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Insilico Design of an SGLT2 Inhibitor in *Diabetes mellitus*

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Abstract: Diabetes Mellitus (DM) is a set of metabolic diseases in which there is increased blood sugar level over an extended period. To control blood glucose, is the supreme and main objective of diabetes treatment, so that the further complication of the disease can be prevented. Medication for DM can function in various ways to control blood sugar levels; viz increasing the insulin sensitivity, increasing glucose excretion, decreasing the absorption of glucose from the gastrointestinal tract or by any other mechanisms. SGLT2 inhibitors provide a unique way to treat Diabetes Mellitus by decreasing hyperglycemia through increased glucosuria. This access decreases renal glucose reabsorption in the proximal renal tubules imparting an insulin independent mechanism to curtail blood glucose. The advent of computer aided drug design has bestowed a lot to the development of newer SGLT2 inhibitors. In this present study, we established a novel SGLT2 inhibitor by means of docking. Docking was carried out using GLIDE XP software, provided by Schrodinger. The molecule (SG1) has a good dock score when compared and analyzed with standard canagliflozin. Binding mode analysis revealed that SG1 exhibited good interactions in the active site of SGLT2. The molecule SG1 is expected to give good *in-vivo* activity and may be considered in the design and discovery of an ideal SGLT2 inhibitor.

Key words: Diabetes Mellitus · SGLT2 Inhibitor · Docking · Glide · Binding Mode Analysis · Canagliflozin

INTRODUCTION

Diabetes mellitus is a metabolic disorder that is rapidly emerging as a global health care problem. Diabetes mellitus results from deficiency in insulin [1] because of impaired pancreatic âcell function or from resistance to insulin in the body, thus leading to abnormally high levels of blood glucose [2]. Diabetes which results from a complete deficiency in insulin secretion is Type 1 diabetes and the diabetes due to resistance to insulin activity together with an inadequate insulin secretion is Type 2 diabetes.

Diabetes is basically treated by diet and exercise therapies [3]. Various antidiabetic agents being currently used include biguanides (Decrease glucose production in the liver and increase sensitivity to insulin), sulfonylureas and meglitinides (Stimulate insulin production), aglucosidase inhibitors (Slow down starch absorption and glucose production), thiazolidinediones (Increase insulin sensitivity) and DPPIV inhibitors (Decrease in inactivation of incretins) [4]. These therapies have various side effects [5, 6].

Sodium glucose co-transporter-2 (SGLT2) inhibitors [7] offer a novel approach to treat diabetes by reducing hyperglycemia via increased glucosuria. In healthy adults, approximately 180 liters of plasma containing 5.5 mm glucose are filtered every day through the kidneys. This means that approximately 180 g of glucose is filtered daily through the glomeruli and lost in the primary urine. Almost of this filtered load (~ 99.9%) is then actively reabsorbed by sodium-coupled transport across the brush border membrane of the proximal tubule and then returned to the circulation by glucose transporters. More than 90% of the glucose that is initially filtered is

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reabsorbed by a low affinity, high capacity system controlled by sodium glucose transporter-2 (SGLT2) in the early convoluted segment of the proximal tubule. Reabsorption of almost all remaining filtered glucose is performed by sodium glucose transporter- 1 (SGLT1), a low affinity, high capacity transporter further down in the straight segment of the descending proximal tubule. So efficient is this glucose reabsorption pathway that, under normal circumstances, less than 0.1 g will ever find its way into the urine of nondiabetic individuals. Phlorizin [8] isolated from the apple tree bark is the first naturally occurring inhibitor of both SGLT1 and SGLT2. Dapagliflozin and Canagliflozin [9, 10] are some examples of drugs that are used as anti diabetic and act by the mechanism of inhibiting the SGLT receptors.

MATERIALS AND METHODS

ACD Lab Chemsketch 12.00 [11, 12]: It is a chemical drawing software used to draw the three dimensional structures, optimize them and to analyze various molecular descriptors of the proposed structures.

Protein Data Bank (PDB): PDB is the only crystallographic database meant for obtaining the 3D structural data of large molecules such as proteins and nucleic acids. The structures were generated after the X –ray crystallography and NMR spectroscopy studies. In this study the protein selected is SGLT2 (PDB code 3DH4) [13].

Protein Preparation: Structures taken from the PDB database could not fit as such for docking studies because it consists of heavy atoms, co-factors, water molecules, metal ions etc. These structures do not show any bond orders, topologies or formal atomic charges. Therefore, the PDB structure should be converted into suitable form for docking. This was done with the protein preparation wizard module of Maestro in which water molecules and peptide substrates (NAG) were deleted. After this, optimized potential for Liquid Simulations – All Atoms (OPLS-AA) force fields were carried out for energy minimization [14].

Ligand Preparation: Ligprep module of the Maestro was used for the development of ligands. It involves various steps –perform conversions, apply corrections to the structures, generate variations on structures, remove unwanted structures and optimize them – which was controlled by selecting options in the ligand preparation panel by specifying command line options.

Molecular Modeling: This was done by using GLIDE (Grid- based Ligand Docking with Energetics) software developed by Schrodinger. Maestro v9.6 Graphical User Interface (GUI) was used for the ligand preparation, protein preparation and docking.

Docking by Glide: All steps involved in docking was performed with GLIDE XP. The prepared ligands were docked into the binding pocket of the protein SGLT-2 (3DH4). Scoring grids were placed at the center of crystal structure of compounds during docking. Glide XP (Extra precision) was used to perform docking calculations. The docked images with least glide score were selected because the perfect binding interactions are given by the most negative glide score.

Induced Fit Docking (IFD): IFD protocol of GLIDE v9.6 from Schrodinger was used to conduct the IFD of prepared ligand molecules with the prepared protein 3DH4. Here, the ligand and the receptor are flexible, which helps to bind the ligand at different sites on the receptor and generate various poses of the receptor- ligand complex, each having unique structural conformations and Glide score (G-score). The derivatives were docked at the active site of 3DH4 individually. The poses generated were ranked on the basis of G score. From this Glide score, the best docked complex can be identified.

G-score [15, 16] is determined by considering the parameters like Hydrogen bonding (H bond) Hydrophobic contacts (Lipo), Vander Waals (vdW), Columbic (Coul), Polar interactions in the binding site (site), Metal binding term (Metal) and the penalty for buried polar group (Bury P) and freezing rotatble bonds (Rot B)

G score = H bond + Lipo + Metal + Site + 0.130 coul + 0.065 vdW – Bury P – Rot B

Visualization and Analysis: Study of hydrogen bond, hydrophobic and pi-pi interactions andpreparation of high resolution images were conducted by PyMol Molecular Graphics system.

(A) (B)

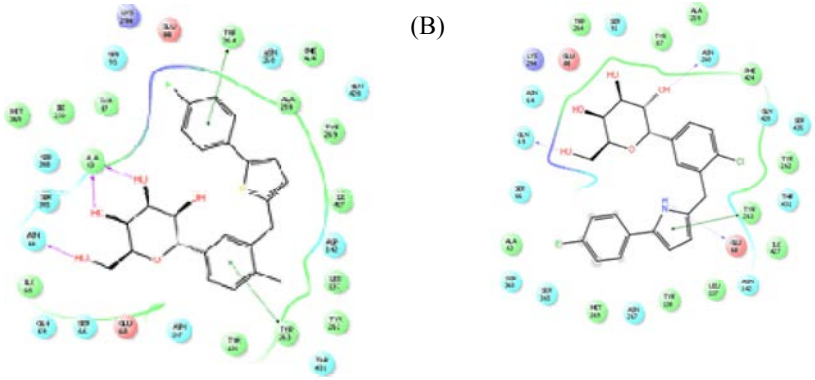


Fig. 1: Schematic 2D representation of standard Canagliflozin (A) and SG 1 (B) in the binding pocket of 3DH4.

(C) (D)

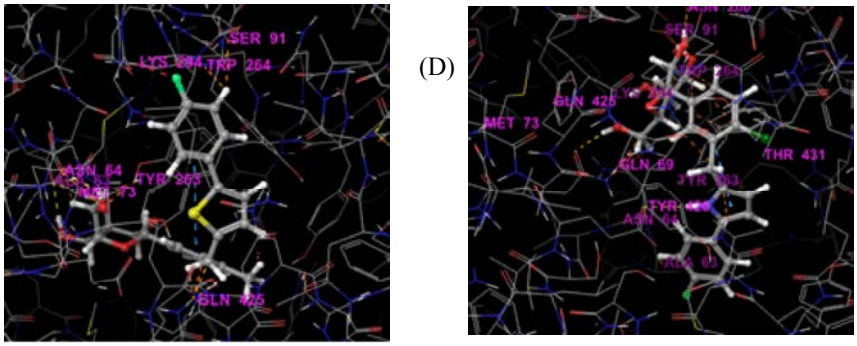
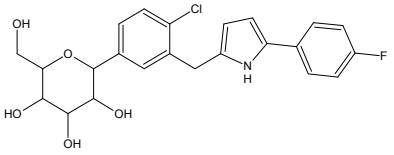
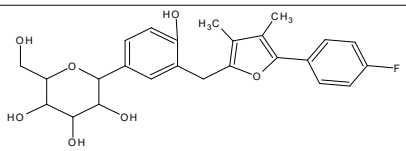
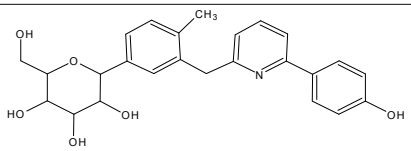
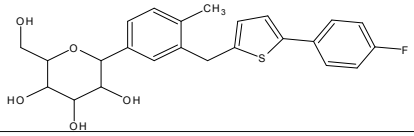


Fig. 2: Docked image of Canagliflozin (C) and SG 1(D) in the active site of 3DH4. The active site of amino acid residues is represented as tubes, while the inhibitor Canagliflozin(C) and SG 1(D) is shown as ball and stick model with the atoms colored as carbon- grey, hydrogen-white, nitrogen-blue, fluorine- light green and chlorine-dark green.

Table 1: Structure and molecular descriptors of top 3 docked compounds with standard Canagliflozin

Compound Code	Structure	MW (g/mol)	HA	HD	Log P	rot b	Violations
SG 1		447.89	6	5	3.15	5	0
SG 2		430.43	7	5	2.81	5	0
SG 3		437.49	7	5	2.18	5	0
Standard Canagliflozin		444.52	5	4	3.92	5	0

MW = Molecular Weight
HA = No. of H_2 bond acceptor groups
HD = No. of H_2 bond donor groups

Table 2: Glide score of top ranked compounds (SG 1 to SG 3 and standard Canagliflozin)

S.No	Compound Code	Glide Score
1	SG 1	-12.68
2	SG 2	-11.08
3	SG 3	-9.19
4	Standard	-11.47

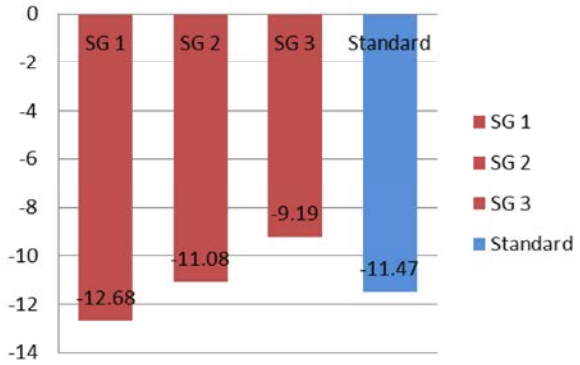


Fig. 3: Representation of Glide score of SG 1, SG 2, SG 3 and Standard Canagliflozin against the receptor 3DH4.

Table 3: Binding mode Analysis of SG 1 and standard in the binding site of 3DH4.

Compound code	Hydrogen bonding	Hydrophobic interaction	π- π interaction
Canagliflozin	ALA 63, ASN 64	LEU 137, TYR 262 TRP 264, ALA 259 TYR 269, ILE 427, ALA 259	TYR 263 TRP 264
SG 1	ASN 260, GLN 69, GLU 68	PHE 424, MET 369, ALA 63 TYR 138, LEU137, TYR 263	TYR 263

RESULTS

A series of structurally related compounds were designed. Those compounds obeying rule of 5 were selected for docking studies (Table 1). Based on the docking score, compounds with higher inhibitory activity were found out. Schematic 2D representation of the docked complex of compound SG 1 in the active site of SGLT2 and that of standard Canagliflozin was visualized in figure 1. The residues involved in inter-atomic contact were shown in figure 2. Docking score of the enlisted compound SG 1 is less than the standard Canagliflozin. It means that the compound SG 1 has higher inhibitory activity, as least binding energy is related with higher activity. The dock score of the ligands and Canagliflozin are plotted in the graph and from the graph, the dock score of all the compounds are observed among which the best ligand is found out (Figure 3). The dock score of compound SG 1 is -12.68 and that of standard Canagliflozin is -11.47. Binding mode analysis of standard Canaglifloxin and compound SG 1 in the active site of 3DH4 explains that the good binding affinity is due to various interactions such as hydrogen bonding, hydrophobic and π-π interactions. The thiazole ring in Canaglifloxin displays hydrophobic interactions with ILE 427, TYR 269 andALA 259, while the methyl benzene moiety interacts favourably with TYR 138, TYR 263, LEU 137 and the flurobenzene moiety displays hydrophobic interaction with ALA 259. The phenyl ring in flurobenzene displays π-π interaction with TYR 263. Canaglifloxin also shows H bond interaction between –OH groups present in lactone ring with ASN 64 and ALA 63. A very similar binding model was observed for SG 1. SG 1 also made similar type of interactions in the active site of SGLT2. SG 1 also had interactions with GLN 69, GLU 68, PHE424, MET 369. This is also shown in Table 3. When methyl group is replaced with electronegative chlorine atom and hydroxyl group in SG 1, hydrophobic interactions were observed. Also pyrrole nucleus in SG 1 had greater π-π interactions in the binding pocket of 3DH4.

DISCUSSIONS

From the 22 compounds designed, those obeying Lipinski rule of five were selected for docking studies. Docking studies were performed on these compounds, in the active site of SGLT2 (3DH4) and based on the results of docking compound SG 1 is selected as the best SGLT2 inhibitor. The dock score was good for SG 1 than the standard. Hydrogen bonding, π-π interaction andhydrophobic interactions are observed in the structure of 3DH4 in complex with Canagliflozin. Similar interactions are observed for compound SG1, so this compound is expected to have good *in-vivo* activity. Electronegative substitutions in SG 1 had higher hydrophobic interactions with the receptor 3DH4 and this may also have attributed to the increase in activity.

CONCLUSION

From the docking studies, we have found out compound SG 1 bound to the active pocket of SGLT2 in a good manner. The good binding affinity of the compound is due to various interactions such as hydrogen bonding, π-π and hydrophobic interactions. So compound SG 1 is expected to give good *in- vivo* activity.

This may be considered in the design and discovery of an ideal SGLT2 inhibitor. Further modification can be carried out to develop better hypoglycemic agent.

Conflict of Interest: The authors confirm that this article content has no conflict of interest.

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