FMN, the control.

Figure 7 illustrates the chemical structures of the best ten ligands, while their physiochemical properties are presented in Table 3. Kelder et al., <sup>(23)</sup> concluded that a drug should have a polar surface area (PSA) of 120 Å<sup>2</sup> or less but require a PSA of 60-70

A<sup>o2</sup> to cross the blood brain barrier and have an action on the brain. Good oral absorption occurs when the molecular weight of a drug candidate is in the range 150-550 and logP values between 0-3 (24). Rutin and vicenin have their molecular weights higher than 550. Four compounds only had their logP values in the range of 0-3. These are scutellarin, apocynin A,

silybin and taspine. Lipinski et al., (25) proposed that a drug will be poorly absorbed when its molecular weight > 500, logP > 5, H-bond donors > 5 and H-bond acceptors > 10; however, antifungals and antibacterial agents seem that do not obey this rule and also those classes of drugs that are substrate for drug transporters inside human body.

**Table 2.** Molecular docking of the model with natural products

Compound	Binding affinity (Kcal/mol) by AutoDock Vina	Residues of the model forming		Total energy (Kcal/mol) by Hex 8.0.0
		Hydrogen bonds (length in A°)	Hydrophobic interactions	
FMN (control)	-8.0	Ser <sup>9</sup> (2.84, 3.17), Arg <sup>45</sup> (2.90), Arg <sup>109</sup> (2.70), Ser <sup>134</sup> (3.06)	Arg <sup>47</sup> , Arg <sup>48</sup> , His <sup>112</sup> , Ala <sup>135</sup> , Arg <sup>136</sup> , Ile <sup>313</sup> , Thr <sup>315</sup> , Asp <sup>339</sup>	-337.60
Amentoflavone	-10.0	Ser <sup>9</sup> (3.20), Arg <sup>39</sup> (2.82,3.05), Asn <sup>251</sup> (2.70), Thr <sup>315</sup> (2.70), Ser <sup>338</sup> (3.15)	His <sup>10</sup> , Arg <sup>45</sup> , Arg <sup>48</sup> , Met <sup>49</sup> , Arg <sup>109</sup> , Leu <sup>130</sup> , Ser <sup>134</sup> , Ala <sup>135</sup> , Arg <sup>136</sup> , Ala <sup>252</sup> , Met <sup>310</sup> , Ile <sup>313</sup> , Asp <sup>339</sup> , Ala <sup>342</sup> , Ala <sup>345</sup>	-351.04
Scutellarin	-9.4	Arg <sup>45</sup> (3.24), Ser <sup>9</sup> (2.76,2.95), Ser <sup>134</sup> (3.04), Asp <sup>339</sup> (2.84)	Arg <sup>48</sup> , Met <sup>49</sup> , His <sup>112</sup> , Ser <sup>133</sup> , Ala <sup>135</sup> , Arg <sup>136</sup> , Asn <sup>251</sup> , Ala <sup>252</sup> , Ile <sup>313</sup> , Pro <sup>314</sup> , Ala <sup>342</sup>	-303.62
Rutin	-9.3	Ser <sup>9</sup> (2.79), Arg <sup>45</sup> (2.58,2.97), Arg <sup>48</sup> (3.30), Ser <sup>134</sup> (3.16), Thr <sup>138</sup> (3.17), Asn <sup>251</sup> (3.08), Thr <sup>315</sup> (2.52), Asp <sup>339</sup> (3.23)	Arg <sup>47</sup> , Met <sup>49</sup> , Ala <sup>135</sup> , Arg <sup>136</sup> , Ile <sup>250</sup> , Phe <sup>253</sup> , Ile <sup>313</sup> , Arg <sup>337</sup> , Ser <sup>338</sup> , Ala <sup>342</sup> , Ala <sup>345</sup> , Ala <sup>346</sup> , Val <sup>349</sup>	-326.52
Hinokiflavone	-9.1	Ala <sup>252</sup> (3.29,2.88)	Arg <sup>45</sup> , His <sup>112</sup> , Ala <sup>113</sup> , Ser <sup>133</sup> , Ala <sup>135</sup> , Val <sup>248</sup> , Ser <sup>249</sup> , Ile <sup>250</sup> , Asn <sup>251</sup> , Ile <sup>313</sup> , Pro <sup>314</sup> , Asp <sup>339</sup> , Ala <sup>342</sup>	-389.63
Isoquercitrin	-8.9	Ser <sup>9</sup> (2.84), Arg <sup>45</sup> (3.15,2.42), Ser <sup>134</sup> (3.02,3.11), Thr <sup>138</sup> (2.82), Asn <sup>251</sup> (3.01), Thr <sup>315</sup> (2.77)	Arg <sup>48</sup> , Met <sup>49</sup> , His <sup>112</sup> , Ala <sup>135</sup> , Arg <sup>136</sup> , Ile <sup>250</sup> , Phe <sup>253</sup> , Met <sup>310</sup> , Ile <sup>313</sup> , Asp <sup>339</sup> , Ala <sup>342</sup> , Ala <sup>346</sup> , Val <sup>349</sup>	-333.32
Apocynin A	-8.8	Arg <sup>39</sup> (3.06,3.34)	Arg <sup>45</sup> , Arg <sup>47</sup> , Met <sup>49</sup> , Arg <sup>109</sup> , His <sup>112</sup> , Leu <sup>130</sup> , Ala <sup>135</sup> , Arg <sup>136</sup> , Ile <sup>313</sup> , Thr <sup>315</sup> , Ile <sup>335</sup> , Asp <sup>339</sup> , Ala <sup>342</sup>	-350.35
Rotenone	-8.7	Arg <sup>109</sup> (2.92), His <sup>112</sup> (3.01), Ser <sup>134</sup> (2.80), Thr <sup>315</sup> (3.01)	Ser <sup>9</sup> , His <sup>10</sup> , Arg <sup>45</sup> , Ala <sup>135</sup> , Met <sup>310</sup> , Ile <sup>313</sup> , Pro <sup>134</sup> , Asp <sup>334</sup> , Ala <sup>342</sup> , Ala <sup>346</sup>	-305.07
Silybin	-8.6	Arg <sup>45</sup> (2.97), Gly <sup>111</sup> (2.86), Ser <sup>133</sup> (2.98), Ser <sup>134</sup> (2.98), Lys <sup>311</sup> (3.08), Asp <sup>339</sup> (2.73)	His <sup>112</sup> , Ala <sup>113</sup> , Ala <sup>135</sup> , Val <sup>248</sup> , Ser <sup>249</sup> , Ile <sup>250</sup> , Asn <sup>251</sup> , Ala <sup>252</sup> , Ile <sup>313</sup> , Pro <sup>314</sup> , Ala <sup>342</sup>	-381.76
Taspine	-8.6	Arg <sup>45</sup> (2.80,3.30), His <sup>112</sup> (2.85), Thr <sup>315</sup> (2.86)	Arg <sup>48</sup> , Ala <sup>135</sup> , Thr <sup>138</sup> , Ile <sup>250</sup> , Asn <sup>251</sup> , Ala <sup>252</sup> , Met <sup>310</sup> , Ile <sup>313</sup> , Pro <sup>314</sup>	-297.38
Vicenin	-8.3	Ser <sup>9</sup> (2.71), Arg <sup>109</sup> (2.74,3.38), His <sup>112</sup> (3.22), Ser <sup>134</sup> (3.0), Thr <sup>315</sup> (3.14)	Asp <sup>339</sup> , Ala <sup>342</sup> , Ala <sup>346</sup> , Val <sup>349</sup> , Arg <sup>48</sup> , Met <sup>49</sup> , Leu <sup>130</sup> , Ala <sup>135</sup> , Arg <sup>136</sup> , Met <sup>310</sup> , Ile <sup>313</sup> , Ile <sup>335</sup> , Ala <sup>342</sup> , Ala <sup>345</sup> , Ala <sup>346</sup>	-342.53

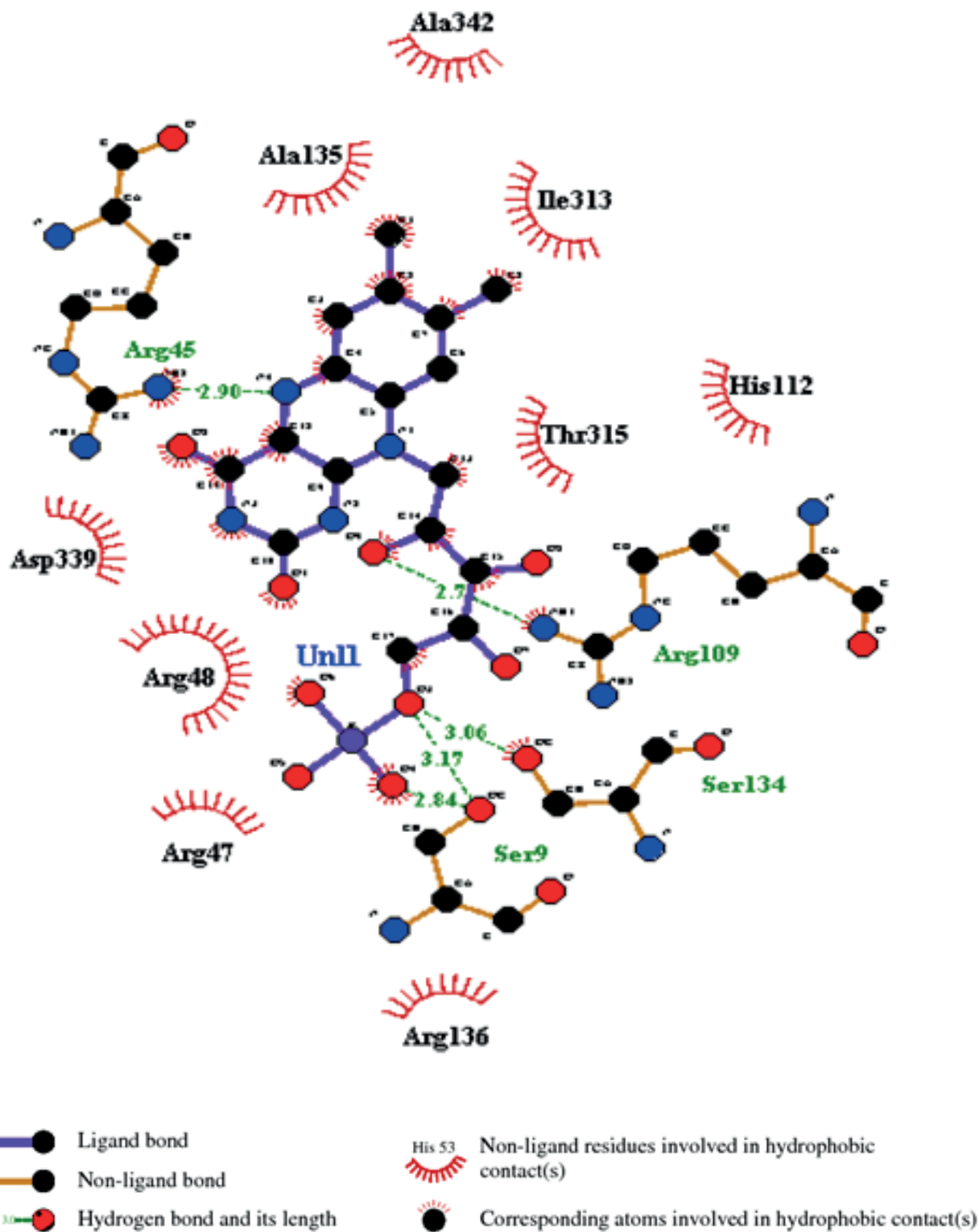
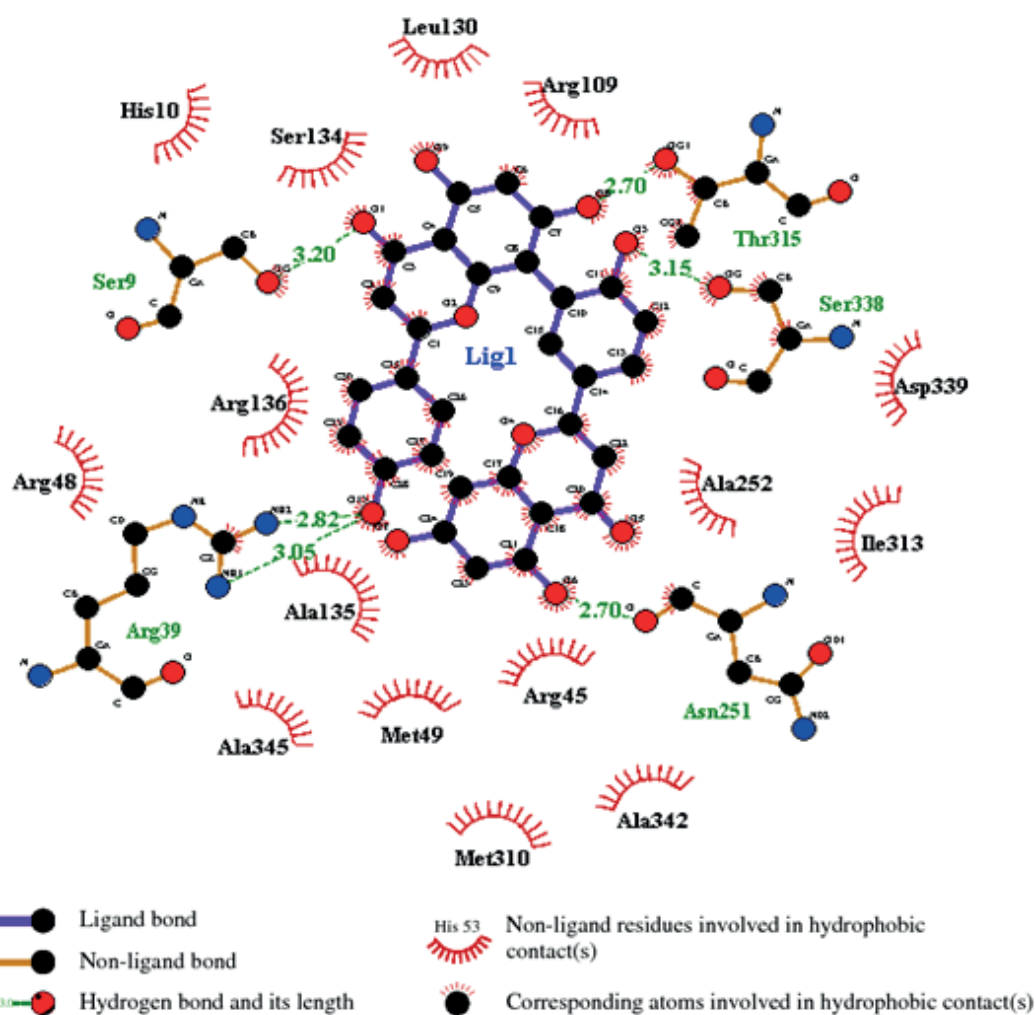


Figure 5. Docking of FMN with the model, visualized by LIGPLOT (1).



**Figure 6.** Docking of amentoflavone with the model, visualized by LIGPLOT [\(11\)](#).

**Table 3.** Physiochemical parameters of the ligands

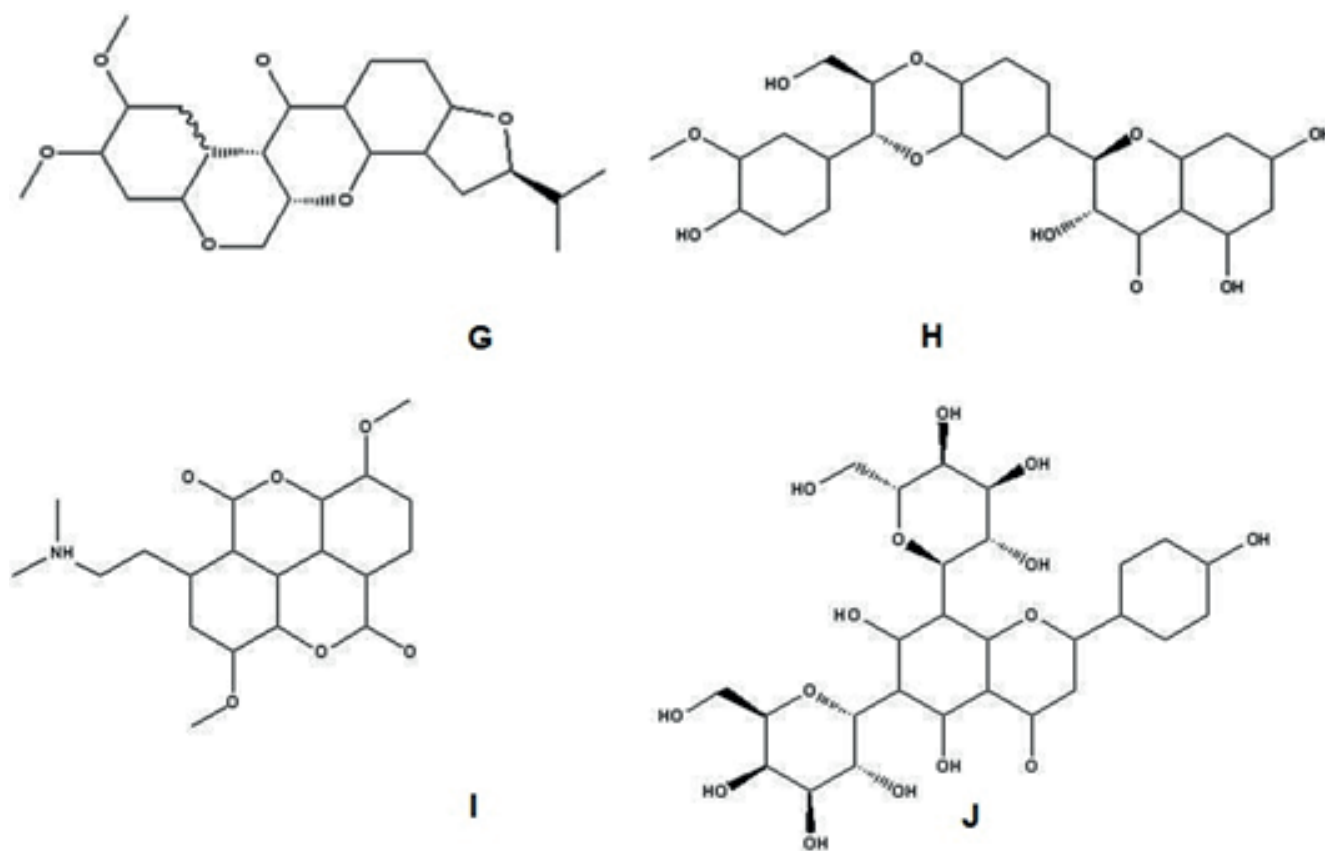
Compound	ZINC data base ID	M. Wt <sup>1</sup> (g/mol)	xlogP <sup>2</sup>	HBD <sup>3</sup>	HBA <sup>4</sup>	PSA <sup>5</sup> (Å <sup>2</sup> )
Amentoflavone	ZINC03984030	538.464	5.61	6	10	182
Scutellarin	ZINC21902916	461.355	0.07	6	12	210
Rutin	ZINC59764511	610.521	-1.06	10	16	269
Hinokiflavone	ZINC04098521	538.464	5.18	5	10	170
Isoquercitrin	ZINC04096845	464.379	-0.36	8	12	211
Apocynin A	ZINC14926814	468.414	1.56	7	10	177
Rotenone	ZINC03860715	394.423	3.59	0	6	63
Silybin	ZINC02033589	482.441	1.47	5	10	155
Taspine	ZINC01702514	370.381	2.67	1	7	83
Vicenin	ZINC98369451	594.522	-2.10	11	15	271

<sup>1</sup>Molecular weight, <sup>2</sup>octanol/water partition coefficient, <sup>3</sup>Hydrogen bond donors, <sup>4</sup>hydrogen bond acceptors, <sup>5</sup>polar surface area.

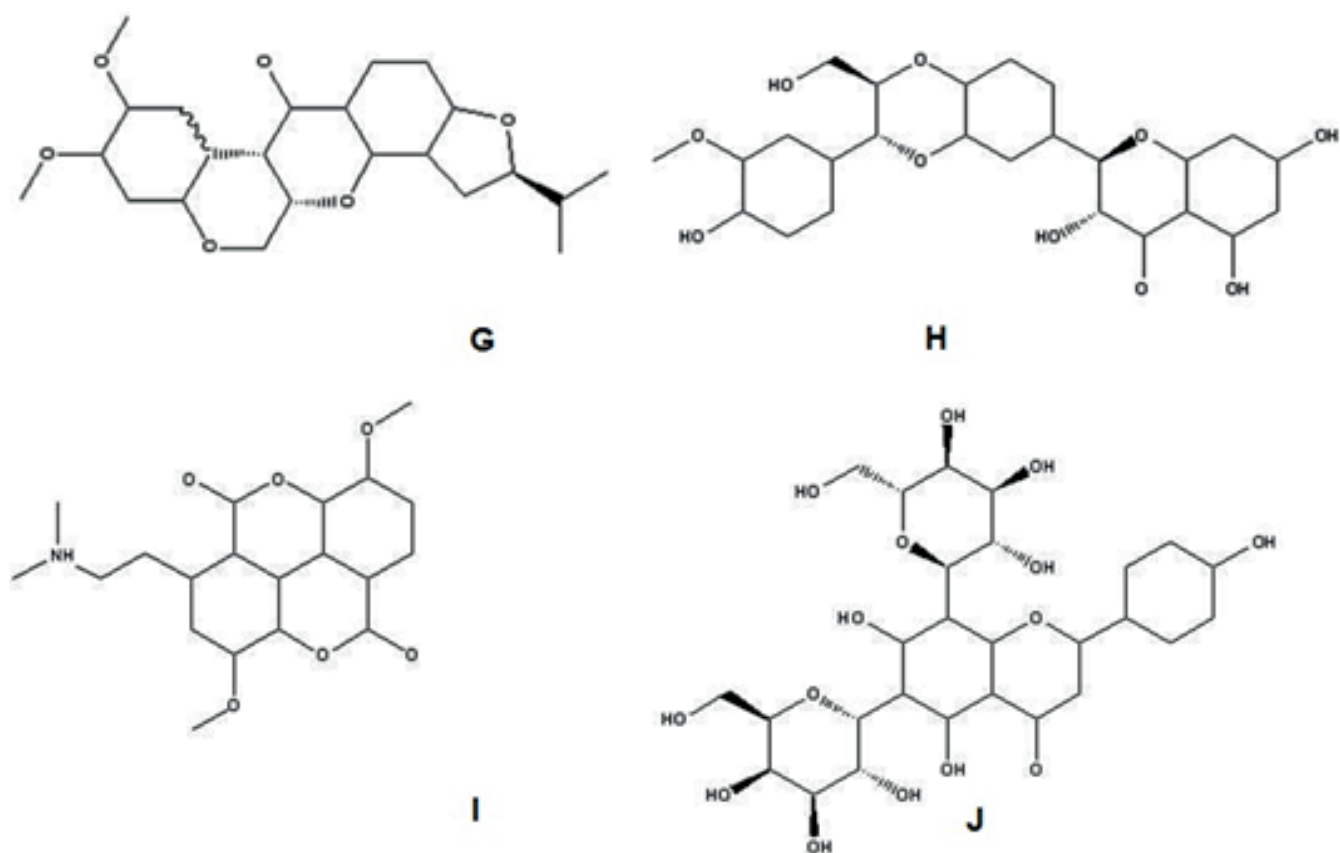


Flavonoids are secondary products of plants present in vegetables and fruits with many medicinal properties (26). Hwang et al., (27) found that amentoflavone, a biflavonoid being extracted from *Selaginella tamariscina*, had an antimicrobial action against Gram positive and Gram negative microorganisms. The compound was active against *Enterococcus faecium*, *S. aureus*, *Streptococcus mutans*, *Escherichia coli* and *Pseudomonas aeruginosa* in a range from 4-36 µg/ml. The study also suggested that amentoflavone also had a synergistic effect when combined with ampicillin, cefotaxime and chloramphenicol.

Hossain et al., (28) used AutoDock 4.2 to screen ZINC database and obtained four compounds designated ZINC03803450, ZINC20149031, ZINC13387711 and ZINC16052528 that had strong binding affinities towards the active site of the enzyme where 5-enolpyruvyl shikimate-3-phosphate binds. In another approach, molecular docking studies on chorismate synthase of *S. pneumoniae* resulted in identification of four substrate analogs of 5-enolpyruvyl shikimate-3-phosphate that had docking scores higher than the original compound (29).



**Figure 7.** Chemical structure of ligands. (A) Amentoflavone, (B) scutellarin, (C) rutin, (D) Hinokiflavone, (E) isoquercitrin, (F) Apocynin A.



**Figure 7.** continued: (G) rotenone, (H) silybin, (I) taspine, (J) vicenin

Pharmacokinetics and toxicity profiles are presented in Table 4. Absorption through the digestive system is predicted by HIA and Caco-2 [\(30\)](#). Except for scutellarin, all the ligands show high probability to be absorbed through intestine as predicted by HIA but only rotenone, silybin and taspine show a similar result in Caco-2. Predicting the permeability of a drug to pass through the blood-brain barrier is of importance in 2 aspects: (a) if the drug is required to exert an action in the central nervous system and (b) if the drug is acting on other organs and may have toxicity on the brain [\(31\)](#). Only amentoflavone, hinokiflavone, rotenone and taspine may cross the blood-brain barrier based on their predicted probabilities.

All ligands are subjected to transport by P-gp since their probabilities indicate that are substrates. However, rotenone inhibits P-gp I, hinokiflavone and silybin act as inhibitors to P-gp II and taspine may inhibit both P-gp I and II. These P-glycoproteins

belong to ATP-binding cassette transporters found in cells of liver, kidney, intestine and blood-brain barrier as well, and function to export substances out from the cells. Thus, decrease the absorption of drugs from intestine while increase the elimination by liver through bile [\(32, 33, 34\)](#).

Cytochromes P450 are responsible for the oxidation step in metabolism of xenobiotics inside body including drugs. Hence, inhibition of these cytochromes would interfere with metabolism of other drugs [\(30\)](#). Amentoflavone, hinokiflavone, rotenone and silybin have a probability of such inhibition. All the compounds were non carcinogens and were not inhibitory to OCT2 which is involved in damage of kidney tissue due to accumulation of nephrotoxic substances [\(35\)](#). Acute rat toxicity profiles showed that rotenone had the highest level among the natural products of 3.787 Mol/Kg and amentoflavone was second to rotenone in this regard.

**Table 4 A.** SARadmet predicted ADMET profile

Compound	HIA (Probability)	BBB (Probability)	Caco-2 (Probability)	P-gp Substrate (Probability)	P-gp I Inhibitor (Probability)	P-gp II Inhibitor (Probability)	OCT2 Inhibitor (Probability)
Amentoflavone	+ (0.974)	+ (0.660)	- (0.509)	S <sup>1</sup> (0.500)	N <sup>2</sup> (0.70)	N (0.775)	N (0.920)
Scutellarin	- (0.805)	- (0.596)	- (0.873)	S (0.565)	N (0.942)	N (0.670)	N (0.945)
Rutin	+ (0.804)	- (0.854)	- (0.917)	S (0.690)	N (0.876)	N (0.855)	N (0.898)
Hinokiflavone	+ (0.956)	+ (0.659)	- (0.731)	S (0.554)	N (0.748)	I <sup>3</sup> (0.771)	N (0.918)
Isoquercitrin	+ (0.786)	- (0.698)	- (0.940)	S (0.591)	N (0.878)	N (0.797)	N (0.892)
Apocynin A	+ (0.928)	- (0.703)	- (0.932)	S (0.587)	N (0.932)	N (0.985)	N (0.956)
Rotenone	+ (0.994)	+ (0.596)	+ (0.735)	S (0.658)	I (0.958)	N (0.506)	N (0.736)
Silybin	+ (0.970)	- (0.768)	+ (0.581)	S (0.614)	N (0.579)	I (0.841)	N (0.855)
Taspin	+ (0.731)	+ (0.634)	+ (0.600)	S (0.806)	I (0.626)	I (0.680)	N (0.687)
Vicenin	+ (0.916)	- (0.687)	- (0.910)	S (0.628)	N (0.919)	N (0.887)	N (0.872)

<sup>1</sup>S: substrate, <sup>2</sup>N: none, <sup>3</sup>I: inhibitor

**Table 4 B.** SARadmet predicted ADMET profile

Compound	CYP 450 substrate (Probability)			CYP 450 Inhibition (probability)			Carcinogen (probability)	ART LD <sub>50</sub> (Mol/Kg)
Amentoflavone	CYP 2C9	N <sup>1</sup> (0.793)	CYP 2C9	I <sup>2</sup> (0.892)	CYP 1A2	I(0.684)	N(0.931)	3.130
	CYP 2D6	N(0.907)	CYP 2D6	N(0.932)	CYP 2C19	I(0.683)		
	CYP 3A4	N(0.715)	CYP 3A4	I(0.617)				
Scutellarin	CYP 2C9	N (0.821)	CYP 2C9	N(0.752)	CYP 1A2	N(0.757)	N(0.947)	2.722
	CYP 2D6	N(0.908)	CYP 2D6	N(0.953)	CYP 2C19	N(0.822)		
	CYP 3A4	N(0.684)	CYP 3A4	N(0.684)				
Rutin	CYP 2C9	N(0.764)	CYP 2C9	N(0.907)	CYP 1A2	N(0.867)	N(0.961)	2.498
	CYP 2D6	N(0.896)	CYP 2D6	N(0.955)	CYP 2C19	N(0.903)		
	CYP 3A4	N(0.537)	CYP 3A4	N(0.925)				
Hinokiflavone	CYP 2C9	N(0.812)	CYP 2C9	I(0.832)	CYP 1A2	I(0.626)	N(0.934)	2.955
	CYP 2D6	N(0.911)	CYP 2D6	N(0.900)	CYP 2C19	I(0.631)		
	CYP 3A4	N(0.682)	CYP 3A4	I(0.500)				
Isoquercitrin	CYP 2C9	N(0.812)	CYP 2C9	N(0.930)	CYP 1A2	N(0.908)	N(0.960)	2.387
	CYP 2D6	N(0.892)	CYP 2D6	N(0.951)	CYP 2C19	N(0.930)		
	CYP 3A4	N(0.604)	CYP 3A4	N(0.919)				
Apocynin A	CYP 2C9	N(0.833)	CYP 2C9	N(0.954)	CYP 1A2	N(0.957)	N(0.964)	2.325
	CYP 2D6	N(0.900)	CYP 2D6	N(0.957)	CYP 2C19	N(0.961)		
	CYP 3A4	N(0.653)	CYP 3A4	N(0.828)				
Rotenone	CYP 2C9	N(0.861)	CYP 2C9	N(0.769)	CYP 1A2	I(0.911)	N(0.941)	3.787
	CYP 2D6	N(0.853)	CYP 2D6	N(0.923)	CYP 2C19	I(0.899)		
	CYP 3A4	S <sup>4</sup> (0.646)	CYP 3A4	I(0.796)				
Silybin	CYP 2C9	N(0.761)	CYP 2C9	I(0.635)	CYP 1A2	N(0.771)	N(0.939)	2.221
	CYP 2D6	N(0.874)	CYP 2D6	N(0.923)	CYP 2C19	N(0.599)		
	CYP 3A4	N(0.550)	CYP 3A4	N(0.550)				
Taspine	CYP 2C9	N(0.809)	CYP 2C9	N(0.806)	CYP 1A2	N(0.565)	N(0.821)	2.549
	CYP 2D6	N(0.694)	CYP 2D6	N(0.828)	CYP 2C19	N(0.870)		
	CYP 3A4	S(0.672)	CYP 3A4	N(0.911)				
Vicenin	CYP 2C9	N(0.812)	CYP 2C9	N(0.918)	CYP 1A2	N(0.880)	N(0.948)	2.200
	CYP 2D6	N(0.871)	CYP 2D6	N(0.945)	CYP 2C19	N(0.910)		
	CYP 3A4	N(0.617)	CYP 3A4	N(0.876)				

<sup>1</sup>N: none, <sup>2</sup>I: inhibitor, <sup>3</sup>W: weak inhibitor, <sup>4</sup>S: substrate

## CONCLUSIONS

Homology modeling and molecular docking reduces cost, time and effort as initial step in screening for new medicines. However, this step should be accompanied by studying pharmacodynamics and pharmacokinetics to provide information about interactions of lead compounds inside the human body as well as co-administered drugs. Antibiotic research may exploit alternative targets in bacteria such as chorismate synthase and other enzymes of the shikimate pathway on which compounds are screened from chemical libraries to identify inhibitors.

## ACKNOWLEDGEMENTS

The author would like to thank Dr. Roman Laskowski for free providing of LIGPLOT+.

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