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RESEARCH ARTICLE

In silico studies for the identification of potential SGLT2 inhibitors

Ritu Bhupal, Subhadip Maity, Vivek Asati

Department of Pharmaceutical Chemistry, ISF College of Pharmacy, Moga, Punjab, India

ABSTRACT

Sodium-glucose cotransporter-2 (SGLT2) inhibitors are used for the lowering the blood glucose level through preventing the reabsorption of glucose from the blood through facilitating glucose excretion in the urine. It is responsible for 90% of reabsorption of glucose through the insulin-independent mechanism. The current study described the screening of potential SGLT2 inhibitors using docking studies. *In silico* studies were carried out with help of the Schrödinger software using protein database ID: 3DH4. Inhibitors were docked which resulted that phlorizin is one of the most potent compounds having highest docking score −12.118 kcaL/moL showing binding interaction with the Asn64, Ser66, Ala63, Ser91, Tyr263, Glu88, Gln 428 (PBD ID: 3DH4) amino acids. Various absorption, distribution, metabolism, and excretion properties were studied and numerous properties were also analyzed. The forecast model can also be used for the further development of the potential compounds against SGLT2.

KEY WORDS: Amino acids, Docking, *In silico,* Protein database, Sodium-glucose cotransporter-2

INTRODUCTION

Sodium-glucose cotransporter-2 (SGLT2) inhibitors are a type of prescription drug that has been licenced by the food and drug administration (FDA) for use in persons with Type 2 diabetes in conjunction with diet and exercise.[1] Canagliflozin, dapagliflozin, and empagliflozin are examples of SGLT2 inhibitors.[2] They come as single-ingredient formulations as well as in combinations with other diabetes medications like metformin. SGLT2 inhibitors reduce blood sugar levels by inducing the kidneys to excrete sugar through urine.[3] The FDA has not approved SGLT2 inhibitors for use in patients with Type 1 diabetes[4] because their safety and efficacy have not been shown. SGLT2 inhibitors are drugs with a distinct mode of action that reduce blood glucose without the use of insulin.[5] These medicines are fast establishing their place in the treatment of diabetes, based on recent data on efficacy and advantages.[6] SGLT2 inhibitors may be another option for people with Type 2 diabetes who require extra glucose reduction[7] and have acceptable risk factor profiles but are unwilling or unable to start insulin. Although there appear to be some favorable effects on cardiovascular endpoints, additional research into the long-term effects of SGLT2 inhibitors is needed.[8] Type 2 diabetes is a chronic illness that often necessitates the use of many drugs to maintain blood glucose control. The FDA recently approved SGLT2 inhibitors as a new class of anti-hyperglycemic drugs.[9] SGLT2 inhibitors work by inhibiting renal tubular glucose reabsorption, lowering blood glucose without triggering insulin release.[10] Other advantages could include lower blood pressure and weight loss. This review will concentrate on clinical studies published in the last year, particularly new safety issues that have resulted in repeated FDA cautions for SGLT2 inhibitors.[11] SGLT2 inhibitors, also known as gliflozins, affect the nephron's critical physiology, as opposed to SGLT1 inhibitors, which control sodium/glucose channels

Address for Correspondence:
Dr. Vivek Asati,
E-mail: vivekasatipharma47@gmail.com

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in the intestinal mucosa.[12] The primary metabolic action appears to suggest that this pharmacological class slows glucose reabsorption in the kidney, lowering blood sugar levels. They work by preventing sodium-glucose transport protein 2 from doing its job (SGLT2).[13] Inhibitors of SGLT2 are used to treat Type 2 diabetes mellitus (T2DM). Gliflozins have been demonstrated to help T2DM patients' cardiovascular health in addition to blood sugar control.[14] Several drugs in this category have been approved or are in the works. Canagliflozin, a member of this family, was found to improve blood sugar control while also lowering body weight and systolic and diastolic blood pressure in studies.[15]

MATERIALS AND METHODS

Docking is a computational simulation method for predicting the preferred orientation of two molecules binding together to produce a stable complex.[16] Docking thus plays a significant part in drug rational design. Using scoring functions, docking is utilized to predict the affinity and activity of small molecule binding to their protein targets.[17] The sensitivity of docking calculations to input ligand geometry demonstrates that even little changes in ligand conformation can result in significant alterations in the geometries and scores of the docked postures.[18] Here, we work with the module grid-based ligand docking from energetics (GLIDE) v3.8 (Schrödinger, LLC, New York)[19] which provide us with detailed docking studies and essential docking parameters with satisfactory results which further can be used to determine the effectiveness of the testing ligand. The chemical structure of the SGLT2 inhibitor was opted for the antidiabetic activity for the Type 2 diabetes. The 2D structure of the SGLT2 inhibitors was retrieved from the PubChem online database. Then, the compound was converted into the 3D structure using LigPrep wizard using Schrödinger, LLC, New York.

Ligand preparation for docking

The Ligprep module was used for the production of the ligand by the Maestro suite.[20] The cleanup wizard converted 2D structures into 3D structures, added hydrogen atoms, removed counterions and water molecules, generated stereoisomers, and performed energy minimization, etc., which are the essential steps of pharmacophore development and docking study [Figure 1].

Protein preparation for docking

The crystal structure was obtained from the protein database (PDB code: 3DH4)[21] and pre-processed using the protein preparation wizard in Maestro Wizard v10.3 (Schrodinger, LLC, New York). The protein structure has been altered into a single unit by removing water molecules and other unwanted subunits. Here, the refinement step involves optimization of hydrogen-bonded groups, removal of water, and constrained minimization using the OPLS_2005 force field. After the optimization process, the generation of the receptor lattice continued at the previously attached ligand site.

Docking method

Molecular docking is a computational simulation method to predict the conformation of the receptor-ligand complex,[22] which is also used for the reproduction of experimental data through the docking validation algorithms, where the protein-ligand or protein-protein conformation is obtained by *in silico* studies. Docking is the main tool for the virtual screening methods, where a library of different compounds is docked to the single drug target and displays the best match. Molecular docking studies were performed with the module GLIDE v3.8 (Schrödinger, LLC, New York) for the synthesized derivatives at the binding site of the glucokinase activator. The GLIDE module demonstrated the filtering of likely positions of the ligands in the binding pocket before docking to reduce the number of pose candidates.[23] A lattice is defined by the shape and properties of the receptor through various sets of fields.[24] In this direction, using standard force field ligand geometries in the receptor's binding pocket is minimized to localize the necessary ligand positions. The distance-dependent dielectric model also aided in locating these poses.[25] To rank the best docked poses, a model energy function combining empirical and force field terms is used. In addition to the standard precision (SP) scoring feature, GLIDE also features a new extra precision (XP) scoring feature that includes new terms and has been shown to improve the selection of actual binding poses.[26] The molecular mechanics generalized born surface area (MM/GBSA)-based rescoring method was applied to reclassify the docked complexes with their relative binding free energies.[27]

GLIDE v3.8 (Schrödinger, LLC, New York) was used to undertake SGLT2 inhibitor molecular docking simulations. The GLIDE module revealed how to decrease the number of pose candidates by screening plausible ligand locations in the binding pocket before docking.[28] The structure and features of the receptor are determined by a lattice through several fields. Standard force field ligand geometries in the receptor's binding pocket are reduced in this direction to locate the required ligand locations.[29] The distance-dependent dielectric model also aids in the identification of these positions. A model energy function integrating empirical and force-field variables is utilized to rank the best docked positions. In addition to the SP scoring function, GLIDE now includes a new XP scoring function, which includes innovative words and has been proved to improve the selection of real binding positions. The docked complexes were re-ranked with their respective binding free energy using the MM/GBSA-based rescoring technique.[30]

MM/GBSA based redocking

MM/GBSA is the molecular mechanics-generalized born surface area.[31] It is a popular method to calculate the free energy of the binding of ligands to proteins. To determine the relative binding affinity of ligands to the receptor, MM/GBSA calculations were used. In theory, MM/GBSA is used to rank ligands belonging to a congeneric series based on their free energy.

Absorption, distribution, metabolism, and excretion (ADME) properties

The Schrodinger ADME QikProp tool has been used to predict the various physicochemical properties of ligand molecules, for the rapid prediction of the ADME properties of drugs QikProp tool is used. Various properties are being predicated such as QP log Po/w, QPP Caco-2, QP logBB, QPPMDCK, Percent human oral absorption, QP logKhsa, including the hydrogen bond donor counting, rotatable bonds involvements, acceptor of hydrogen bonds, and molecular weight.

Polar surface area (PSA) is one of the molecular properties and one of the vital parameters in the design of drug, for the optimization of drug's ability to permeate cells.[32] PSA is defined as the surface sum all over the polar atoms or molecules, primarily oxygen and nitrogen also including hydrogen. Lipophilicity is one of the major molecular properties of the molecule to mix with an oily phase rather than water. It is evaluated as partition coefficient P, between the two phases and is typically represented as logP. Therefore, in the present study, we calculated the logP using Schrodinger software to know the lipophilicity of the compounds and antidiabetic activity.

The apparent permeability across the Caco-2 cell membrane is represented by descriptors like QPPCaco.[33] The brain/blood partition coefficient is represented by QPlogBB, and the apparent permeability across MDCK cells is represented by QPPMDCK, which can be used as an excellent non-active transport which mimic for the blood-brain barrier. Using a 1.4-radius probe, solvent accessible surface area (SASA) represents the total SASA in square angstroms.[34] The SASA values should fall between 300.0 and 1000.0.

RESULTS AND DISCUSSION

Molecular docking

Molecular docking studies were performed on GLIDE v5.8 (Schrodinger, LLC, New York, NY) to investigate the potential interactions SGLT2 inhibitors with PDB ID: 3DH4.[35] All the inhibitors were docked to active sites for the required interactions of compounds with protein to produce anti-diabetic activity. The docking studies were performed using GLIDE in the allosteric site of protein (PDB entry: 3DH4) and validated by docking of 3DH4 ligand in the allosteric site.

The SGLT2 inhibitors were docked, and the GLIDE scores by SP and XP as well as the inhibitors GLIDE energy, are shown in Table 2. The compounds docking investigations revealed a complementary match in the protein's allosteric location. Further inhibitors were examined in PyMOL to study the binding manner and docking contacts of the developed compounds[36] with the amino acid residues in the allosteric region of protein based on the GLIDE score, lowest GLIDE energy (kcaL/moL), and docking interactions.

The inhibitors showed the similar binding interactions with the receptors as shown by the crystal ligand. The compound phlorizin showed the best glucose lowering effect with the docking score (−12.118). Compound interaction with active site residue Asn64, Ser66, Ala63, Ser91, Tyr263, Glu88, Gln 428 in Figure 1 the compound empagliflozin Figure 2 showed the interaction with the active site Ala63, Tyr263, Ser91. The compound canagliflozin Figure 3 showed the interaction with active site Asn267.

In silico prediction of ADME properties SGLT2 inhibitors

Table 3 displayed ADME attributes. The surface and rotatable bond of all SGLT2 inhibitors were within the permitted range of medication resemblance attributes. The QikProp module of Schrodinger software was used to assess physicochemical properties. In this study, PSA, predicted brain/blood partition coefficient (QPlogBB), predicted apparent MDCK cell permeability (QPPMDCK), SASA, percent human oral absorption, QPlogPo/w, and predicted apparent Caco-2 permeability were all investigated *in silico* (QPPCaco). Pharmacokinetic parameters were crucial

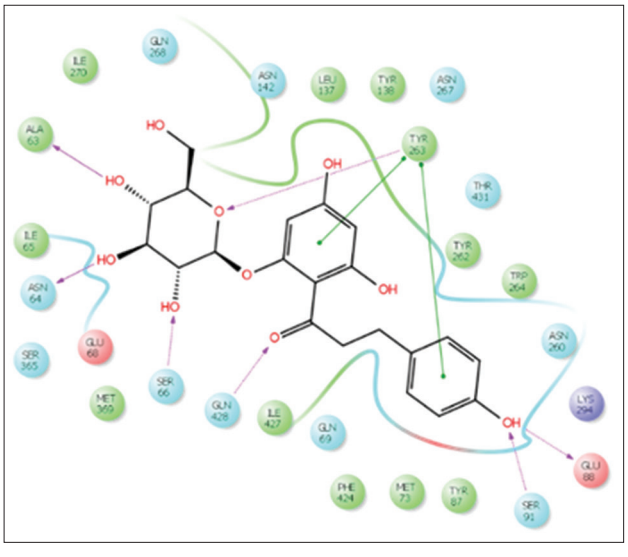
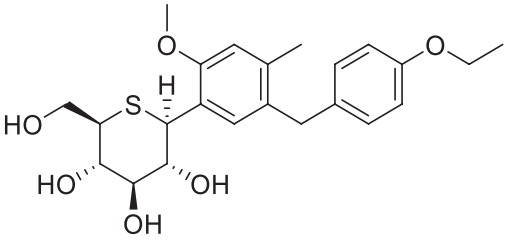
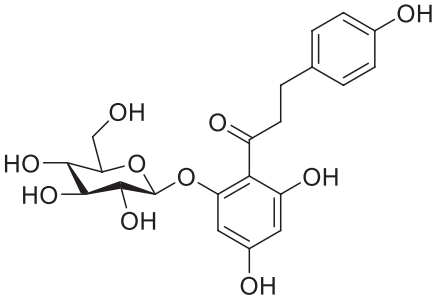
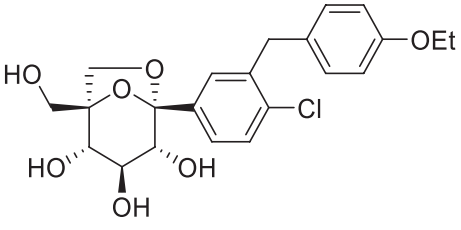
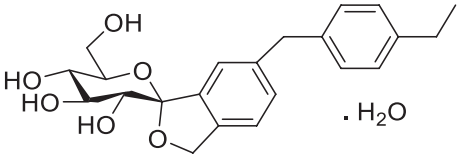
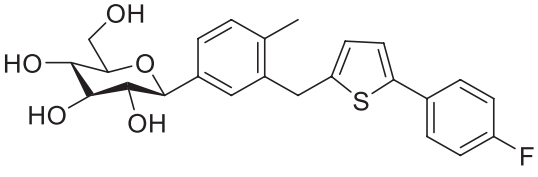
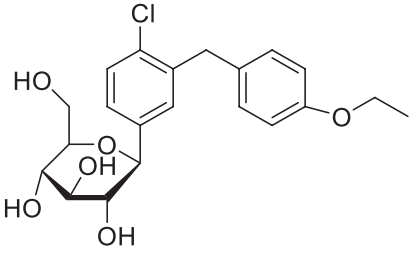
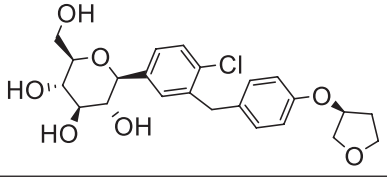


Figure 1: 2D interaction of phlorizin with protein database ID: 3DH4

Table 1: The compounds used as SGLT2 inhibitors

S. No.	Compounds	Chemical structure	Reference code
1	Luseogliflozin		11988953
2	Phlorizin		6072
3	Steglatro		44814423
4	Tofogliflozin		46908929
5	Canagliflozin		24812758
6	Dapagliflozin		9887712
7	Empagliflozin		11949646

SGLT2: Sodium-glucose cotransporter-2

in relating biological activity to its physicochemical characteristics.

Here, in this present study, we have studied various physicochemical properties such as PSA is one of the

Table 2: Docking scores (using HTVS, SP and XP methodologies) with binding energy (using MM/GBSA) of potent compounds

Compounds name	PDB ID: 3DH4		
	Docking score (SP) kcaL/moL	Docking score (XP) kcaL/moL	MM/GBSA dG bind (XP complex) kcaL/moL
Phlorizin	−7.761	−12.118	−42.3791
Empaglifozin	−7.664	-	-
Canaglifozin	−5.901	−10.289	−57.1803

HTVS: High throughput virtual screening, SP: Standard precision, XP: Extra precision, MM/GBSA: molecular mechanics generalized born surface area

Table 3: *In silico* predicted LogP and ADME properties SGLT2 inhibitors

Compounds	Mol. Wt.	PSA	QPlogPo/w[a]	QPP Caco[b]	QPlog BB[c]	QPP MDCK[d]	SASA[e]	Percent human oral absorption[f]
Luseogliflozin	448.57	94.87	3.051	321.22	−1.66	172.63	757.904	89.67
Phlorizin	436.41	184.75	−0.545	8.69	−3.27	2.931	653.4	27.60
Steglatro	436.88	107.90	2.442	268.35	−1.58	224.14	712.43	84.71
Tofogliflozin	384.47	91.89	2.55	270.29	−1.56	120.29	681.70	85.4
Canagliflozin	444.51	90.98	3.15	256.92	−1.49	272.64	747.41	88.55
Dapagliflozin	408.878	100.63	2.13	223.40	−1.66	183.47	700.32	81.46
Empagliflozin	450.915	109.82	1.95	223.38	−1.66	183.51	729.07	80.40

PSA: Polar surface area, ADME: Absorption, distribution, metabolism, and excretion, SGLT2: Sodium-glucose cotransporter-2 [a]Predicted octanol/water partition coefficient (Range=−2.0–6.5); [b]Predicted apparent Caco-2 cell permeability in nm/sec. Caco-2 cells are a model for the gut blood barrier (<25% is poor, >500 great). [c]predicted brain/blood partition coefficient. [d]Predicting apparent passive permeability of Caco-2 and MDCK cell. eTotal solvent accessible surface area in square angstroms using a probe with a 1.4 Å radius (range=300–1000); [f]Predicted human oral absorption on 0–100% scale. >80% is high <25% is poor

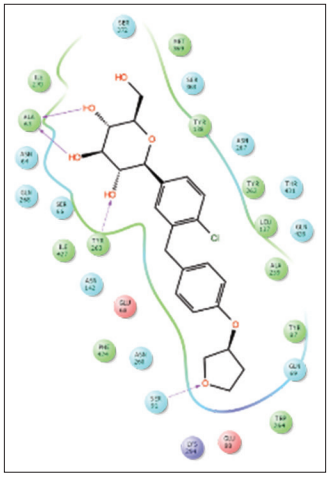


Figure 2: 2D interaction of empagliflozin with protein database ID: 3DH4

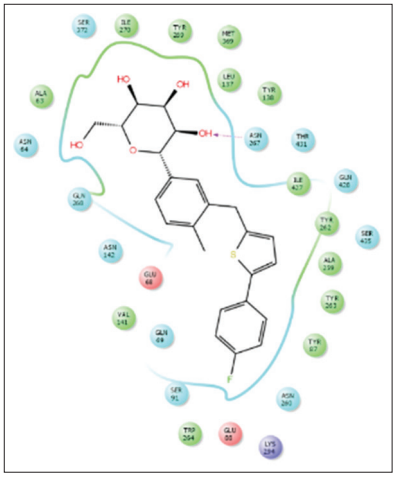


Figure 3: 2D interaction of Canaglifozin with protein database ID: 3DH4

vital parameters for the optimization of the drug's ability to permeate the cells. Some compounds having value >140 Å^2 which is the reason for the poor cell membrane permeability.

Descriptor like QPPCaco signifies the apparent permeability across the Caco-2 cell, whereas in this study all the compounds were in a range 25–500 showing moderate to good Caco-2 values.

SASA is a SASA in square angstroms within 1.4 Å radius. The compound canagliflozin showed the highest value whereas the phlorizin showed the lowest value. As per Qikprop projections, inhibitors have optimal properties for antidiabetic action and can be used as a lead molecule for further development.

MM/GBSA- based redocking determined the efficacy of binding of ligands with receptors in term of energy. In the

present study, the compound phlorizin, canagliflozin (complex with the PDB ID: 3DH4) found with the highest binding free energy as the dG bind = −42.3791, −57.1803 kcaL/moL, respectively [Table 2]. The compound canagliflozin showed the highest dG binding (dGbind = −57.1803 kcaL/moL) in MM/GBSA data redocking.

CONCLUSION

In the present *in silico* study, potential SGLT2 inhibitors have been identified. SGLT2 inhibitors are the blood glucose level lowering inhibitors through an insulin-independent mechanism for Type 2 diabetes. Using GLIDE v5.8 (Schrodinger, LLC, New York, NY) selected SGLT2 inhibitors were docked and resulted in the good potency of the compound and desired ADME properties with help of XP, SP, and MM/GBSA redocking. From the above study, it has been concluded that phlorizin having highest docking score −12.118 kcaL/moL showing binding interaction with the Asn64, Ser66, Ala63, Ser91, Tyr263, Glu88, Gln 428 and other compounds empagliflozin showed the interaction with Ala63, Tyr263, Ser91, canagliflozin showed the interaction with Asn267 (PBD ID: 3DH4) amino acid. The result of ADME studies were also good making the compound well-suited for the further studies. The present study may be helpful for the further development of the potent SGLT2 inhibitors.

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