Plant Archives Vol. 20, Supplement 1, 2020 pp. 3768-3771 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

IN SILICO DOCKING STUDIES OF COMPOUNDS FROM PERSIAN SHALLOT AS ALLOSTERIC GLUCOKINASE ACTIVATORS

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Abstract

Glucokinase (GK) is an emerging target for the treatment of diabetes and allosteric activators of human GK were showed hypoglycaemic potential in various preclinical and clinical studies. Persian shallot extract (hydro-alcoholic) was reported to potentiate GK activity. The present study is designed to evaluate *in silico* some compounds (alliogenin, gitogenin, kaempferol, quercetin and shallomin) found in Persian shallot in order to explore their binding mode and interactions with GK. These compounds showed analogous binding pattern with the allosteric site of GK as that of the co-crystallised GK activator. These compounds displayed good binding free energy and significant binding interactions with the allosteric site residues of GK and this docking study supported the *in vitro* GK activity of Persian shallot extract. This information can be used to design potent and non-toxic natural GK activators for the diabetes therapeutics.

Key words: Allosteric, Diabetes, Docking, Glucokinase, GK activators, Persian shallot.

Introduction

Glucokinase (GK) is a cytoplasmic enzyme expressed predominantly in pancreatic β-cells and liver hepatocyte; and catalyzes the conversion of glucose to glucose-6-phosphate with the help ATP (Pal, 2009; Coghlan and Leighton, 2008). In pancreatic β-cells of pancreas, GK regulates glucose-stimulated insulin release and in liver hepatocytes of liver, it controls the breakdown of sugars. GK acts as an emergent drug target for treatment and management of type 2 diabetes due to its key function in controlling sugar breakdown. Small molecule allosteric activators of human GK are the unique class of therapeutic candidates which allosterically activate GK and show their hypoglycemic potential (Coghlan and Leighton, 2008; Perseghin, 2010; Matschinsky *et al.*, 2011). Several GK activators had been progressed into clinical trials (phase II) including AZD6370, AZD1656, MK-0941, Piragliatin, and AMG151; even though strong decrease in blood sugar was observed, potential adverse reactions were also reported, such as hypoglycemia and elevated levels of triglycerides (Grewal *et al.*, 2014; Singh *et al.*, 2017; Charaya *et al.*, 2018).

Persian shallot (*Allium hirtifolium* Boiss) is a nutritive plant used as spices, native to Iran with special taste that belongs to Alliaceae family and also used in medicine (Jafariana *et al.*, 2003). For many years, fresh and dry bulbs of A. hirtifolium are used in traditional medicine for treatment of rheumatic, inflammatory, arthritis, diarrhoea and stomach pain in Iran (Asili *et al.*, 2010).

Persian shallot possesses various pharmacological activities including antioxidant, hypoglycaemic, anticancer, anti-platelet aggregation, antihyperlipidemic, antimicrobial, antifungal, anti-parasitic, hepato-protective and immunomodulatory effects (Asgarpanah and Ghanizadeh, 2012; Moradi *et al.*, 2013; Panahandeh *et al.*, 2016; Fasihzadeh *et al.*, 2016). Various types of phytoconstituents such as flavonoids and other phenolic compounds (kaempferol, quercetin and shallomin), fatty acids (palmitic acid, stearic acid, oleic acid, linolenic acid and linoleic acid), saponins and sapogenins (furostanol and spirostanol), steroidal compounds (alliogenin and gitogenin), sulphur compounds (thiosulfinates such as allicin, diallyl disulphide, S-allylcysteine, diallyl trisulfide and ajoene), flavonol glycosides, folic acid, vitamin C and minerals (Cu, Zn and Mn) are present in different parts of the plant (Moradi *et al.*, 2013; Panahandeh *et al.*, 2016; Ali *et al.*, 2017).

The hydro-alcoholic extract of Persian shallot was reported to increase GK expression and potentiate GK activity (Mahmoodi *et al.*, 2013; Grewal *et al.*, 2014). Recently, flavonoids (eupatilin and mangiferin) and steroidal derivative (coaglunide) were reported as allosteric GK activators (Kang *et al.*, 2008; Singh *et al.*, 2012; Grewal *et al.*, 2014; Grewal *et al.*, 2019). In the current investigation some steroidal and flavonoid derivatives (alliogenin (AG), gitogenin (GT), kaempferol (KP), quercetin (QC) and shallomin (SL)) were selected for the *in-silico* evaluation using molecular docking studies to explore their binding mode and interactions with GK enzyme (Fig. 1).

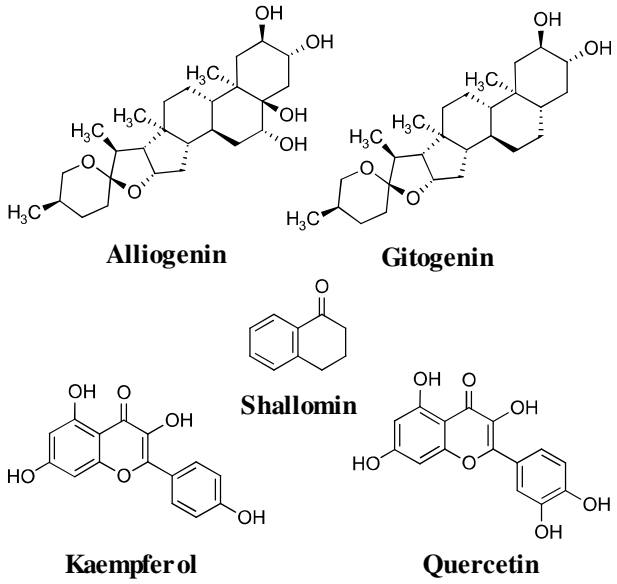

Fig. 1: Chemical structure of compounds selected for docking with GK enzyme.

Materials and Methods

Prediction of pharmacokinetic parameters

All the compounds selected for molecular docking studies were analyzed for the prediction of pharmacokinetic parameters related to absorption, distribution, metabolism, and excretion (ADME) by employing FAF-Drugs4 server; and accessed for drug-likeness using Lipinski's rule (Miteva *et al.*, 2006; Lagorce *et al.*, 2017).

Molecular docking studies

Molecular docking investigations were performed for the synthesized derivatives in the allosteric binding site of the GK employing AutoDock Vina (Trott and Olson, 2010) and AutoDock Tools (ADT) (Morris *et al.,* 2009).

Preparation of ligand files

The 2D chemical structures ("sdf" format) of all the ligands were prepared by MarvinSketch 18.5.0 (ChemAxon) followed by conversion to 3D ("mol2" format) by Frog2 server (Miteva *et al.*, 2010). The ligands ("mol2" format) were converted to "pdbqt" files using ADT (Morris *et al.*, 2009).

Preparation of protein file

After assessing a number of the co-crystallized structures for the target protein available in the protein data bank; the best ligand bound complex was selected based on higher resolution and key binding interactions between the GK and small molecule activators. The "pdb" file of the GK protein was edited using PyMOL (Schrödinger, LLC.) by removing the co-crystallized activator, all the water molecules along with other non-interacting species. The "pdbqt" file of GK protein was generated from "pdb" file using ADT (Rathee *et al.*, 2019).

Preparation of grid box

The "Grid" tool of ADT was used to calculate the grid parameters and all the information concerning target protein, ligand, grid size and geometry were saved in "txt" file (Charaya *et al.*, 2018; Rathee *et al.*, 2018; Rathee *et al.*, 2019).

Docking run

Docking was performed using command line on Windows. The reference ligand was docked in the allosteric binding site of GK and compared with that of co-crystallized GK activator for determining accuracy of docking protocol. The 3-D optimized ligands were docked in the allosteric site of the refined GK protein and scored by 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 allosteric site of the GK protein were analysed using PyMOL (Charaya *et al.*, 2018; Grewal et al., 2018; Grewal *et al.*, 2019a; Rathee *et al.*, 2019).

Results and Discussion

Prediction of ADME properties

ADME properties including molecular weight (MW), partition coefficient (log P), distribution coefficient (log D), water solubility (log S_w), topological polar surface area (tPSA), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), solubility (mg/L) and number of rotatable bonds (NRB) were calculated for the compounds selected for docking studies. Almost all of the compounds selected for in silico studies showed good pharmacokinetic parameters for oral bioavailability (Table 1) and drug-likeness as contrived by Lipinski's rule of five.

In silico docking studies

In silico molecular docking studies were carried out to explore the affinity and binding interactions of the selected compounds using AutoDock Vina in the allosteric site of GK (PDB ID: 3IMX). The reference ligand of PDB 3IMX produced an analogous binding pattern and overlay on the binding mode of the co-crystallized activator with ΔG of -9.0 kcal/mol validating accuracy of docking method. Based on the lowest binding free energy (ΔG) and docking interactions in the allosteric site of GK enzyme, KP and QC were further analyzed in details using PyMOL for exploring binding interactions of these molecules with allosteric site residues of GK protein (Table 2).

Table 1: Predicted ADME properties of the selected compounds.

Comp.	AG	GT	KT	QC	SL
MW	464.63	432.64	286.24	302.24	146.19
log P	3.12	5.52	1.90	1.54	2.02
log D	1.95	4.26	1.35	1.01	2.28
log S_w	-4.69	-6.00	-3.13	-2.99	-2.23
tPSA	99.38	58.92	110.80	131.03	17.07
HBA	6	4	6	7	1
HBD	4	2	4	5	0
Solubility	4284	1072	12543	15228	15731
NRB	0	0	1	1	0

Table 2: Binding interactions and docking score (ΔG) of the selected compounds with GK.

Comp.	H-bond (Distance)	Hydrophobic interactions	ΔG
AG	Ser69 (3.19 Å)	Trp99, Tyr215	-7.2
GT	Ser69 (3.08 Å)	Trp99, Tyr215, Val455	-7.4
KP	Arg63 (2.71 Å) Ser69 (4.29 Å)	Val62, Trp99, Ile211, Tyr214, Val455, Ala456, Lys459	-8.1
QC	Arg63 (3.08 Å and 4.49 Å)	Val62, Pro66, Ile159, Ile211, Val455, Ala456, Lys459	-8.4
SL	Ser69 (3.05 Å)	Trp99, Tyr215, Leu451	-6.3

Super-positioning of the docked poses of KP and QC with that of the ligand of PDB ID: 3IMX (i.e., (2R)-3-cyclopentyl-N-(5-methoxy[1,3]thiazolo[5,4-b]pyridin-2-yl)-2-{4-[(4-methylpiperazin-1-yl)sulfonyl]phenyl}propanamide)) in the allosteric site of GK protein showed that the selected molecules had the similar binding and orientation pattern in the allosteric site of GK protein as that of the co-crystallized synthetic GK activator (Fig. 2 and 3).

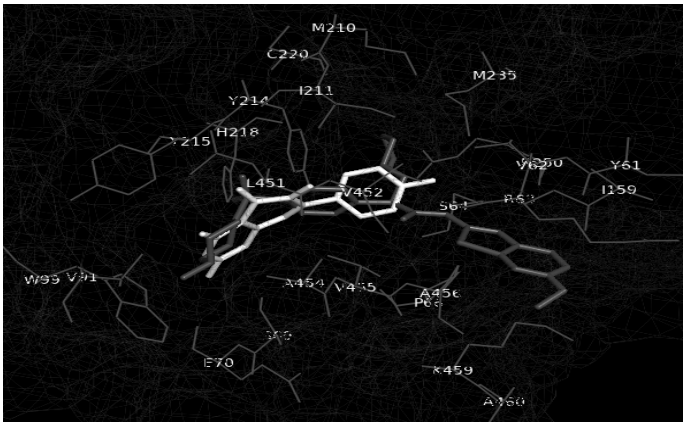

Fig. 2 : Super-positioning of the docked pose of KP (white) with that of PDB ligand 3IMX (grey) in the allosteric site of GK.

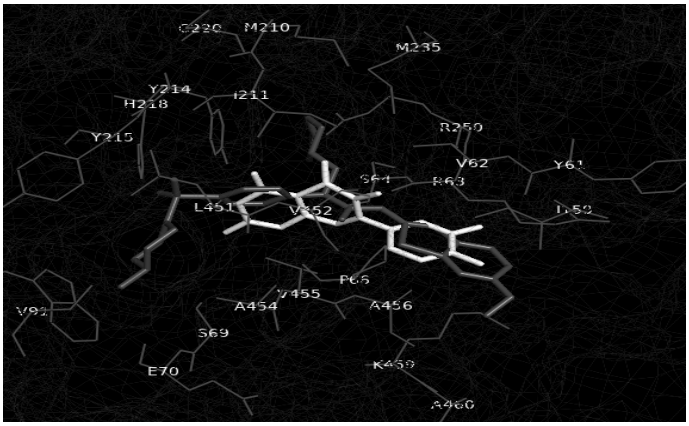

Fig. 3 : Super-positioning of the docked pose of QC (white) with that of PDB ligand 3IMX (grey) in the allosteric site of GK.

Docked pose of KP showed H-bond interactions between 'OH' of 4-hydroxyphenyl group and amide 'NH' of Arg63 residue; and 'OH' of chromene-4-one moiety and backbone 'carbonyl' of Ser69 residue on GK with bond length of 2.71 and 4.29 Å, respectively. The 4-hydroxyphenyl ring of KP projected in the hydrophobic pocket displaying interactions with Val455 and Lys459 of the R13 helix, as well as Pro66 of connecting region I and Ile211, chromene-4-one moiety packs between Tyr214 and Val455 residues of allosteric site of GK (Fig. 4).

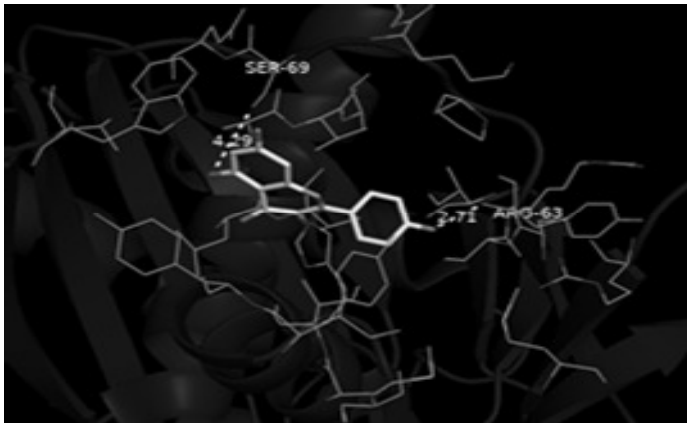

Fig. 4: Best docked pose showing H-bond interactions of KP with the allosteric site residues of GK.

Docked pose of QC showed H-bond interactions between 'OH' of 3,4-dihydroxyphenyl and amide 'NH' of Arg63 residue; and 'OH' of chromene-4-one moiety and backbone 'carbonyl' of Arg63 residue on GK with bond length of 3.08 and 4.49 Å, respectively. The 3,4-dihydroxyphenyl ring of QC projected in the hydrophobic pocket displaying interactions with Val455 and Lys459 of the R13 helix, as well as Pro66 of connecting region I and Ile211, chromene-4-one moiety packs between Tyr214, Val455 and Ala456 residues of in allosteric site of GK (Fig. 5).

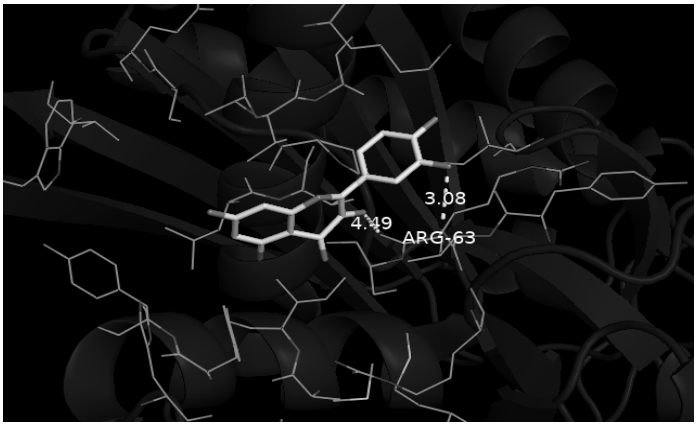

Fig. 5 : Best docked pose showing H-bond interactions of QC with the allosteric site residues of GK.

Conclusions

In summary, five compounds found in Persian shallot were evaluated in silico using docking studies for exploring binding interactions of these compounds with allosteric site residues of GK. Amongst these compounds, KP and QC displayed strong and stable interactions with allosteric site residues of GK supporting the in vitro GK activity of Persian shallot extract. Structural modifications and further studies on these natural compounds are required to develop safe and potent allosteric GK activators for the treatment of diabetes.

Acknowledgements

The authors are thankful to Chitkara College of Pharmacy, Chitkara University, Rajpura, Punjab (India) for their support and encouragement for this research work.

References

Asgarpanah, J. and Ghanizadeh B. (2012), Pharmacologic and medicinal properties of Allium hirtifolium Boiss, African Journal of Pharmacy and Pharmacology, 6: 1809-1814.

Ali, S.E.; Chehri, K.; Karimi N. and Karimi I. (2017). Computational approaches to the in vitro antibacterial activity of Allium hirtifolium Boiss against gentamicin-resistant Escherichia coli: focus on ribosome recycling factor, In Silico Pharmacology, 5: Article 7.

Asili, A.; Behravan, J.; Naghavi M.R. and Asili J. (2010), Genetic diversity of Persian shallot (*Allium hirtifolium*) ecotypes based on morphological traits, allicin content

and RAPD markers, Open Access Journal of Medicinal and Aromatic Plants, 1: 1-6.

Charaya, N.; Pandita, D.; Grewal, A.S. and Lather V. (2018), Design, synthesis and biological evaluation of novel thiazol-2-yl benzamide derivatives as glucokinase activators, Computational Biology and Chemistry, 73: 221-229.

Coghlan, M. and Leighton, B. (2008), Glucokinase activators in diabetes management, Expert Opinion on Investigational Drugs, 17(2): 145-167.

Fasihzadeh, S.; Lorigooini, Z. and Jivad, N. (2016), Chemical constituents of *Allium stipitatum* regel (persian shallot) essential oil, Der Pharmacia Lettre, 8(1): 175-180.

Grewal, A.S.; Sekhon, B.S. and Lather V. (2014). Recent updates on glucokinase activators for the treatment of type 2 diabetes mellitus, Mini-Reviews in Medicinal Chemistry, 14(7): 585-602.

Grewal, A.S.; Sharma, K.; Singh, S.; Singh, V.; Pandita, D. and Viney, L.V. (2018), Design, synthesis and antidiabetic activity of novel sulfamoyl benzamide derivatives as glucokinase activators. Journal of Pharmaceutical Technology, Research and Management, 6(2): 113-122.

Grewal, A.S.; Sharma, N. and Singh, S. (2019), Molecular docking investigation of compounds from Sapium ellipticum (Hochst) Pax as allosteric activators of human glucokinase. International Journal of Pharmaceutical Quality Assurance, 10(4): 588-596.

Grewal, A.S.; Dua, J.S.; Prasad, D.N.; Kharb, R. and Lather, V. (2019a). N-Pyridin-2-yl benzamide analogues as allosteric activators of glucokinase: design, synthesis, *in vitro*, in silico and in vivo evaluation. Chemical Biology and Drug Design, 93(3): 364-372.

Jafariana, A.; Ghannadib, A. and Elyasia, A. (2003). The effects of *Allium hirtifolium* Boiss. on cell-mediated immune response in mice, Iranian Journal of Pharmaceutical Research, 2: 51-55.

Kang, Y.; Jung, U.; Lee, M.; Kim, H.; Jeon, S.; Park, Y.; Chung, H.; Baek, N.; Lee, K.; Jeong, T. and Choi, M. (2008). Eupatilin, isolated from Artemisia princeps Pampanini, enhances hepatic glucose metabolism and pancreatic beta-cell function in type 2 diabetic mice, Diabetes Research and Clinical Practice, 82(1): 25-32.

Lagorce, D.; Bouslama, L.; Becot, J.; Miteva, M.A. and Villoutreix, B.O. (2017). FAF-Drugs4: Free ADME-Tox filtering computations for chemical biology and early stages drug discovery, Bioinformatics, 33: 3658-3660.

Matschinsky, F.M.; Zelent, B.; Doliba, N.; Li, C.; Vanderkooi, J.; Naji, A.; Sarabu, R. and Grimsby, J. (2011). Glucokinase activators for diabetes therapy, Diabetes Care, 34: S236-S243.

Mahmoodi, M.; Zarei, S.; Rezaeian, M.; Arababadi, M.K.; Ghasemi, H.; Khoramdelazad, H.; Rezayati, N.; Hasanshahi, G. and Hosseini-Zijoud, S.M. (2013). Persian Shallot (*Allium hirtifolium* Boiss) extract elevates glucokinase (GCK) activity and gene expression in diabetic rats, American Journal of Plant Sciences, 4(7): 1393-1399.

Miteva, M.; Violas, S.; Montes, M.; Gomez, D.; Tuffery, P. and Villoutreix, B. (2006). FAF-Drugs: free ADME/Tox filtering of compound collections, Nucleic Acids Research, 34: W738-W744.

Miteva, M.; Guyon, F. and Tufféry P. (2010). Frog2: efficient 3D conformation ensemble generator for small compounds, Nucleic Acids Research, 38: W622-W627.

Moradi, Y.; Moradi-Sardareh, H.; Ghasemi, H.; Mohamadi, N.; Moradi, M.-N. and Hosseini-Zijoud S.-M. (2013), Medicinal properties of Persian Shallot, European Journal of Experimental Biology, 3(1): 371-379.

Morris, G.M.; Huey, R.; Lindstrom, W.; Sanner, M.F.; Belew, R.K.; Goodsell, D.S. and Olson, A.J. (2009). AutoDock4 and AutoDockTools4: automated docking with selective receptor flexiblity, Journal of Computational Chemistry, 30(16): 2785-2791.

Pal, M. (2009), Medicinal chemistry approaches for glucokinase activation to treat type 2 diabetes. Current Medicinal Chemistry, 16(29), p. 3858-3874.

Panahandeh, J., Farhadi, N., Azar, A.M. and Salte, S.A. (2016), Evaluation of Persian shallot (Allium hirtifolium) ecotypes for phytochemical components and antioxidant activity, Journal of Medicinal Plants and By-products, 2, p. 217-226

Perseghin, G. (2010), Exploring the in vivo mechanisms of action of glucokinase activators in type 2 diabetes, Journal of Clinical Endocrinology and Metabolism, 95(11), 4871-4873.

Rathee, D., Lether, V., Grewal, A.S. and Dureja, H. (2018), Targeting matrix metalloproteinases with novel diazepine substituted cinnamic acid derivatives: design, synthesis, in vitro and in silico studies, Chemistry Central Journal, 12(1), Article 41.

Rathee, D., Grewal, A.S. Dureja, H. and Lather, V. (2019), Enzymatic inhibitory activity of Iridoid glycosides from Picrorrhiza kurroa against matrix metalloproteinases: correlating in vitro targeted screening and docking, Computational Biology and Chemistry, 78, P. 28-36.

Singh, A.B., Singh, N., Akanksha, Jayendra, Maurya, R. and Srivastava, A.K. (2012), Coagulanolide modulates hepatic glucose metabolism in C57BL/KsJ-db/db mice, Human & Experimental Toxicology, 31(10), p. 1056-1065.

Singh, R., Lather, V., Pandita, D., Judge, V., Arumugam, K.N. and Grewal, A.S. (2017), Synthesis, docking and antidiabetic activity of some newer benzamide derivatives as potential glucokinase activators. Letters in Drug Design & Discovery, 14(5), p. 540-553.

Trott, O. and Olson, A.J. (2010), AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. Journal of Computational Chemistry, 31, p. 455-461.