

In silico Antidiabetic Screening of Borapetoside C, Cordifolioside A and Magnoflorine

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Khanal *et al.*: *Tinospora* Species as a Source of Antidiabetic Molecules

The current study was aimed to screen borapetoside C, cordifolioside A, and magnoflorine against targets that are involved in the pathogenesis of type 2 diabetes mellitus. All active compounds were retrieved from PubChem database and protein molecules were downloaded from Protein Data Bank. Each protein was prepared using Discovery studio, and the binding pocket was predicted using castP. Each ligand was docked against the target proteins using AutoDock Vina 4.0. Drug-likeness, toxicity, PK-PD and probable antidiabetic activity of each compound were also predicted. Among the three phytoconstituents, magnoflorine scored highest drug-likeness score and also predicted to be a potent antidiabetic molecule. Comparisons were made with sitagliptin and repaglinide two clinically approved antidiabetic drugs.

Key words: Borapetoside C, cordifolioside A, docking, *in silico*, magnoflorine, *Tinospora*

Diabetes mellitus (DM) is a chronic metabolic disorder due to defective insulin secretion or resistance, or a combination of both that leads to various health complications^[1]. According to a global report of World Health Organization (WHO) 2017, the prevalence of diabetes in adults aged 20-79 y was estimated to be 8.8 % in 2015 and expected to rise to 10.4 % in 2040^[2]. Synthetic molecules are prescribed for the pharmacotherapy of type 2 diabetes mellitus (T2DM) but they possess well-known side effects^[3,4]. Since active phytoconstituents from traditional medicinal plants are believed to possess lower side effects^[5], these could be used to discover new drug molecules. The WHO has also recommended investigating hypoglycaemic agents from folk medicinal plants as antidiabetic drugs^[6].

Species of genus *Tinospora* are widely used as a folk medicine for various purposes^[7]. *Tinospora* species have been reported to possess antidiabetic, antioxidant, immunostimulatory and antiosteoporosis activities. Further, Ayurveda has listed *T. cordifolia* in the

management of diabetes. *T. capillipes* and *T. sinensis* are also officially listed in Chinese Pharmacopoeia^[7,8]. Borapetoside C is one of the active phytoconstituents from *T. crispa* while cordifolioside A and magnoflorine are the major phytoconstituents from *T. cordifolia* and were reported to cause a reduction of oxidative stress and regularise carbohydrate metabolism^[9-11] in experimental models of T2DM. However, the molecular mechanism responsible for the blood glucose lowering effect has not been understood.

Hence, an *in silico* study was performed to screen borapetoside C, cordifolioside A, and magnoflorine as antidiabetics against known targets such as 17 β -hydroxysteroid dehydrogenase, retinol binding protein, C-jun N-terminal kinase, cholesteryl ester

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transfer protein, Lamin A/C, protein kinase B, adiponectin, insulin degrading enzyme, PPAR γ , human glucose transporter and adenylate cyclase, which have been implicated in the pathogenesis of T2DM and associated complications. The results were compared to those of repaglinide and sitagliptin, 2 clinically used antidiabetic drugs.

All 2D and 3D structures of borapetoside C, cordifolioside A, magnoflorine, repaglinide, and sitagliptin were retrieved from PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) in .sdf format and converted into .pdb and .mol using Discovery studios 2016. Canonical SMILES were also retrieved from PubChem database. Canonical SMILES explains the molecular structure as a graph with optional chiral indications. The energy of ligand molecules was minimized using mmff94 (<https://openbabel.readthedocs.io/en/latest/Forcefields/mmff94.html>) force field and conjugate gradients as an optimization algorithm. Energy minimization is an important step in the preparation of a ligand to abolish clashes within the atoms of a ligand molecule and produce a reasonable starting pose. The energy minimized ligand molecules were then converted into AutoDock ligand in the format of .pdbqt. All the 2D structures of compounds are shown in fig. 1.

All the targets were retrieved from Protein Data Bank (<https://www.rcsb.org>). The retrieved 3D structures of proteins were combined with water and other hetero molecules. Discovery studio 2016 was used to remove water molecules and heteroatoms. Water molecules and other heteroatoms were removed to clear the binding pocket and make computations easier so that ligand can create satisfying interactions with the protein. The chain was selected based on their completeness of amino acid residue and presence of the active site. This modified protein was then saved in .pdb format. An online tool, Computed Atlas of Surface Topography of proteins^[12] was used to predict a number of mouth opening, pocket MS area, pocket MS volume, mouth

MS area and mouth MS circumference sum of each protein under the radius probe of 1.4 angstroms.

Drug-likeness character (Lipinski's rule of five) is associated with absorption, bioavailability, and biodistribution of the drug molecule from the human intestinal tract. This prediction is made before the molecule is synthesized in the laboratory or extracted from the natural source. Hence, molsoft (<http://molsoft.com/>), an online server was used to predict the drug-likeness character of all the selected ligand molecules.

Various pharmacokinetic parameters of drug molecules and probable toxicity in different models were predicted by using an online server, admetSAR. Blood-brain barrier permeability, human intestinal absorptivity, caco-2 permeability, AMES toxicity, aqueous solubility, rat acute toxicity, and fish toxicity were predicted. The server was also used to predict the possible interaction of drug molecule with various isoenzymes. The prediction was based on a vector machine classification algorithm and in-house substructure pattern recognition method, which were built via regression methods^[13]. Similarly, *Escherichia coli* toxicity of each molecule was predicted using abSYNTH^[14]. The probable antidiabetic activity of these 3 molecules was also predicted via the PASS online. PASS online predicts about 4000 probable pharmacological activities with an average probability of 0.95 based on structure-activity relationships^[15].

Docking was performed using AutoDock Vina. It uses sophisticated gradient optimization method to calculate grid maps and provides the cluster of results^[16]. After the completion of docking, 10 docking poses of ligand were obtained. The pose with minimum binding energy was selected for visualizing the ligand-protein interaction.

Among the targeted protein molecules, insulin-degrading enzyme scored the maximum number of mouth openings, pocket MS area, pocket MS volume, mouth MS area, and mouth MS circumference sum compared to other target molecules (Table 1).

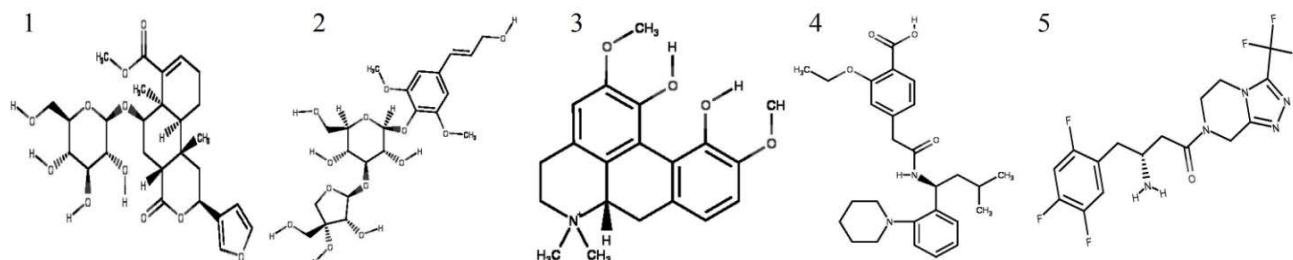


Fig. 1: Chemical structures
(1) Borapetoside C, (2) cordifolioside A, (3) magnoflorine, (4) repaglinide and (5) sitagliptin

All the selected molecules were able to interact with targeted protein molecules. The interaction between the ligand and protein is explicated by the binding energy and number of interactions; defined by the hydrogen bonds and pi interactions. Binding energy and number of hydrogen bond interaction of all compounds with each protein are shown in Table 2.

Borapetoside C scored the highest binding affinity with two proteins i.e. retinol binding protein (-5.1 kcal/mol) and human glucose transporter (-6.7 kcal/mol), cordifolioside A scored highest binding affinity with 17 β -hydroxysteroid dehydrogenase (-9.1 kcal/mol), retinol binding protein (-5.1 kcal/mol), Lamin A/C (-6.6 kcal/mol), insulin degrading enzyme (-7.5 kcal/mol) and PPARG (-6.4 kcal/mol). Further, magnoflorine scored highest binding affinity with only one protein molecule cholesteryl ester transfer protein (-5.4 kcal/mol). Sitagliptin scored the highest binding

affinity with five protein molecules, C-jun N-terminal kinase (-6.1 kcal/mol), protein kinase B (-7.1 kcal/mol) and adiponectin (-7.1 kcal/mol), PPARG (-6.4 kcal/mol) and adenylate cyclase (-5.8 kcal/mol). Binding affinity of individual compounds with each target and a respective number of hydrogen bond interactions are summarized in Table 2. Among the phytoconstituents tested, magnoflorine scored highest drug-likeness value of 0.80 without any violation of the rule of five. However, borapetoside C violated two rules, the molecular weight and the number of hydrogen bond acceptors, while cordifolioside A violated three rules, the molecular weight, the number of hydrogen bond acceptors and the number of hydrogen bond donors (Table 3). Most drug molecules under development fail in clinical trials due to poor pharmacokinetics and high toxicity^[17]. Hence, each individual molecule was predicted for pharmacokinetic, i.e. absorption,

TABLE 1: NUMBER OF BINDING POCKET/OPENINGS, MOUTH VOLUME AREA AND CIRCUMFERENCE OF EACH OF THE PROTEIN

Protein	PDB ID	NMO	PMSA	PMSV	MMSA	MMSCS
17 β -hydroxysteroid dehydrogenase	1BHS	2	1452.215	2214.501	156.5	80.50
Retinol binding protein	1GGL	1	2096.563	2779.102	9.81	11.28
C-Jun N-terminal kinase	1JNK	3	1630.603	3342.965	947.35	321.01
Cholesteryl ester transfer protein	2OBD	3	3303.066	5518.994	122.36	77.92
Lamin A/C	3GEF	5	1680.576	3682.228	764.62	309.74
Protein kinase B	3QKK	2	1079.742	1770.330	87.37	59.78
Adiponectin	4DOU	4	682.933	1110.525	144.90	99.10
Insulin degrading enzyme	4PF9	8	11882.211	32149.483	995.70	402.56
PPARG	4Y29	2	1492.833	1932.907	83.82	57.43
Human glucose transporter	5C65	2	1613.297	3310.845	668.11	194.83
Adenylate cyclase	5IV4	3	1108.655	1795.905	184.61	104.47

NMO: Number of mouth openings, PMSA: pocket MS area, PMSV: pocket MS volume, MMSA: mouth MS area and MMSCS: mouth MS circumference sum

TABLE 2: BINDING ENERGY AND NUMBER OF HYDROGEN BONDS OF LIGAND PROTEIN INTERACTION

PDB ID	Binding energy and number of hydrogen bond of protein molecules with each ligand									
	Borapetoside C		Cordifolioside A		Magnoflorine		Repaglinide		Sitagliptin	
	BE (kcal/mol)	NHB	BE (kcal/mol)	NHB	BE (kcal/mol)	NHB	BE (kcal/mol)	NHB	BE (kcal/mol)	NHB
1BHS	-6.9	2	-9.1	1	-7.6	0	-8	1	-8.7	7
1GGL	-5.1	4	-5.1	0	-4.9	0	-4.1	2	-4.6	3
1JNK	-5.9	3	-5.7	1	-5.3	1	-5.3	3	-6.1	2
2OBD	-5	1	-5	1	-5.4	0	-4.7	1	-5.3	3
3GEF	-6.4	6	-6.6	3	-5.6	2	-5.4	1	-6.4	6
3QKK	-6.9	2	-6.9	0	-5.9	2	-6.1	2	-7.1	4
4DOU	-6.6	3	-6.7	0	-6.3	1	-6.5	2	-7.1	1
4PF9	-7.4	4	-7.5	1	-6.7	2	-6.5	1	-7.2	3
4Y29	-6.1	3	-6.4	0	-6.2	4	-5.3	3	-6.4	1
5C65	-6.7	4	-6.5	3	-5.5	3	-5.2	1	-6.4	3
5IV4	-5.1	1	-5.4	2	-5.1	1	-5	1	-5.8	4

BE: binding energy, NHB: number of hydrogen bond

distribution, metabolism, excretion and toxicity (ADMET) using regression models^[13]. Magnoflorine was predicted to cross blood brain barrier but not the human intestinal tract. All compounds were predicted to be non-mutagenic and non-carcinogenic. Toxicity of each compound and interaction with p-gp and various isoenzymes are shown in Table 4.

Cordifolioside A scored highest binding affinity to the majority of the selected protein molecules, but this parameter alone could not be considered for the

drug development process. Hence, Lipinski's rule of five, a qualitative parameter, was used to understand the drug-likeness character of each molecule. Magnoflorine scored highest drug-likeness value compared to other compounds tested. Similarly, *E. coli* toxic concentration of magnoflorine. 0.45 g/l was predicted to be very close to that of repaglinide, 0.43 g/l and sitagliptin, 0.46 g/l. Hence, based on *E. coli* toxicity prediction, magnoflorine could be considered as a safe molecule compared to borapetoside C and

TABLE 3: DRUGLIKENESS AND TOXICITY PREDICTION ON *E. COLI*

Ligand molecules	PubChem CID	Lipinski rule of five				DLS	ECT	Log S (mg/l)
		M. W.	NHBA	NHBD	Log P			
		<500	<10	<5	<5			
Borapetoside C	101697033	536.23	11	4	0.63	0.15	0.19	235.03
Cordifolioside A	45359937	504.18	13	7	-2.60	0.57	0.17	166434.48
Magnoflorine	73337	342.17	4	2	2.77	0.80	0.45	52245.2
Repaglinide	65981	452.27	4	2	5.80	0.92	0.43	1.34
Sitagliptin	4369359	407.12	4	2	1.43	0.49	0.46	6.77

MW: molecular weight, NHBA: number hydrogen bond acceptors, NHBD: number hydrogen bond donors, DLS; drug-likeness score and ECT: *E. coli* toxicity IC₅₀ (g/l)

TABLE 4: PK-PD AND TOXICITY ASSESSMENT OF LIGAND MOLECULES

Model	Ligand molecule									
	Borapetoside C		Cordifolioside A		Magnoflorine		Repaglinide		Sitagliptin	
	R	P	R	P	R	P	R	P	R	P
BBB	-	0.8034	-	0.6879	+	0.8895	-	0.7101	+	0.9438
HIA	+	0.8334	+	0.8742	-	0.9462	+	0.9172	+	1.0000
Caco-2 P	-	0.8812	-	0.8501	+	0.7275	-	0.5891	-	0.6415
AMEST	-	0.8376	-	0.8113	-	0.9133	-	0.8136	-	0.5487
Carcinogens	-	0.9644	-	0.9694	-	0.9348	-	0.8004	-	0.7973
P-gp substrate	S	0.8619	S	0.8560	S	0.8891	S	0.8145	S	0.6509
P-gp inhibitor 1	I	0.5712	I	0.5396	NI	0.9000	I	0.6044	I	0.7141
P-gp inhibitor 2	I	0.8884	I	0.7389	NI	0.8391	I	0.6868	I	0.5248
CYP450 2C9 substrate	NS	0.8370	NS	0.8575	NS	0.7778	NS	0.8288	NS	0.9277
CYP450 2D6 substrate	NS	0.8863	NS	0.8825	NS	0.5854	NS	0.9117	NS	0.7228
CYP450 3A4 substrate	S	0.7316	S	0.7200	S	0.8015	S	0.7019	S	0.6412
CYP450 1A2 inhibitor	NI	0.8674	NI	0.8534	NI	0.6378	NI	0.9206	NI	0.7178
CYP450 2C9 inhibitor	NI	0.8690	NI	0.8791	NI	0.8997	NI	0.9070	I	0.6111
CYP450 2D6 inhibitor	NI	0.9401	NI	0.9264	NI	0.5128	NI	0.9213	NI	0.5380
CYP450 2C19 inhibitor	NI	0.9059	NI	0.9075	NI	0.8657	NI	0.9026	I	0.7048
CYP450 3A4 inhibitor	NI	0.7578	NI	0.7261	NI	0.9015	NI	0.9264	I	0.5358
ROCT	NI	0.8195	NI	0.7788	I	0.5000	NI	0.8490	I	0.5920
RAT (LD50, mol/kg)	5.0822		4.7687		2.6986		2.4497		2.8093	
FT (pLC50, mg/l)	0.5094		0.6158		1.1536		1.1109		1.5115	
TPT (pIGC50, µg/l)	1.2413		1.1436		0.8194		0.5514		0.5432	

BBB: blood brain barrier, HIA: human intestinal absorption, Caco-2 P: Caco-2 permeability, AMEST: AMES toxicity, p-gp: p-glycoprotein, R: result, P: probability, '-' negative, '+' positive, I: inhibitor, S: substrate, NS: non-substrate, NI: non-inhibitor, ROCT: renal organic cation transporter, RAT: rat acute toxicity, FT: fish toxicity and TPT: *Tetrahymena pyriformis* toxicity

cordifolioside A. Further, pharmacological activity prediction of all the phytoconstituents suggested that magnoflorine to be a potent antidiabetic molecule as shown in fig. 2. Personalized medicine concept is based on mutagenicity involving the genes that encode CYP2D6 and CYP2C19^[18]. Current study showed that the selected phytoconstituents were not a substrate for CYP2D6 nor inhibitors of CYP2D6 and CYP2C19. However, predicted result showed that CYP2C19 mutagenicity test is necessary for sitagliptin.

The target protein molecules play a crucial role in the pathogenesis of diabetes. Alteration of these proteins or inhibition of their synthesis might be help in the management of diabetes. Hence, the current study demonstrated that magnoflorine to be a potential molecule that could alter majority of the proteins that have been implicated in the pathogenesis of diabetes. The summary of the current study is shown in fig. 3.

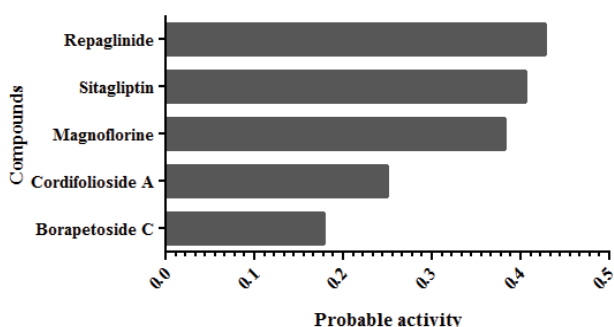


Fig. 2: Probable antidiabetic activity of borapetoside C, cordifolioside A, magnoflorine, repaglinide and sitagliptin

Borapetoside C, cordifolioside A and magnoflorine are the three molecules reported to possess antidiabetic activity from various modes. Borapetoside C is reported to lower the blood glucose by enhancing insulin sensitivity and GLUT2 expression followed by Akt phosphorylation^[10]. Similarly, cordifolioside A is reported to improve insulin signaling and hepatic metabolism during insulin resistance. Further, it is reported to lower the blood glucose and cholesterol level^[19]. Magnoflorine is reported to have a direct effect on carbohydrate metabolism, increase the insulin sensitivity and reverse diabetes-associated alterations in lipid profile and oxidative stress^[20]. Repaglinide is reported to stimulate the release of insulin from beta cells of the pancreas by increasing the intracellular calcium level^[21]. Sitagliptin is a selective DPP-4 inhibitor to minimize the inactivation of various incretin hormones, which are involved in the regulation of glucose homeostasis^[22].

In conclusion, the current study projected magnoflorine as a potential molecule to interact with majority of proteins that are implicated in the pathogenesis DM. ADMET prediction also demonstrated magnoflorine to be a non-toxic molecule compared to borapetoside C and cordifolioside A. The current study also reported that these predictions were comparable to those of repaglinide and sitagliptin.

Conflicts of interest:

The authors declare that there is no conflict of interest.

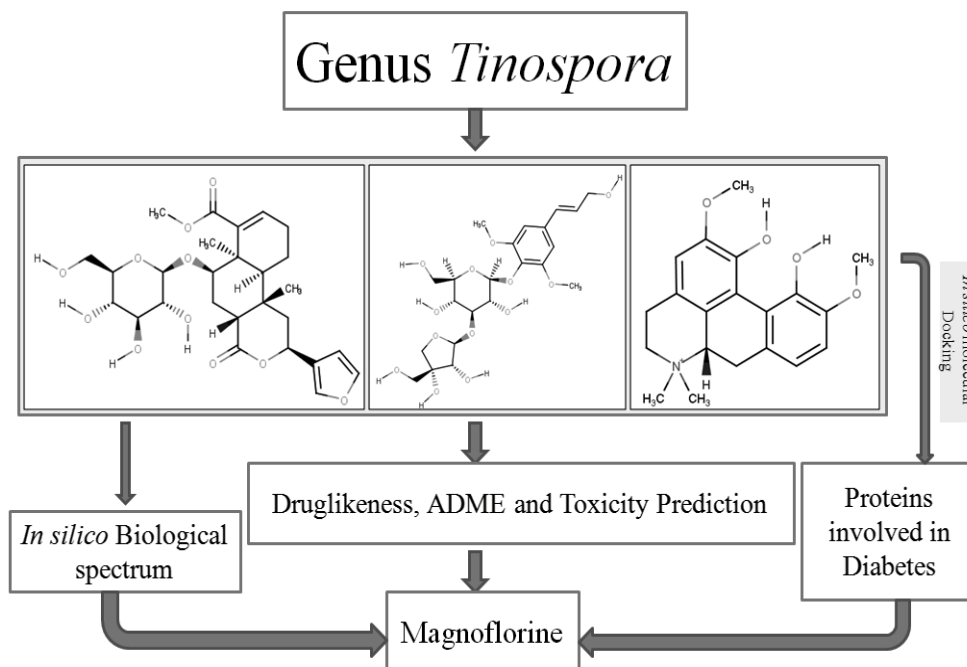


Fig. 3: Summary of the study

REFERENCES

- Hameed I, Masoodi SR, Mir SA, Nabi M, Ghazanfar K, Ganai BA. Type 2 diabetes mellitus: From a metabolic disorder to an inflammatory condition. *World J Diabetes* 2015;6(4):598-612.
- Ogurtsova K, Fernandes JDR, Huang Y, Linnenkamp U, Guariguata L, Cho NH, *et al.* IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pract* 2017;128:40-50.
- Upadhyay J, Polyzos SA, Perakakis N, Thakkar B, Paschou SA, Katsiki N, *et al.* Pharmacotherapy of type 2 diabetes: An update. *Metabolism* 2018;78:13-42.
- Krentz AJ, Bailey CJ. Oral antidiabetic agents: Current role in type 2 diabetes mellitus. *Drugs* 2005;65(3):385-411.
- Moreira DDL, Teixeira SS, Monteiro MHD, De-oliveira ACAX, Paumgarten FJR. Traditional use and safety of herbal medicines. *Rev Bras Farmacogn* 2014;24(2):248-57.
- Diabetes mellitus. Report of a WHO expert committee. *World Health Organization*. 1965. p. 1-44. Available from: https://apps.who.int/iris/bitstream/handle/10665/38442/WHO_TRS_310.pdf;jsessionid=2C4D0847578CDB3E30D98B6B54F12241?sequence=1.
- Chi S, She G, Han D, Wang W, Liu Z, Liu B. Genus *Tinospora*: Ethnopharmacology, Phytochemistry, and Pharmacology. *Evid Based Complement Alternat Med* 2016;2016:1-32.
- Nadig PD, Revankar RR, Deth SM, Narayanswamy SB, Aliyar MA. Effect of *Tinospora cordifolia* on experimental diabetic neuropathy. *Indian J Pharmacol* 2012;44(5):580-83.
- Sangeetha MK, Balaji Raghavendran HR, Gayathri V, Vasanthi HR. *Tinospora cordifolia* attenuates oxidative stress and distorted carbohydrate metabolism in experimentally induced type 2 diabetes in rats. *J Nat Med* 2011;65(3-4):544-50.
- Ruan CT, Lam SH, Chi TC, Lee SS, Su MJ. Borapetoside C from *Tinospora crispa* improves insulin sensitivity in diabetic mice. *Phytomedicine* 2012;19(8-9):719-24.
- Patel MB, Mishra S. Hypoglycemic activity of alkaloidal fraction of *Tinospora cordifolia*. *Phytomedicine* 2011;18(12):1045-52.
- Dundas J, Ouyang Z, Tseng J, Binkowski A, Turpaz Y, Liang J. CASTp: Computed atlas of surface topography of proteins with structural and topographical mapping of functionally annotated residues. *Nucleic Acids Res* 2006;34:W116-8.
- Cheng F, Li W, Zhou Y, Shen J, Wu Z, Liu G, *et al.* admetSAR: A Comprehensive Source and Free Tool for Assessment of Chemical ADMET Properties. *J Chem Inf Model* 2010;52(11):3099-105.
- Planson AG, Carbonell P, Paillard E, Pollet N, Faulon JL. Compound Toxicity Screening and Structure-Activity Relationship Modeling in *Escherichia coli*. *Biotechnol Bioeng* 2012;109(3):846-50.
- Filimonov DA, Lagunin AA, Glorizova TA, Rudik AV, Druzhilovskii DS, Pogodin PV, *et al.* Prediction of the biological activity spectra of organic compounds using the pass online web resource. *Chem Heterocycl Compd* 2014;50(3):444-57.
- Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *J Comput Chem* 2010;31(2):455-61.
- Kennedy T. Managing the drug discovery/development interface. *Drug Discov Today* 1997;2(10):436-44.
- Annemans L, Redekop K, Payne K. Current methodological issues in the economic assessment of personalized medicine. *Value Heal* 2013;16(6S):S20-6.
- Stohs SJ, Ray S. Anti-diabetic and Anti-hyperlipidemic Effects and Safety of *Salacia reticulata* and Related Species. *Phyther Res* 2015;29(7):986-95.
- Ajiboye T, Uwazie JN, Haliru FZ, Ibitoye OB, Sunmonu T, Yakubu M, *et al.* Mechanisms of action of columbamine, jatrorrhizine and magnoflorine as antidiabetic agents. *Recent Progress Med Plant* 2017;45:221-32.
- Ambavane V, Patil R, Ainapure SS. Repaglinide: a short acting insulin secretagogue for postprandial hyperglycaemia. *J Postgrad Med* 2002;48:246.
- Gallwitz B. Review of sitagliptin phosphate: a novel treatment for type 2 diabetes. *Vasc Health Risk Manag* 2007;32):203-10.