Molecular Docking-Guided Screening of Phytoconstituents from Artemisia princeps as Allosteric Glucokinase Activators

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Research Article

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Abstract

Background

Glucokinase (GK) occurs in pancreatic β-cells and liver cells. GK plays a crucial role in whole-body glucose homeostasis. GK is often referred to as a glucose sensor in the β-cells. Small molecule GK activators not only reduce fasting and basal blood glucose levels but also improve glucose tolerance.

Objective

The present investigation was proposed to screen some phytoconstituents (from *Artemisia princeps*) as allosteric activators of the human GK enzyme using *in silico* molecular docking.

Methods

A library of phytoconstituents reported in *Artemisia princeps* was evaluated for the prediction of drug-like properties by *in silico* approach. Molecular docking studies of the phytoconstituents with GK were performed using AutoDock vina in order to explore binding interactions between the phytoconstituents and GK enzyme followed by *in silico* prediction of toxicity of these phytoconstituents.

Results

The selected phytoconstituents showed good pharmacokinetic parameters for oral bioavailability and drug-likeness as contrived by Lipinski's rule of five. Four compounds (rutin, 5,4'-dihydroxy-6,7,3'-trimethoxyflavone, daucosterol and methyl commate D) showed appreciable binding interactions with the allosteric site residues of the GK enzyme as per docking results.

Conclusion

These screened phytoconstituents may serve as promising leads for further development of clinically useful and safe allosteric activators of the human GK enzyme.

Introduction

Diabetes mellitus is probably one of the oldest diseases known to man. It was first reported in an Egyptian manuscript about 3000 years ago (Olokoba et al. 2012). Diabetes mellitus (DM) is often simply regarded as diabetes, a syndrome of disordered metabolism with abnormally high blood glucose levels resulting from a defect in either insulin secretion or insulin action or both. Based on aetiology the term type 1 DM (T1DM) and type 2 DM (T2DM) were widely used to describe insulin-dependent diabetes

mellitus and non-insulin-dependent diabetes mellitus, respectively (Sharma et al. 2022; Amos et al. 2010). T2DM is far more common than T1DM; accounting for about 90% of all cases of DM. Currently, North America, Europe and Japan have the highest prevalence of diabetes and India is supposed to surpass them with 79 million cases of diabetes by 2030. Current anti-diabetic drugs include oral hypoglycaemics, bromocriptine, insulin and insulin analogues (Guyton and Hall 2006; Grewal and Lather 2022). Despite the availability of several options to treat T2DM currently, no single oral antidiabetic drug is capable of achieving acceptable, long-lasting blood glucose control in the majority of patients thus making it necessary to combine 2–3 drugs to achieve adequate control. So, the scientific community is currently focusing on developing new, safe and clinically different anti-diabetic agents that can be used as mono-drug therapy with improved efficacy (Grewal et al. 2020a).

Glucokinase (GK) is a type IV isoenzyme that belongs to the family of hexokinases. GK plays a role in the glucose metabolism of the liver and is a glucose sensor in pancreatic β-cells involved in glucose-dependent insulin release (Sarabu et al. 2008). GK catalyze the conversion of glucose to glucose-6-phosphate (G-6-P), the first step of glucose metabolism. GK is selectively expressed in pancreatic β-cells and liver parenchymal cells (hepatocytes). Indeed, studies in transgenic animals have confirmed that GK plays a crucial role in whole-body glucose homeostasis. GK is often referred to as a 'glucose sensor' in β-cells. Hepatic GK activity and the intracellular location of GK are controlled by a protein produced in hepatocytes called GK regulatory protein (GKRP). Small molecules may activate GK either directly or by destabilizing the GK-GKRP complex (Matschinsky and Porte 2010). GK activators not only reduce fasting and basal blood glucose levels but also improve glucose tolerance. Although various types of GK activators have been reported in the literature but they suffer from various serious side effects, especially hypoglycaemia (Pal 2009; Grewal et al. 2020a; Sarabu et al. 2008). Various natural/plant-derived drugs with potential GK action have recently been reported in the literature (Grewal et al. 2014, Grewal et al. 2020a; Grewal and Lather 2022).

Hikino et al. (1989) investigated the mechanism of action of ganoderan B (a glycan derived from an aqueous extract of *Ganoderma lucidum* fruit). Ganoderan B boosted hepatic GK, phosphofructokinase, and glucose-6-phosphate dehydrogenase activity while decreasing glycogen synthetase activity. Qian-Cutrone et al. (1999) reported GK activators glucolipsin A and B extracted from alcoholic extracts of "*Streptomyces purpurogeniscleroticus*" and "*Nocardia vaccinia*", respectively. Kang et al. (2008) examined the effect of "eupatilin" obtained from "*Artemisia princeps*" on blood glucose homeostasis and pancreatic β-cell activity. Eupatilin administration improved hepatic GK activity, which resulted in lower fasting sugar levels and elevated hepatic glycogen metabolism. Singh et al. (2012) investigated the mode of action of coagulanolide (extracted from "*Withania coagulans*" fruits) on hepatic glucose-regulating enzymes in type 2 diabetic mice. After administration of coagulanolide, hepatic GK activity has risen greatly (approximately 37%) compared to the untreated group. Tatanans A, B, and C, three unique sesquilignans, were extracted from *Acorus tatarinowii* Schot rhizomes and tested for GK-activating potential. With $EC_{1.5}$ values ranging from 0.16 to 1.85 μM, these compounds showed different structural features and a substantial GK-activating effect (Ni et al. 2011; Xiao et al. 2013). A comparison of the

mechanisms of GK activation of five mulberry (*Morus alba*) bioactive components suggested that they might be used to improve postprandial glucose disposal by acting as GK activators. At 12.5 µm, both 1-deoxynojirmycin and resveratrol were observed to increase GK dislocation (He et al. 2016). Mahmoodi et al. (2013) studied the impact of an extract from a hydro-alcoholic content of Persian shallot (*Allium hirtifolium* Boiss) on the glycaemic content of blood, insulin, GK activity and GK expression. In a molecular docking study, quercetin from Persian shallot showed two H-bonds with Arg63 residue (bond lengths of 3.08 Å and 4.49 Å) in the allosteric site of GK (Grewal et al. 2020b). Angadi et al. (2013) docked "guggultetrol" isolated from the foliage of the waterlily (*Nymphaea pubescens*) in GK's allosteric site. Mangiferin, a C-glycosylxanthone polyphenol derived from the mango tree (*Mangifera indica*), exhibits anti-glycaemic effects. Using a structure-based drug design, they found mangiferin, a C-glycosyl xanthonoid, as a GK activator with an EC_{50} value of 156 µM (Min et al. 2017). Camptothecin showed considerable binding interactions with PPARγ, GK and insulin receptor in docking studies (Jeyabaskar et al. 2017). Ethyl alcohol extract of *Sapium ellipticum* leaves activated GK in induced diabetic rats (Ighodaro et al. 2017). Kaempferol (obtained from *Syzygium cumini*) demonstrated two H-bonds (4.9 Å and 4.7 Å) with Arg63 GK protein (Grewal et al. 2018).

In pursuit of the fact that GK plays an important role in the treatment of T2DM and the role of natural products in GK activation and decreasing blood sugar levels, the present study was planned to screen phytoconstituents from *Artemisia princeps* as allosteric GK activators using molecular docking studies.

Materials & Methods

Design of database

A database of the phytoconstituents present in *Artemisia princeps* including organo-sulphur compounds, alcohols, alkaloids, amino acids, carboxylic acids, esters, fatty acids, fatty acid ester, terpenes, phenols, saponins steroids, and phenolic compounds was prepared for further *in silico* studies (Table 1).

Prediction Of Drug-like Properties

In silico methods for the determination of absorption, distribution, metabolism, and excretion (ADME) parameters depends on theoretically derived statistical models. The compounds selected for this study were evaluated for their ADME parameters by employing the Swiss online ADME web tool (Gleeson et al. 2011; Daina et al. 2017) and accessed using Lipinski's rule of five for drug-likeness.

Molecular Docking

In silico docking studies of the selected compounds were carried out using AutoDock vina and AutoDock tools (Morris et al. 2009). The crystal structure of GK (PDB ID: 3IMX) was retrieved from the protein data bank (www.rcsb.org). The crystal structure was prepared individually by removing existing ligand and

water molecules while missing hydrogens were added using AutoDock tools. Thereafter, non-polar hydrogens were merged while polar hydrogens were added to the protein file and then saved into docking ready PDBQT format. 2-D chemical structures of all the ligands (standard as well as test compounds) were retrieved from PubChem and converted into 3D conformation (mol2 format) using Frog2 server (Miteva et al. 2010). The ligand molecules were further saved into the docking-ready PDBQT format using AutoDock tools (Morris et al. 2009). Docking of the ligands with GK and determination of binding affinities was carried out using AutoDock vina (Trott and Olson 2010). The grid center for docking was detected as X = 0.380, Y = 2.721, Z = -16.334 with the dimension of the grid box 30 × 30 × 30. The reference ligand was docked with GK and compared with that of the reference activator for determining accuracy of the docking protocol. The 3-D optimized ligands were docked with the refined protein and scored using the scoring function. The binding free energy (ΔG, kcal/mol) for each compound was reported in log file and the binding interactions of the ligands in the binding site of GK protein were analysed using PyMOL molecular graphics tool and Discovery Studio (Rathee et al. 2019).

Prediction Of Toxicity

All the compounds were evaluated *in silico* for the prediction of the possible toxicity of these compounds using the “pkCSM” online computer program (Pires et al. 2018; Salgueiro et al. 2016).

Results & Discussion

Prediction of drug-likeness

“Drug-like” molecules usually follow these five criteria: molecular weight up to 500 (MW), logP = 5 (milog), up to 10 and 5 hydrogen bond acceptors (HBAs) and donors (HBDs), respectively. The compounds that do not violate these rules can be further analysed to select the best drug candidate. The results obtained from the ADME study revealed that the selected compounds showed good pharmacokinetic parameters for oral bioavailability (Table 2) and drug-likeness as contrived by Lipinski’s rule of five. However, rutin failed the Lipinski’s rule of five for drug-likeness (with 3 violations, i.e., Mol. Wt, HBAs and HBDs). Amongst the compounds tested, only a few compounds (rutin, stigmasterol, β-sitosterol, daucosterol, γ-sitosterol, friedelin, β-amyrin, β-amyrin acetate, wrightial acetate, 27-norcycloart-20(21)-ene-25-al-3β-ol acetate, ursolic acid, 3-keto-urs-12-ene and α-amyrin) were predicted to be having low GI absorption.

Table 2

ADME properties of the selected phenolic compounds predicted using SwissADME online web server.

Sr. No.	Mol. Wt.	Log P	HBAs	HBDs	NRBs	TPSA	GI ab.	BBB permeant	Lipinski
1	358.34	3.45	7	1	5	87.36	High	No	Yes
2	330.29	2.36	7	3	3	109.36	High	No	Yes
3	284.26	2.56	5	2	2	79.90	High	No	Yes
4	284.26	2.48	5	2	2	79.90	High	No	Yes
5	344.32	3.11	7	2	4	98.36	High	No	Yes
6	316.26	2.13	7	4	2	120.36	High	No	Yes
7	270.24	1.89	5	3	1	90.90	High	No	Yes
8	300.26	2.27	6	3	2	100.13	High	No	Yes
9	374.34	3.36	8	2	5	107.59	High	No	Yes
10	360.31	2.58	8	3	4	118.59	High	No	Yes
11	374.34	3.39	8	2	5	107.59	High	No	Yes
12	608.54	0.21	15	10	6	260.20	Low	No	No
13	344.32	3.07	7	2	4	98.36	High	No	Yes
14	218.25	2.28	3	0	1	43.37	High	Yes	Yes
15	354.35	3.46	6	0	2	55.38	High	Yes	Yes
16	138.12	1.13	3	2	1	57.53	High	Yes	Yes
17	138.12	0.86	3	2	1	57.53	High	Yes	Yes
18	138.12	0.85	3	2	1	57.53	High	Yes	Yes
19	168.15	1.40	4	2	2	66.76	High	No	Yes
20	154.12	0.49	4	3	1	77.76	High	No	Yes
21	154.12	0.66	4	3	1	77.76	High	No	Yes
22	198.17	1.54	5	2	3	75.99	High	No	Yes
23	126.11	0.66	3	3	0	60.69	High	Yes	Yes
24	150.22	2.24	1	1	1	20.23	High	Yes	Yes

HBAs: No. of H-bond acceptors; HBDs: No. of H-bond donors; NRBs: No. of rotatable bonds; TPSA: Topological surface area; GI ab.: Gastro-intestinal absorption; BBB permeant: Blood-brain barrier permeation.

Sr. No.	Mol. Wt.	Log P	HBAs	HBDs	NRBs	TPSA	GI ab.	BBB permeant	Lipinski
25	220.35	3.33	1	1	2	20.23	High	Yes	Yes
26	94.11	1.24	1	1	0	20.23	High	Yes	Yes
27	110.11	1.13	2	2	0	40.46	High	Yes	Yes
28	110.11	0.92	2	2	0	40.46	High	Yes	Yes
29	110.11	0.96	2	2	0	40.46	High	Yes	Yes
30	154.16	1.85	3	1	2	38.69	High	Yes	Yes
31	222.24	2.26	4	0	6	52.60	High	Yes	Yes
32	278.34	2.97	4	0	10	52.60	High	Yes	Yes
33	292.37	3.86	4	0	10	52.60	High	Yes	Yes
34	390.56	5.42	4	0	16	52.60	High	No	Yes
35	211.13	0.09	6	2	3	120.42	High	No	Yes
36	412.69	5.08	1	1	5	20.23	Low	No	Yes
37	414.71	5.05	1	1	6	20.23	Low	No	Yes
38	428.65	4.60	3	1	4	38.69	High	No	Yes
39	576.85	5.17	6	4	9	99.38	Low	No	Yes
40	414.71	5.07	1	1	6	20.23	Low	No	Yes
41	426.72	4.49	1	0	0	17.07	Low	No	Yes
42	426.72	4.63	1	1	0	20.23	Low	No	Yes
43	468.75	4.86	2	0	2	26.30	Low	No	Yes
44	400.64	4.10	2	1	4	37.30	High	No	Yes
45	442.67	4.50	3	0	6	43.37	Low	No	Yes
46	468.67	4.77	3	0	7	43.37	Low	No	Yes
47	456.70	3.95	3	2	1	57.53	Low	No	Yes
48	486.73	4.08	4	2	2	66.76	High	No	Yes
49	424.70	4.58	1	0	0	17.07	Low	No	Yes

HBAs: No. of H-bond acceptors; HBDs: No. of H-bond donors; NRBs: No. of rotatable bonds; TPSA: Topological surface area; GI ab.: Gastro-intestinal absorption; BBB permeant: Blood-brain barrier permeation.

Sr. No.	Mol. Wt.	Log P	HBAs	HBDs	NRBs	TPSA	GI ab.	BBB permeant	Lipinski
50	426.72	4.80	1	1	0	20.23	Low	No	Yes

HBAs: No. of H-bond acceptors; HBDs: No. of H-bond donors; NRBs: No. of rotatable bonds; TPSA: Topological surface area; GI ab.: Gastro-intestinal absorption; BBB permeant: Blood-brain barrier permeation.

Molecular Docking

In silico virtual screening is an effective technique to find a safe and effective solution to major disorders like T2DM. The main objective of the present research was to identify potential allosteric activators of human GK (an enzyme involved in T2DM). *In silico* molecular docking studies were performed to explore the affinity and binding interactions of the designed molecules using AutoDock vina in the active site of GK (PDB ID: 3IMX). The docking protocol used in this study was first validated by redocking the co-crystallized ligand of GK. The re-docked GK activator produced pose similar to those of the co-crystallized GK activator in the allosteric site of GK with ΔG of -11.1 kcal/mol, validating the accuracy of the docking methodology used (Fig. 1A). The 3IMX ligand showed strong hydrogen bond interactions with Ser69 (3.20 Å) and Arg63 (3.50 and 3.20 Å) residues in the allosteric site of GK protein (Fig. 1B).

The selected phytoconstituents were docked in the allosteric site of GK, which is surrounded by the b1 strand and a5 helix of the large domain, the C-terminal a13 helix of the small domain, and the GK specific connecting region I (Ser64-Gly72). The allosteric site of GK comprise of Arg63, Ser69, Tyr215, Met210, Tyr214, Val452 and Val455 residues. Binding energy (ΔG) is an important factor for predicting a potential drug candidate against any disease. The lower the binding energy, the more stable will be the complex. Some of the docked compounds showed significant binding interactions in the allosteric site of GK as established by analysing their bonding interactions and ΔG (kcal/mol) of the best-docked poses (Table 3).

Table 3
Hydrogen bond interactions and docking score (ΔG, kcal/mol) of the best docked poses of phytoconstituents with GK.

Sr. No.	Ligand	ΔG (kcal/mol)	No. of H-bonds	Residue(s) involved in H-bonds	Bond length (Å)
1	5-Desmethyl-sinensetin	-7.4	1	Arg63	3.25
2	Jaceosidin	-7.5	1	Arg63	2.88
3	Acacetin	-7.2	0	-	-
4	Genkwanin	-6.9	0	-	-
5	Eupatilin	-7.6	1	Arg63	2.70
6	Eupafolin	-7.2	1	Arg63	2.80
7	Apigenin	-7.2	1	Arg63	3.55
8	Hispidulin	-7.4	1	Arg63	2.77
9	Chrysoplenetin	-7.0	1	Arg63	3.08
10	3,5,4'-Trihydroxy-6,7,3'-trimethoxy flavone	-7.0	1	Arg63	2.71
11	5,4'-Dihydroxy-6,7,3',5'-tetramethoxyflavone	-7.2	1	Arg63	2.94
12	Rutin	-8.0	3	Ser64, Glu67 and Ser69	2.74, 3.18 and 3.25
13	5,4'-Dihydroxy-6,7,3'-trimethoxyflavone	-8.4	2	Arg63 Ser69	2.86 2.97
14	6-Acetyl-2,2-dimethylchroman-4-one	-7.6	1	Arg63	3.47
15	Sesamin	-7.6	1	Arg63	3.83
16	Salicylic acid	-5.1	1	Arg63	3.03
17	m-Hydroxy benzoic acid	-5.2	1	Ser69	3.08
18	p-Hydroxy benzoic acid	-5.0	1	Arg63	4.01
19	Vanillic acid	-5.1	1	Arg63	2.84
20	Gentisic acid	-5.4	1	Arg63	2.78
21	Protocatechuic acid	-5.2	1	Arg63	2.84

*Reference GK activator: (2R)-3-cyclopentyl-N-(5-methoxy[1,3]thiazolo[5,4-b]pyridin-2-yl)-2-{4-[(4-methylpiperazin-1-yl)sulfonyl]phenyl}propanamide.

Sr. No.	Ligand	ΔG (kcal/mol)	No. of H-bonds	Residue(s) involved in H-bonds	Bond length (Å)
22	Syringic acid	-5.2	1	Arg63	3.01
23	Phloroglucinol	-4.6	1	Arg63	3.21
24	6-Methyl-3-isopropylphenol	-5.7	1	Arg63	3.10
25	2,6-Di-tert-butyl-4-methylphenol	-6.8	0	-	-
26	Phenol	-4.5	0	-	-
27	1,2-Benzenediol	-4.4	1	Arg63	2.99
28	1,4-Benzenediol	-4.3	1	Arg63	3.25
29	1,3-Benzenediol	-4.4	1	Arg63	3.16
30	2,6-Dimethoxy phenol	-4.6	1	Arg63	3.14
31	Diethyl phthalate	-6.3	0	-	-
32	Dibutyl phthalate	-6.6	0	-	-
33	Butyl isobutyl phthalate	-6.9	0	-	-
34	Diisooctyl phthalate	-6.6	0	-	-
35	3-Nitrophthalic acid	-5.9	0	-	-
36	Stigmasterol	-7.6	0	-	-
37	β-Sitosterol	-6.9	0	-	-
38	Ergosterol peroxide	-7.0	0	-	-
39	Daucosterol	-8.6	1	Tyr214	2.97
40	γ-Sitosterol	-7.5	0	-	-
41	Friedelin	-7.6	0	-	-
42	β-Amyrin	-7.7	0	-	-
43	β-Amyrin acetate	-7.8	0	-	-
44	Wrightial	-6.9	0	-	-
45	Wrightial acetate	-7.0	0	-	-
46	27-Norcycloart-20(21)-ene-25-al-3β-ol acetate	-6.5	1	Ser69	3.09

*Reference GK activator: (2R)-3-cyclopentyl-N-(5-methoxy[1,3]thiazolo[5,4-b]pyridin-2-yl)-2-{4-[(4-methylpiperazin-1-yl)sulfonyl]phenyl}propanamide.

Sr. No.	Ligand	ΔG (kcal/mol)	No. of H-bonds	Residue(s) involved in H-bonds	Bond length (Å)
47	Ursolic acid	-7.6	0	-	-
48	Methyl commate D	-8.5	1	Arg63	3.04
49	3-Keto-urs-12-ene	-7.5	0	-	-
50	α-Amyrin	-6.8	0	-	-
51	Reference GK activator*	-11.1	3	Arg63 and Ser69	3.20, 3.50 and 3.20

*Reference GK activator: (2R)-3-cyclopentyl-N-(5-methoxy[1,3]thiazolo[5,4-b]pyridin-2-yl)-2-{4-[(4-methylpiperazin-1-yl)sulfonyl]phenyl}propanamide.

Out of the phytoconstituents docked with GK in this study, rutin, 5,4'-dihydroxy-6,7,3'-trimethoxyflavone, daucosterol and methyl commate D showed appreciable binding in the allosteric site of GK as determined by analyzing the hydrogen bond and hydrophobic interactions of the best-docked poses. On the basis of their lowest binding free energy (kcal/mol) and docking interactions in the allosteric site, these compounds were further analyzed in detail by PyMOL and Discovery Studio visualiser. An overlay of the docked pose of these compounds (rutin, 5,4'-dihydroxy-6,7,3'-trimethoxyflavone, daucosterol and methyl commate D) with that of the 3IMX ligand showed that these compounds had a similar binding pattern in the allosteric site of GK enzyme as that of the co-crystallized ligand (Figure 2). The docking studies of these molecules suggested a complementary fit in the allosteric site of GK.

Hydrogen bond interactions of the selected phytoconstituents (rutin, 5,4'-dihydroxy-6,7,3'-trimethoxyflavone, daucosterol and methyl commate D) with the allosteric site residues of GK (PDB ID: 3IMX) are presented in Fig. 3. Rutin showed three hydrogen bonds with Ser64, Glu67 and Ser69 residues of GK (with bond distances of 2.74, 3.18 and 3.25 Å, respectively). 5,4'-Dihydroxy-6,7,3'-trimethoxyflavone showed two hydrogen bonds with Arg63 and Ser69 residues of GK (with bond distances of 2.86 and 2.97 Å, respectively). Daucosterol showed one hydrogen bond with Tyr214 residue of GK (with bond distance of 2.97 Å). Methyl commate D also showed one hydrogen bond with Arg63 residue of GK (with bond distance of 3.05 Å).

Hydrophobic interactions of the selected phytoconstituents with the allosteric site residues of human GK protein (PDB ID: 3IMX) are presented in Fig. 4. These 4 phytoconstituents protruded in the hydrophobic pocket of GK to form strong hydrophobic interactions. Rutin showed hydrophobic interactions with Trp99, Ile211, Tyr214, Tyr215, Leu451 and Val455 residues in the allosteric site of GK. 5,4'-Dihydroxy-6,7,3'-trimethoxyflavone displayed strong hydrophobic interactions with Val91, Trp99, Met210, Ile211, Tyr214 and Val455 residues in the allosteric site of GK. Daucosterol showed hydrophobic interactions with Val62, Arg63, Pro66, Ile159, Val452, Val455, Ala456, Lys459 and Ala460 residues in the allosteric site of GK. Methyl commate D showed hydrophobic interactions with Trp99, Tyr214, His218 and Val455 residues in

the allosteric site of GK. These molecular docking studies thus helped us in predicting that these phytoconstituents could act as potential allosteric activators of the human GK enzyme.

Artemisia genus has been reported as a rich source of flavonoids, including eupatilin, eupatorine and rutin. Several lines of evidence suggest that flavonoids that originated from vegetables and medicinal plants have beneficial effects on diabetes by improving glycaemic control, lipid profile, and antioxidant status. Eupatilin showed strong hydrogen bond interaction with Arg63 residue of GK (binding energy of -7.6 kcal/mol) *in silico* supporting the *in vitro* GK activation and *in vivo* glucose lowering activity reported earlier (Kang et al. 2008). Rutin is a flavonoid found in many plants and shows a wide range of biological activities including anti-inflammatory, antioxidant, neuroprotective, nephroprotective, and hepatoprotective effects (Prince et al. 2006; Ghorbani 2017; Habtemariam and Lentini 2015; Hasanein et al. 2020). Administration of 5,4'-dihydroxy-6,7,3'-trimethoxyflavone (cirsilineol) at 7.5 and 15 mg/kg for 28 days reduced the blood glucose level to a significant level in streptozotocin-induced diabetic animals (Shah et al. 2019; Ekiert et al. 2022). Daucosterol, a natural phytosterol-like compound isolated from *Costus pictus* (after enteric coating) significantly reduced blood glucose levels and increased insulin release in rats at a very low dose (Ivorra et al. 1988; Benny et al. 2020).

Prediction Of Toxicity

The possible toxicity (mutagenic, carcinogenic, cardiotoxicity, immunotoxicity, skin irritation and reproductive toxicity) for the selected compounds was accessed using the pkCSM online tool (Tables 4). Mutagenicity (AMES toxicity) was predicted for eupatilin and sesamin. Cardiac toxicity (hERG II inhibition) was predicted for 17 compounds (5-desmethyl-sinensetin, acacetin, eupatilin, chrysoplenetin, rutin, 5,4'-dihydroxy-6,7,3'-trimethoxyflavone, diisooctyl phthalate, stigmasterol, β-sitosterol, γ-sitosterol, friedelin, β-amyrin, β-amyrin acetate, wrightial acetate, 27-norcycloart-20(21)-ene-25-al-3β-ol acetate, 3-keto-urs-12-ene and α-amyrin. Hepatotoxicity was predicted for 6-methyl-3-isopropylphenol, ergosterol peroxide and 27-norcycloart-20(21)-ene-25-al-3β-ol acetate). Skin sensitivity was predicted for 6-methyl-3-isopropylphenol, 2,6-di-tert-butyl-4-methylphenol, phenol, 1,2-benzenediol, 1,4-benzenediol and 1,3-benzenediol.

Table 4
Predicted toxicity (probability for presence or absence of toxicity) for the selected compounds obtained using pkCSM.

Sr. No.	AMES toxicity	Max. tolerated dose	hERG I inhib.	hERG II inhib.	Rat acute tox.	Rat chronic tox.	Hepato-toxicity	Skin toxicity
1	No	0.679	No	Yes	2.744	1.576	No	No
2	No	0.989	No	No	2.310	2.476	No	No
3	No	0.573	No	Yes	2.350	1.448	No	No
4	No	0.006	No	No	1.923	1.843	No	No
5	Yes	0.993	No	Yes	2.473	2.364	No	No
6	No	0.868	No	No	2.059	2.288	No	No
7	No	0.555	No	No	2.225	1.653	No	No
8	No	0.555	No	No	2.225	1.653	No	No
9	No	0.733	No	Yes	2.142	1.532	No	No
10	No	0.538	No	No	2.020	2.725	No	No
11	No	0.419	No	No	1.960	1.516	No	No
12	No	0.49	No	Yes	2.482	5.126	No	No
13	No	0.547	No	Yes	2.572	1.513	No	No
14	No	0.801	No	No	1.652	2.096	No	No
15	Yes	0.299	No	No	3.170	1.614	No	No
16	No	0.605	No	No	2.048	2.213	No	No
17	No	0.993	No	No	1.863	2.978	No	No
18	No	1.002	No	No	1.862	2.971	No	No
19	No	1.408	No	No	2.203	1.982	No	No
20	No	0.027	No	No	2.016	2.366	No	No
21	No	1.379	No	No	2.180	1.950	No	No
22	No	1.652	No	No	2.086	2.470	No	No

AMES toxicity: Mutagenic (or carcinogenic) potential; Max. tolerated dose: Maximum tolerated human dose (log mg/kg/day); hERG I inhib.: hERG I inhibition (cardio-toxicity); hERG I inhib.: hERG II inhibition (cardio-toxicity); Rat acute tox.: Oral rat acute toxicity (LD50) (mol/kg); Rat chronic tox.: Oral rat chronic toxicity (log mg/kg_bw/day); Skin toxicity: Skin sensitization.

Sr. No.	AMES toxicity	Max. tolerated dose	hERG I inhib.	hERG II inhib.	Rat acute tox.	Rat chronic tox.	Hepato-toxicity	Skin toxicity
23	No	0.456	No	No	1.891	2.241	No	No
24	No	0.522	No	No	2.334	2.229	Yes	Yes
25	No	0.201	No	No	2.207	2.255	No	Yes
26	No	1.113	No	No	1.910	1.981	No	Yes
27	No	0.610	No	No	2.144	2.172	No	Yes
28	No	0.610	No	No	2.144	2.172	No	Yes
29	No	1.020	No	No	1.983	2.769	No	Yes
30	No	1.245	No	No	1.924	2.249	No	No
31	No	1.136	No	No	1.950	2.680	No	No
32	No	1.289	No	No	1.855	2.291	No	No
33	No	1.265	No	No	1.655	2.249	No	No
34	No	1.112	No	Yes	1.249	2.695	No	No
35	No	0.635	No	No	2.039	2.513	No	No
36	No	0.318	No	Yes	2.454	1.125	No	No
37	No	0.272	No	Yes	2.474	1.108	No	No
38	No	0.013	No	No	2.041	1.868	Yes	No
39	No	0.546	No	No	2.598	3.023	No	No
40	No	0.272	No	Yes	2.474	1.108	No	No
41	No	0.260	No	Yes	2.454	1.063	No	No
42	No	0.018	No	Yes	2.373	1.274	No	No
43	No	0.375	No	Yes	2.322	2.149	No	No
44	No	0.735	No	No	2.702	2.222	No	No
45	No	0.275	No	Yes	2.441	2.036	No	No
46	No	0.172	No	Yes	2.293	2.178	No	No

AMES toxicity: Mutagenic (or carcinogenic) potential; Max. tolerated dose: Maximum tolerated human dose (log mg/kg/day); hERG I inhib.: hERG I inhibition (cardio-toxicity); hERG I inhib.: hERG II inhibition (cardio-toxicity); Rat acute tox.: Oral rat acute toxicity (LD50) (mol/kg); Rat chronic tox.: Oral rat chronic toxicity (log mg/kg_bw/day); Skin toxicity: Skin sensitization.

Sr. No.	AMES toxicity	Max. tolerated dose	hERG I inhib.	hERG II inhib.	Rat acute tox.	Rat chronic tox.	Hepato-toxicity	Skin toxicity
47	No	0.650	No	No	4.086	2.043	Yes	No
48	No	0.017	No	No	2.382	1.763	No	No
49	No	0.140	No	Yes	2.071	1.038	No	No
50	No	0.186	No	Yes	2.383	1.280	No	No

AMES toxicity: Mutagenic (or carcinogenic) potential; Max. tolerated dose: Maximum tolerated human dose (log mg/kg/day); hERG I inhib.: hERG I inhibition (cardio-toxicity); hERG I inhib.: hERG II inhibition (cardio-toxicity); Rat acute tox.: Oral rat acute toxicity (LD50) (mol/kg); Rat chronic tox.: Oral rat chronic toxicity (log mg/kg_bw/day); Skin toxicity: Skin sensitization.

Conclusions

Molecular docking studies using AutoDock vina were performed to explore the binding mechanism of the phytoconstituents of *Artemisia princeps* with the human GK enzyme (an enzyme involved in T2DM). In current *in silico* docking studies, results clearly demonstrated that amongst the phytoconstituents tested in silico, some compounds (rutin, 5,4'-dihydroxy-6,7,3'-trimethoxyflavone, daucosterol and methyl commate D) showed appreciable binding in the allosteric site of GK enzyme. *In silico* study is actually an added advantage for screening allosteric GK activators and natural compounds may serve as useful leads for the development of clinically useful and safe GK activators. However, structural modifications and further studies on these phytoconstituents are required to develop safe and effective activators of human GK.

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Table 1

Table 1 is available in Supplementary Files section.

Figures

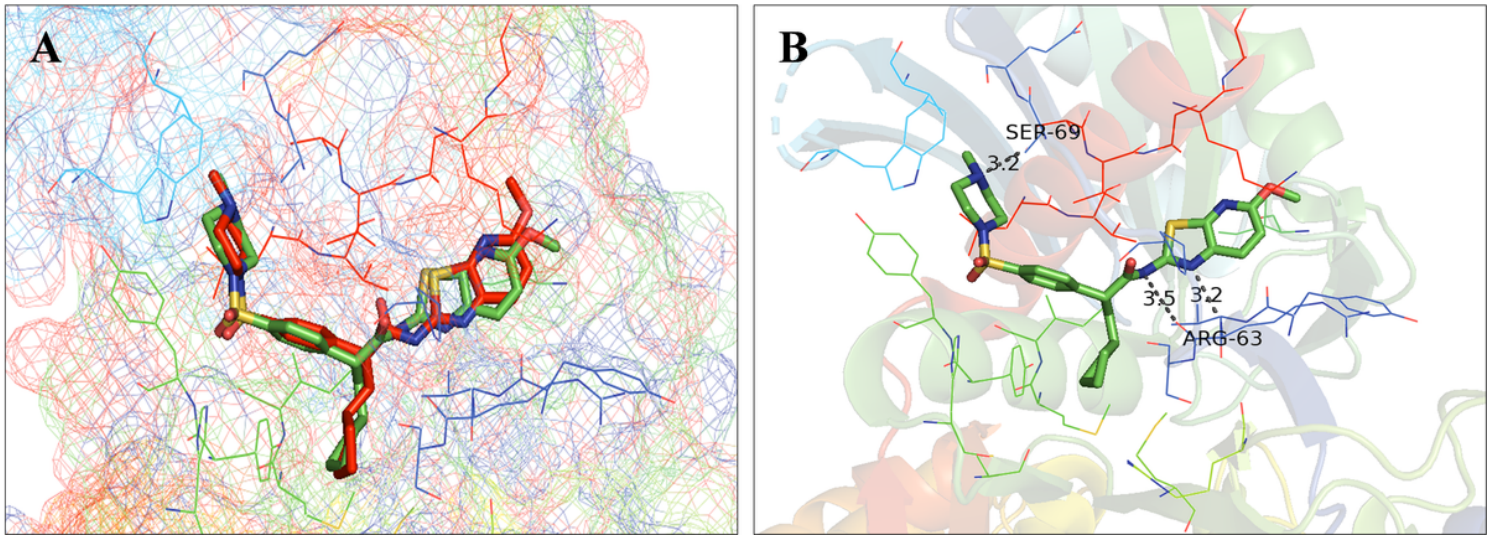

Figure 1

A. Overlay of the docked pose of the 3IMX ligand (green sticks) on that produced by the co-crystallized ligand (red sticks) in the allosteric site of GK. B. Docked pose showing hydrogen bond interactions of the 3IMX ligand (reference GK activator) with allosteric site residues of GK protein.

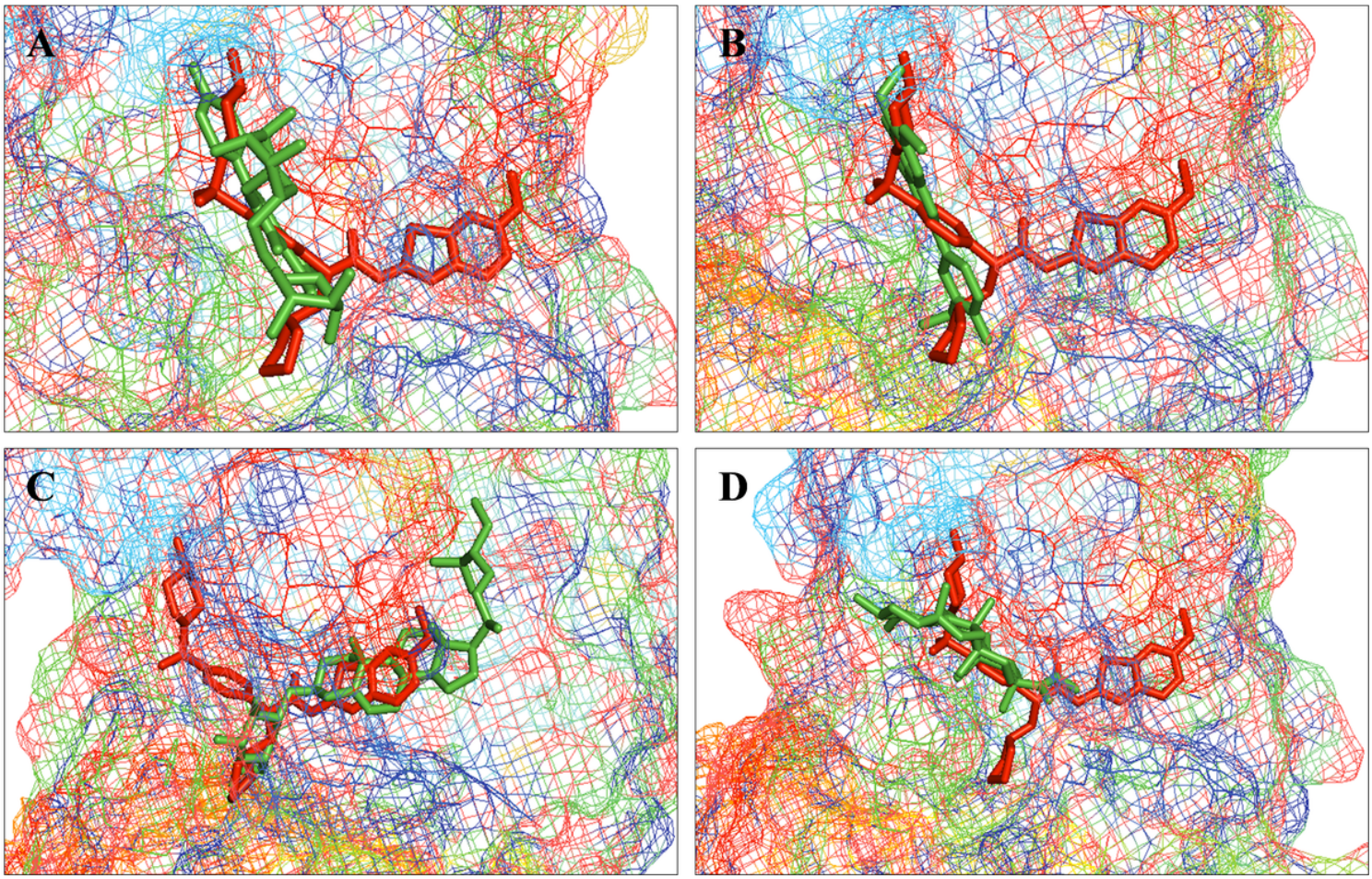

Figure 2

Overlay of the docked poses of rutin (A), 5,4'-dihydroxy-6,7,3'-trimethoxyflavone (B), daucosterol (C) and methyl commate D (D) (green sticks) with that of 3IMX ligand (red sticks) in the allosteric site of GK protein.

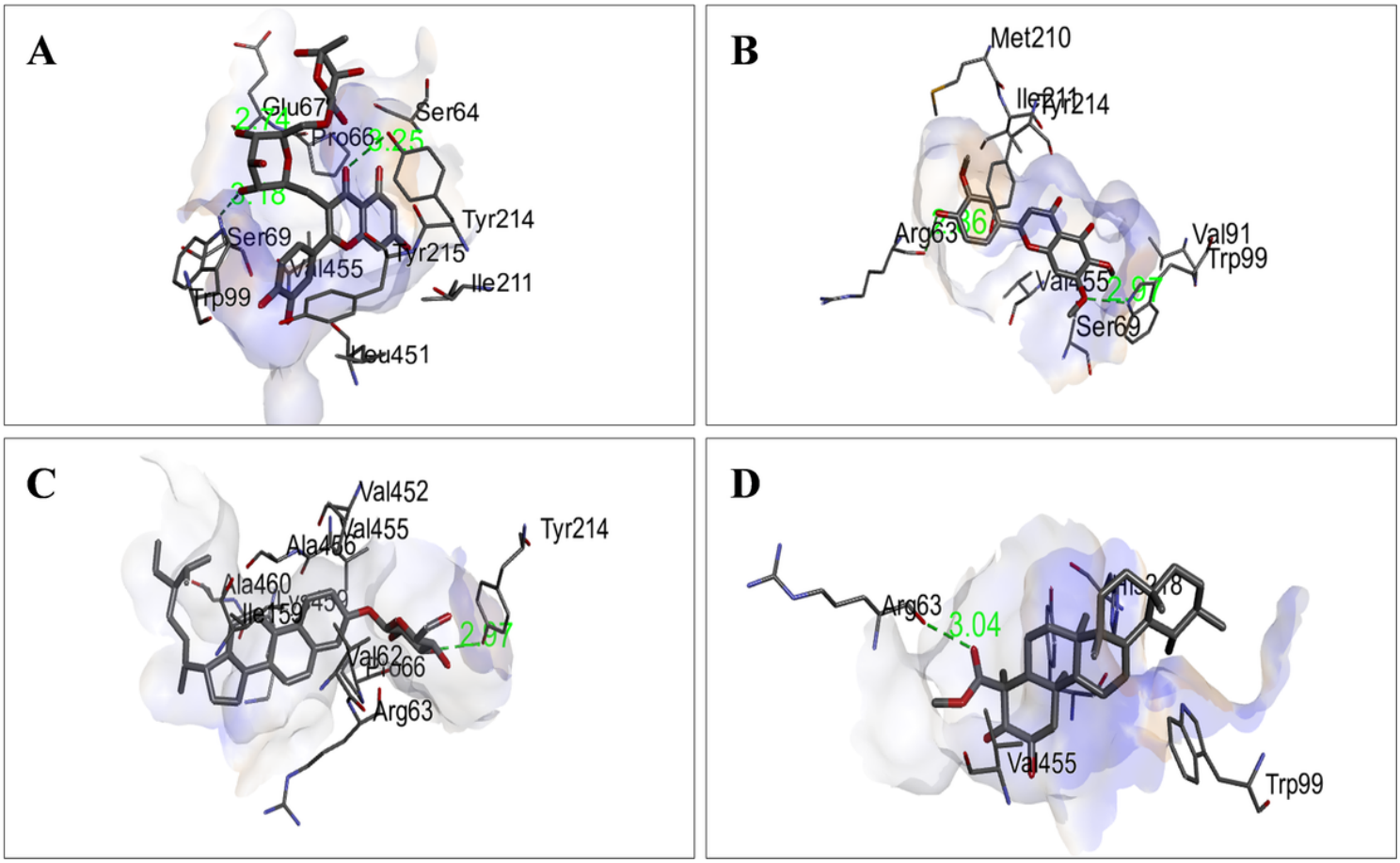

Figure 3

Docked poses showing hydrogen bond interactions of rutin (A), 5,4'-dihydroxy-6,7,3'-trimethoxyflavone (C), daucosterol (C) and methyl commate D (D) in the allosteric site of human GK enzyme (PDB ID: 3IMX).

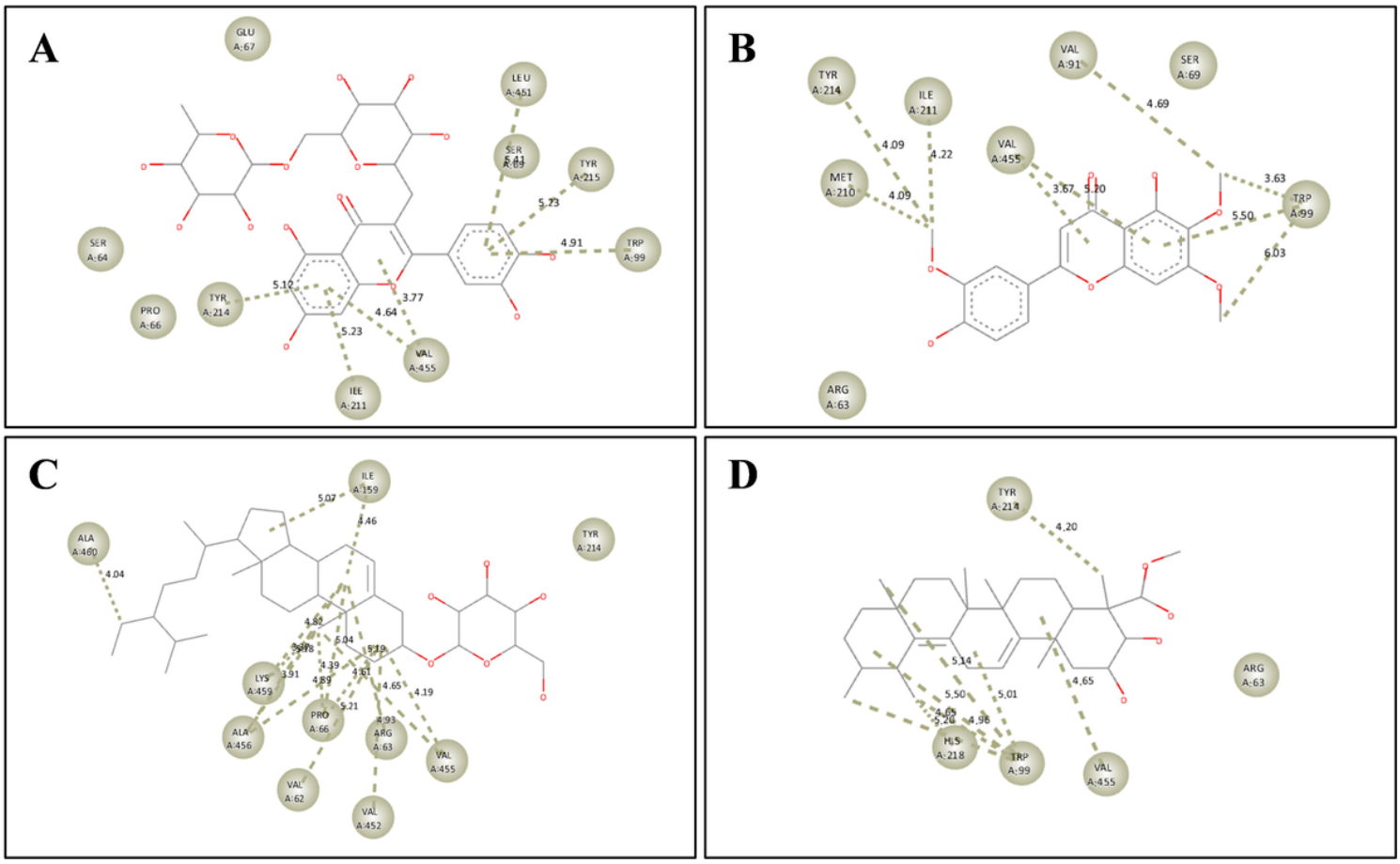

Figure 4

Docked poses showing hydrophobic interactions of rutin (A), 5,4'-dihydroxy-6,7,3'-trimethoxyflavone (C), daucosterol (C) and methyl commate D (D) in allosteric site of human GK enzyme (PDB ID: 3IMX).

Supplementary Files

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- Table1.docx