Original Ariticle:

Molecular docking assessment of pyridone derivatives as glucokinase activators

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ABSTRACT

Background: Mutations in glucokinase (GK) gene results in maturity onset diabetes of the young 2 (MODY2). It has been observed that GK activators (GKAs) can activate GK structure and promote glucose phosphorylation and bring blood glucose levels to normal condition. The present study is aimed to identify the binding mode of pyridone derivatives (PDs) as GKAs through molecular docking study.

Methods: GK structure was retrieved from the Protein Data Bank (PDB), protonated and energy minimized. A database was constructed with 29 PDs and docked into the allosteric site specified with Y61, R63, S69 and Y215 residues using Molecular Operating Environment (MOE) software. Docking conformations were generated using triangle match algorithm and ranked by London dG scoring function. The binding orientations and strength of interactions were evaluated by ligand interaction module of MOE.

Results: Molecular docking of 29 PDs in allosteric site of GK gave reliable docking scores, interestingly arene cationic interactions were observed with the compounds PD1, PD12, PD20 and PD21. R63 residue of allosteric site played a predominant role in binding with PDs.

Conclusions: PDs can be potentially useful agents in future management strategies of type 2 diabetes mellitus.

Key Words: *Glucokinase, Maturity onset diabetes of the young 2, Molecular docking, Glucokinase activators*

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INTRODUCTION

Glucokinase (GK) plays a key role in whole-body glucose homeostasis by catalyzing the phosphorylation of glucose in cells, such as pancreatic β-cells and hepatocytes. Pancreatic GK acts as rate limiting enzyme for glucose utilization where it determines the rate of glucose-induced insulin secretion[1] where as liver GK determines the rate of glucose utilization and glycogen synthesis.[2] These functions of GK makes it a key metabolic regulator of blood glucose levels. In type 2 diabetes mellitus, hepatic glucose output is more than hepatic glucose utilization contributing to the elevated glucose levels in circulation.[3]

The mutations in GK results in its altered activities of glucose utilization causing abnormal glycemic control. This kind of loss and or alteration of the activity of GK by mutations is linked up to maturity onset diabetes of the young 2 (MODY2), which is characterized by early onset and mild chronic fasting hyperglycaemia.[4] MODY2 patients display an impaired glucose regulation with decreased accumulation of glycogen and increased hepatic glucose production.[5] Mutations in GK causing MODY2 conditions results in decreased enzymatic activity due to reduction in its maximal velocity (V_{max}) and or reduced affinity towards glucose and adenosine triphosptate (ATP).[6]

A GK activator has the promise of potentially affecting both the β-cells of the pancreas, by improving glucose sensitive insulin secretion, as well as the liver, by reducing uncontrolled glucose output and restoring post-prandial glucose uptake and storage as glycogen.[7] Activation of GK results in a rise in insulin secretion from the pancreas and also stimulation of glucose uptake and glycogen synthesis in

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liver.[8] These two functions lead to an increase in glucose disposal and the return of blood sugar to basal levels. This provides a rational expectation that enhancement of GK activity would be a novel therapeutic strategy for type 2 diabetes mellitus. Consistent with this rationale, recently small molecules that are acting as allosteric modulators and can activate GK by binding to allosteric site on the GK have been discovered and its number has been enormously increasing.[9,10] They have the ability to enhance the GK activity and hence called as GK activators (GKAs). They can be the potential and promising molecules for an effective treatment of type 2 diabetes mellitus. To investigate the mechanism of action of GKAs, the mode of interaction between activator molecules and GK is a key aspect. Hence in the present study we describe the binding mode of a few recently published pyridone derivatives as GKAs[11] *by in silico* means to find out their efficacy to interact with the allosteric site of GK.

MATERIAL AND METHODS

All the computational studies were carried out using Molecular Operating Environment (MOE), 2011.10; Chemical Computing Group Inc. software.[12]

Protein processing and preparation

The X-ray Crystallographic structure of GK was retrieved from Protein Data Bank (PDB)[13] (ID: 1V4S) with a resolution of 2.30 Å and loaded into MOE working environment ignoring water molecules and heteroatoms. The structure was protonated in generalized Born implicit solvated environment at a temperature of 300K, pH of 7 and a salt concentration of 0.1. Electrostatic potential was applied to a cut off value of 1.5 Å at a dielectric value of 1. A non-bonded cut off value of 8 Å was applied to the Leonard-Jones terms. After successful protonation, the structure was energy minimized in CHARM27[14] force field applying a gradient cut-off value of 0.05. Kcal/mol/Å.

Molecular dynamics simulations[15] were carried out at a constant temperature of 300 K for a heat time of 10 pico seconds and a total run time of 10 nano seconds. The temperature relaxation time was set to 0.2 pico seconds (ps) by the time step consideration of 0.001. The energy terms of GK were plotted as a graph to observe stability of the trajectories generated during simulations.

Construction and preparation of ligand database

A total of 29 pyridone derivatives (PDs) that can act as GKAs were obtained from literature survey[11] and their three dimensional structure were constructed in MOE working environment using MOE builder tool. A ligand database (.mdb file) was constructed using all the ligands and subjected to energy minimization in implicit solvated environment under AMBER99[16] force field. Related potential energy terms were enabled for all bonded and non bonded interactions at a gradient of 0.05 Kcal/mol/Å and force field partial charges were enabled during minimization process.

Allosteric site identification

GKAs can bind in the allosteric regulatory site situated near the catalytic site of GK and increase the rate of glucose phosphorylation. The allosteric site residues were identified from the previously reported glucose-GKA complex structures in PDB (IDs: 3VF6, 3ID8, 3H1V, 4DHY, 3IMX, 3F9M, 3S41, 3FRO, 3A0I and 3GOI). Their ligand plots were studied from PDBSum[17] using their PDB IDs and the residues that are in direct contact with GKAs were identified. The group of these residues was defined as binding site for the docking of ligands.

Molecular Docking

Molecular docking was carried out between the GK and 29 PDs to know their binding orientations. The simulated stabilized trajectory obtained at the end of molecular dynamics simulations was loaded into MOE and the binding site was defined with the residues Y61, R63, S69 and Y215. The database containing 29 PDs was docked into the binding site using triangle matcher docking placement

methodology and poses were generated by aligning the ligand triplets on the alpha sphere triplets of receptor. Thirty docking conformations were generated for each ligand and these conformations were ranked based on the free binding energies that were generated by London dG scoring function.[18,19] The conformations were refined and rescored in the same force filed to remove the duplicate conformations. From the final list of docked conformation the pose with least docking score was chosen for each ligand for the analysis.

Molecular dynamics of GK - PD24 complex

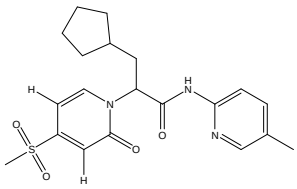
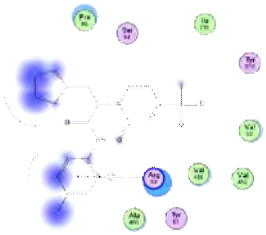
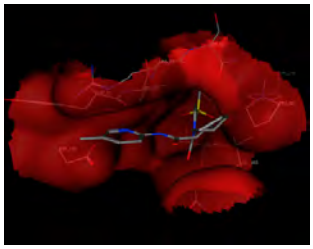
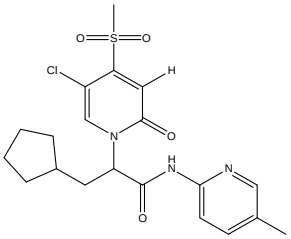
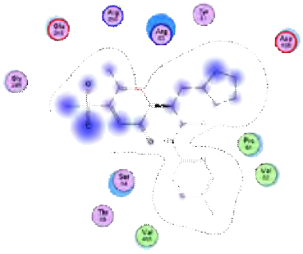
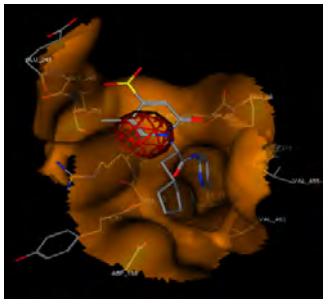
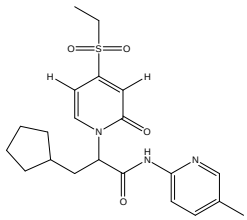
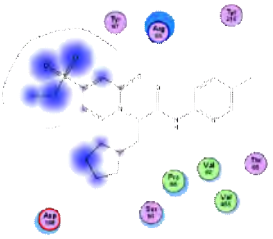
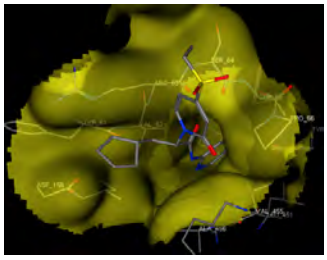
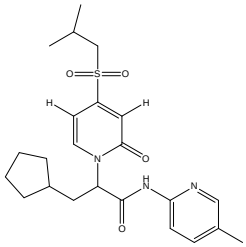
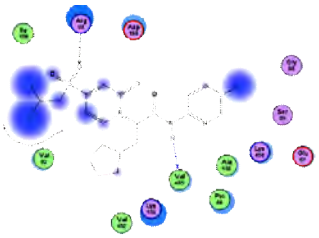
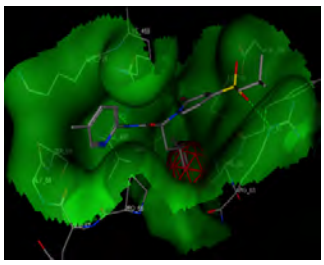
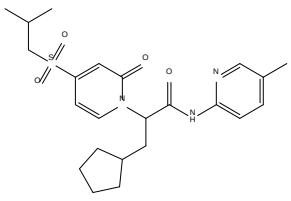
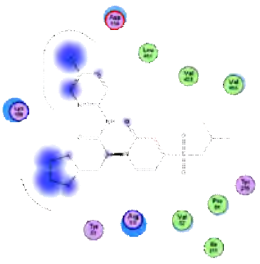
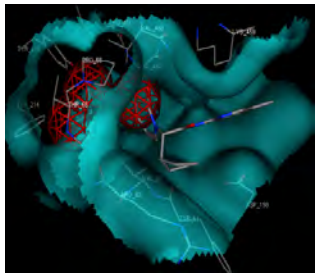
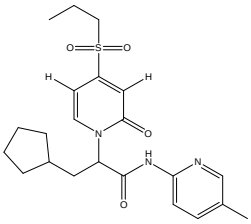
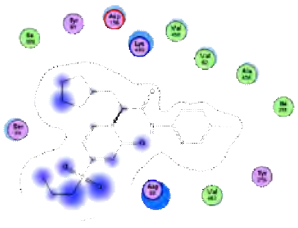
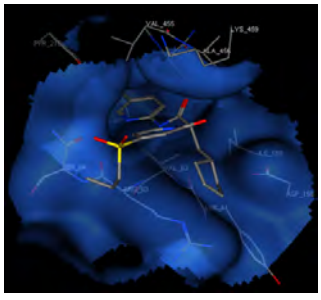
Among all the PDs, PD24 showed the best binding score with satisfactory hydrogen bond interactions. The strength of ligand to bind with GK and stability of this receptor-ligand complex was determined by molecular dynamics simulations. The glucokinase-pyridone derivative 24 (GK-PD24) docking conformation with lowest docking score was subjected to molecular dynamics simulation for a 10 ns of run time with the conditions specified above for GK simulations alone. The energy variations of the complex during simulations were plotted as graph to find out the stability of the complex through out the simulation period.

RESULTS

The three dimensional X-ray crystallographic structure of GK was obtained from PDB and the structure was optimized by energy minimization (Supplementary Figure 1). The molecular dynamics simulations of this structure have generated a total of 20000 conformations for a period of 10 ns. Their energy plot of the dynamics simulations showed that the energy fluctuated up to 2 ns, afterwards found to be stabilized and generated stable trajectories. This stability was found through out simulations after 2 ns (Supplementary Figure 2). The stabilized conformation was obtained at the end of the simulations is used for the docking purpose. The binding site residues were identified as Y61, R63, S69 and Y215 from the ligand plots of all GK entries with GKAs (Supplementary Figures 3-12).

The ligand database was constructed for all the ligands and all the structures were optimized to a gradient cut off value of 0.05 Kcal/mol/Å. This database was docked into the specified binding domain and docking conformations were generated. The binding orientations and interaction mechanisms along with 29 ligand structures are shown in Table 1 and the docking score information is shown in Table 2. The docking results indicated that among all the ligands, PD24 was found to be showing least docking score and forming four hydrogen bonds with the allosteric site among which two bonds are formed with R63 and two bonds with T65. Because of best docking score and good hydrogen bond interactions PD24 is considered as an efficient GK activator among all PDs. Its ability to stay bound with the GK at binding site should be stable through out its life time, then only it can exhibit its role and promotes GK activation, so to find out this the GK-PD24 docking complex was subjected to molecular dynamics simulations for 10 ns run time and it was found that the complex is stable during simulation period (Supplementary Figure 13). This indicates PD24 can act as a best GK activator with its best binding efficiency. All other ligands are also showing good docking scores and the highest docking score was found to be –9.353 for PD10. Among all the binding site residues defined during docking process R63 was found to be playing a major role to bind with the ligands indicating its significance and catalytic role of GK activation. The aromatic ring structures of PD1, PD12, PD20 and PD21 were found to be forming arene cationic interactions with the receptor. These are the strong interactions than hydrogen bonds and hold the receptor-ligand complex tightly. The sulphate moiety, methyl groups and cyclo pentyl rings of the PDs are found to be contacting with solvent exposing out from the binding site cavity. The hydrogen bonding is confined to the sulphonyl oxygen atoms and the amide hydrogens of pyrimidine rings of PDs to pair with allosteric site. These reactive groups

Table 1: Molecular docking interaction of PDs with GK allosteric site

Compound	Structure of ligand*	2D dock image†	3D dock image‡
PD1			
PD2			
PD3			
PD4			
PD5			
PD6			

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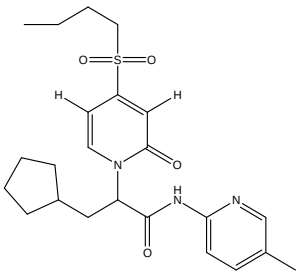

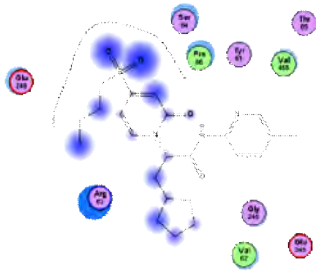

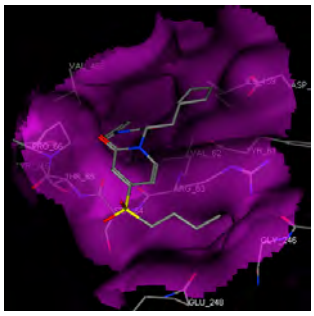

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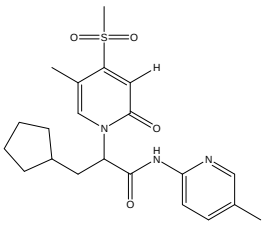

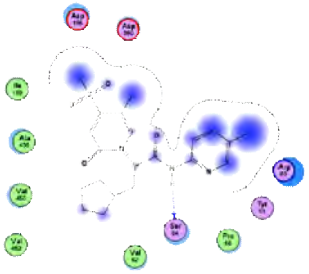

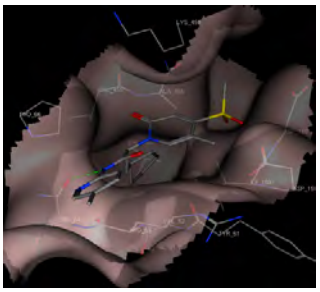

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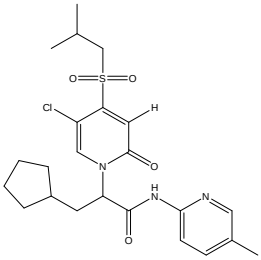

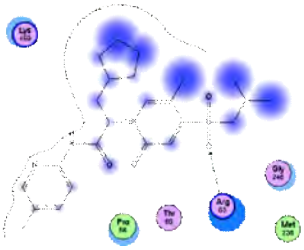

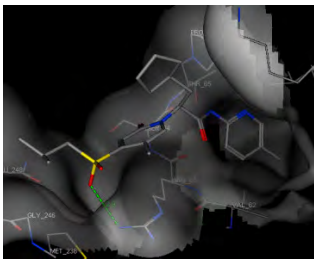

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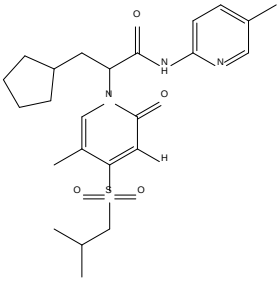

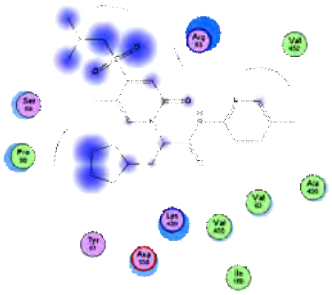

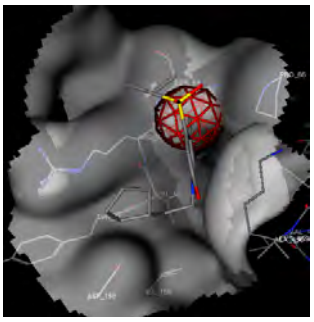

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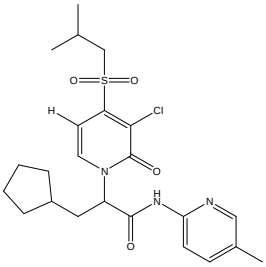

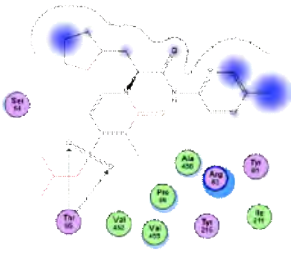

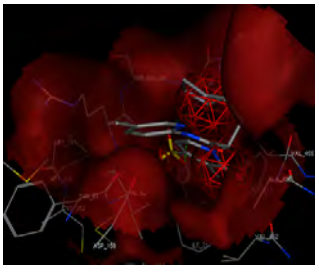

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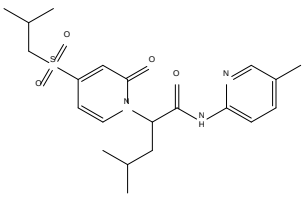

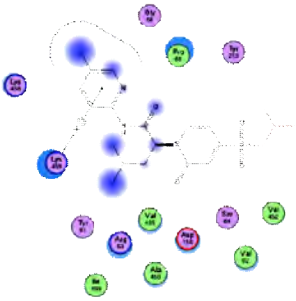

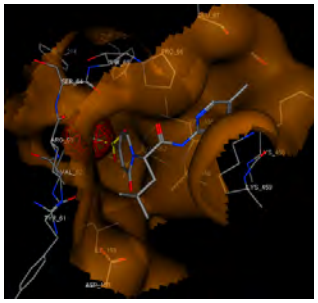

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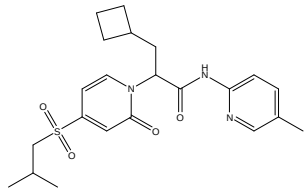

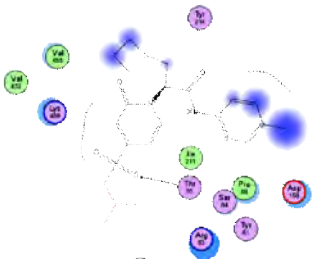

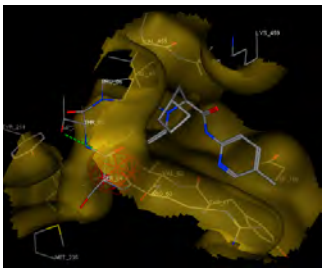

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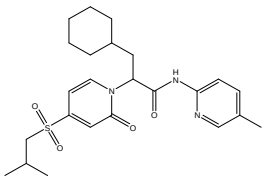

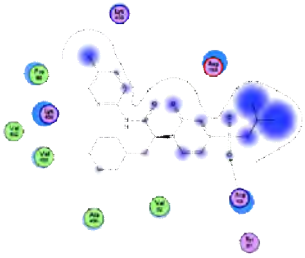

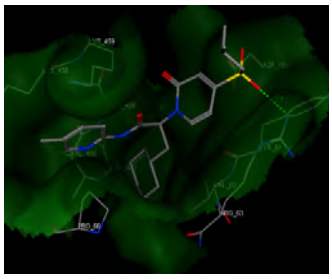

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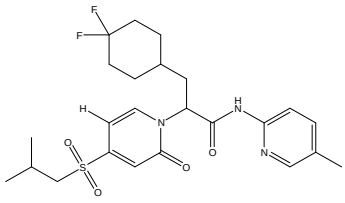

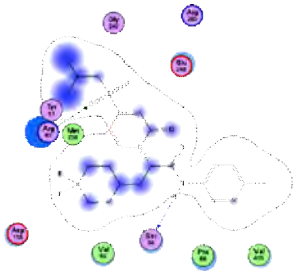

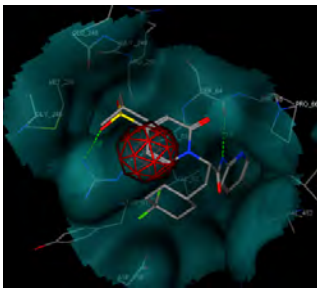

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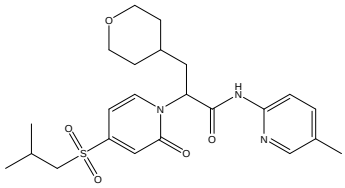

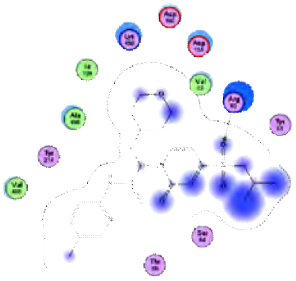

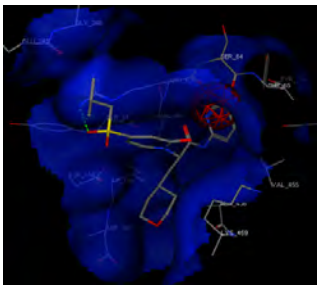

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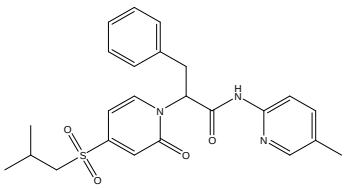

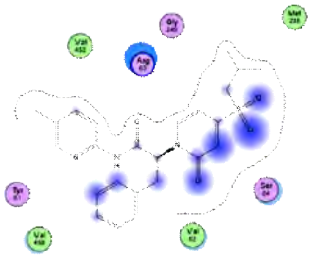

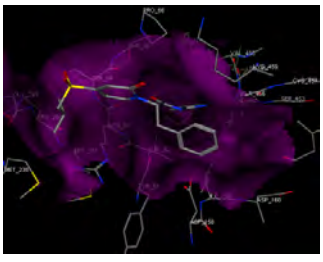

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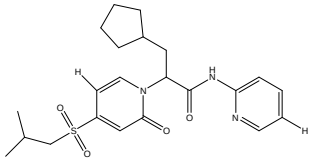

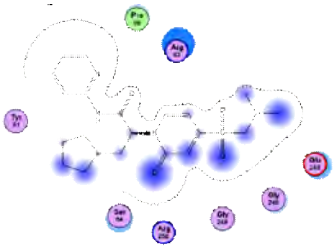

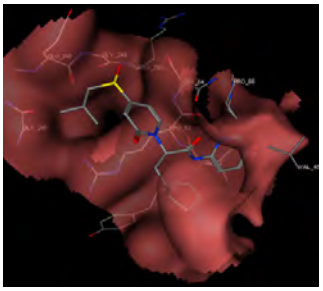

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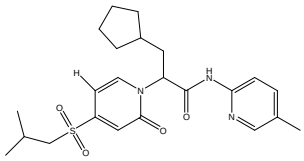

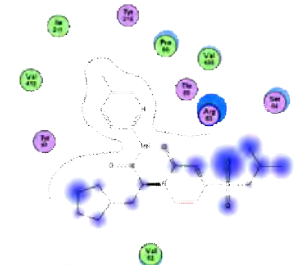

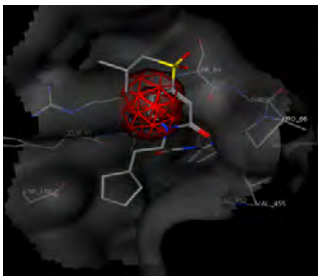

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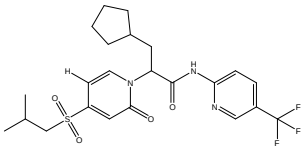

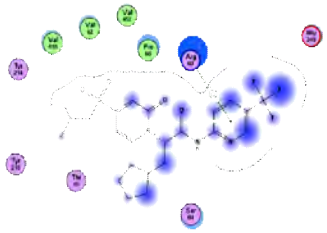

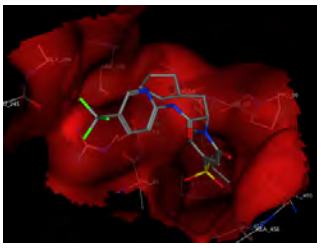

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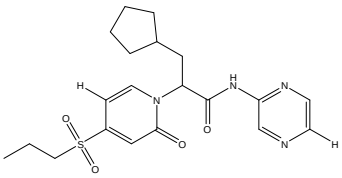

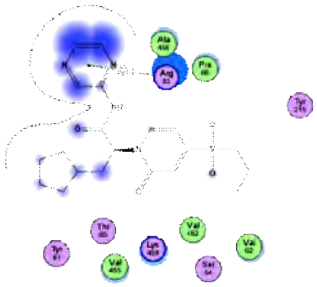

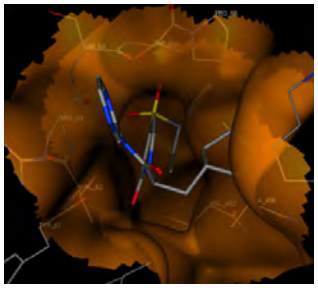

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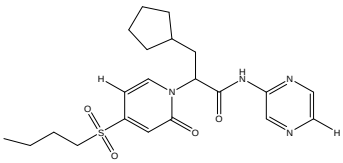

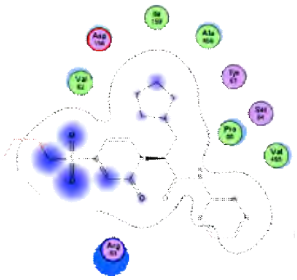

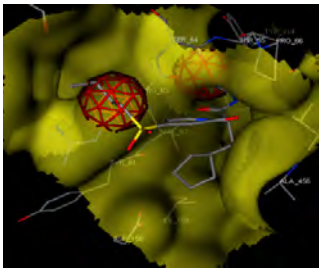

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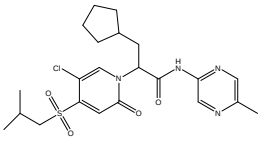

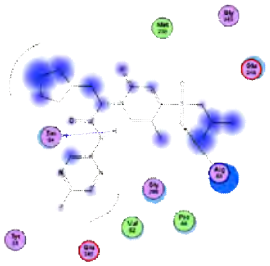

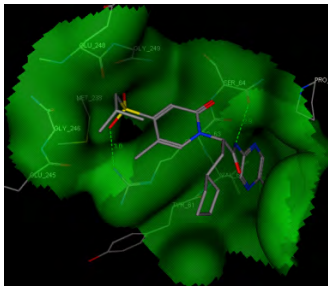

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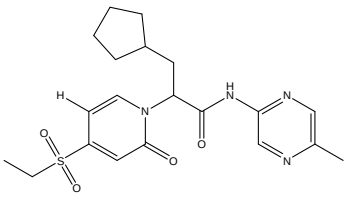

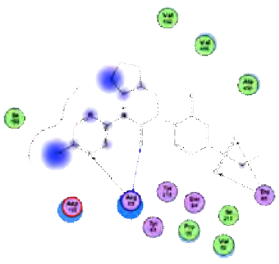

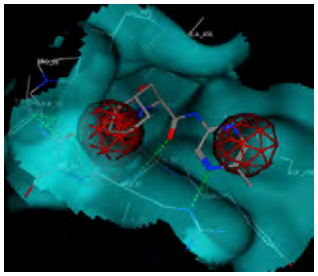

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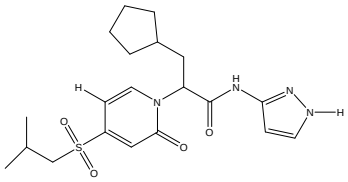

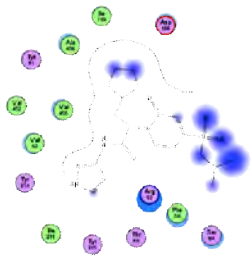

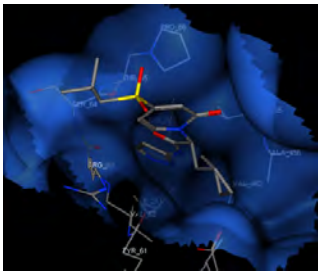

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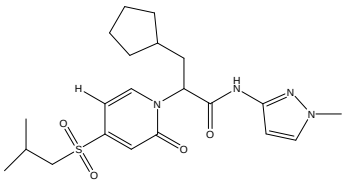

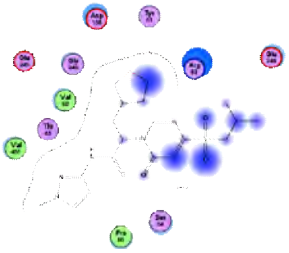

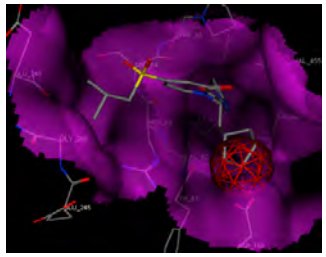

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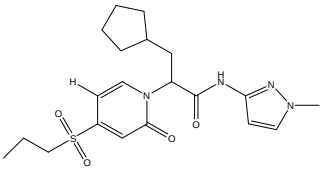

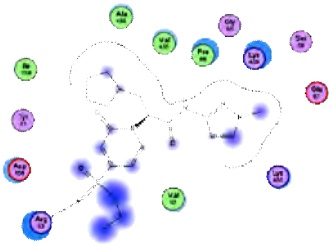

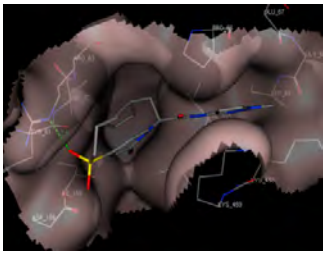

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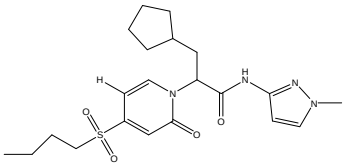

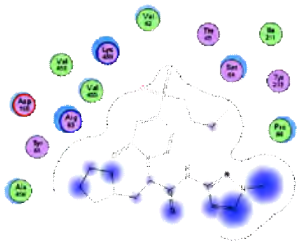

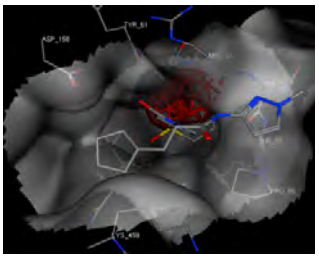

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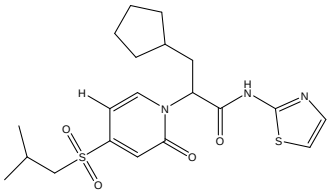

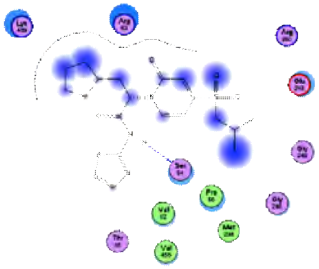

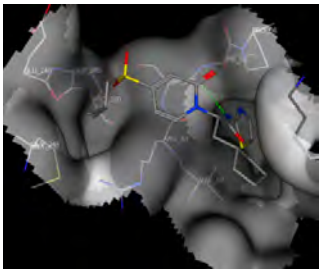

*Structure of PDs, †two dimensional linear representation of docking conformations of respective PDs with receptor. Arrow symbol indicates the hydrogen bond interactions and blue shaded region indicates the solvent contacts made by ligands. ‡Three dimensional graphical representations of docking conformations of respective PDs with receptor. The positional variations in the binding mode of PDs within the allosteric cavity of GK are depicted

PD = pyridone derivatives; GK = glucokinase

Table 2: Molecular docking information of PDs with GK allosteric site

Compound	Docking score* (Kcal/mol)	No of. H-bonds†	Interactive residues of allosteric site‡	H-bond length (Å)
PD1	–10.3673	Arene cationic interaction	R63	-
PD2	–10.225	-	-	-
PD3	–10.204	-	-	-
PD4	–10.384	2	R63	2.4
			V455	2.1
PD5	–10.073	-	-	-
PD6	–10.354	-	-	-
PD7	–10.708	-	-	-
PD8	–10.076	1	S64	2.2
PD9	–10.161	1	R63	2.9
PD10	–9.353	-	-	-
PD11	–10.329	2	T65	2.4
			T65	3.1
PD12	–10.515	Arene cationic interaction	K459	-
PD13	–11.101	1	T65	2.7
PD14	–10.929	1	R63	2.7
PD15	–10.047	2	R63	2.6
			S64	2.8
PD16	–10.833	1	R63	2.0
PD17	–9.745	-	-	-
PD18	–10.195	-	-	-
PD19	–10.383	-	-	-
PD20	–10.582	Arene cationic interaction	R63	-
PD21	–9.885	Arene cationic interaction	R63	-
PD22	–9.720	-	-	-
PD23	–9.355	2	R63	3.0
			S64	2.9
PD24	–12.192	4	R63	2.6
			R63	3.0
			T65	2.1
			T65	3.0
PD25	–10.042	-	-	-
PD26	–9.883	-	-	-
PD27	–9.991	1	R63	2.5
PD28	–9.974	-	-	-
PD29	–10.536	1	S84	2.7

*Docking scores of respective PDs and GK docking complexes. †The total number of hydrogen bonds formed between the corresponding PDs and GK allosteric site residues. ‡The amino acid residues of allosteric site that are involved in the interaction with PDs.

PD = pyridone derivative; GK = glucokinase

were found to be present inside the cavity of the allosteric site indicating their role in the catalytic mechanism. The arene cationic interactions were found between the pyridine rings of PDs and the R-group of basic amino acid residues lysine (K459) and arginine (R63). Both of them are charged amino acid residues and these arene cationic interactions were found with the pyridine rings especially making solvent contacts. This might be a key factor for the induction of charge in the allosteric sight which in turn promotes the activation of GK and results in the phosphorylation of glucose in the catalytic site situated very near to the allosteric site. Finally it is clear that this docking study helped to predict the binding mode and their role in the activation of GK where their efficacy of activation was already proved[9] but not the binding mode and catalytic mechanism.

DISCUSSION

Blood glucose levels are dynamically regulated in the body via multiple mechanisms among which GK plays a major role in all the metabolically responsive organs like liver, pancreas, brain and gut.[20] The X-ray crystal structure of GK along with activators shows a palm shape topology where two unequal size of domains are observed that can be separated by a deep cleft. This cleft forms the active site for glucose phosphorylation and is situated about 20 Å away from the hydrophobic allosteric site. GKAs can bind to this allosteric site and activates GK.[21] The docking studies of present PDs as GKAs revealed the involvement of R63 as predominant residue in hydrogen bond formation with almost all ligands. Few of the ligands showed arene cationic interactions which contributed to the hydrophobic interaction with allosteric site. Ligands having such interactions with the residues like lysine and arginine shows stronger binding affinities and contribute to the potentiality of ligands. All the PDs in the present study have a pyridone core moiety in which the amide nitrogen shows high reactivity with allosteric site residues forming hydrogen bond interactions and helps for the GK activation. Hence, it may be an ideal choice focusing the enzyme like GK having high control strength to maintain basal glucose levels and can also be a plausible rationale for narrowing the options to advance the diabetic therapy by means of GKAs. On the basis of the binding efficiency of present explained GKAs we can anticipate that they have the ability to better interact with the allosteric site there by enhancing the GK activity so as to maintain the glucose homeostasis. These results support the concept that GKAs represent a new class of compounds that increase both insulin secretion and hepatic glucose use by means of GK activation and in turn prove to be effective agents for the control and management of blood glucose levels in type 2 diabetes mellitus.

The present study gave a better understanding and insight into the interaction of PDs as GKAs and also pointed out the contribution of arene cationic interactions and hydrophobic interactions by the aromatic pyridone structures to the docking score. Exploration of these ligand interactions expelled the key residues of allosteric site to activate GK. Finally it can be concluded that the present ligands could be the potential agents for activation of GK which is likely to be the future of management of type2 diabetes mellitus.

ACKNOWLEDGEMENTS

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