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Investigation of SGLT2 Variant Interaction with Empagliflozin and Sotagliflozin

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ABSTRACT

Postprandial hyperglycemia, a hallmark of type 2 diabetes mellitus, is often mitigated through the use of sodium-glucose co-transporter 2 (SGLT2) inhibitors, which function to lower blood glucose levels by promoting glucose excretion in the urine. Empagliflozin and sotagliflozin are examples of such inhibitors. The molecular mechanism and efficiency of these drugs on SGLT2 variants are less understood. In this study, the effectiveness of empagliflozin and sotagliflozin on the SGLT2 protein variants, including native, V95I, V157A, L283M, and F453A, has been investigated to explore the extent and mechanism of action of these drugs on the protein's function. The molecular docking technique was used to investigate the interactions between empagliflozin, sotagliflozin, and the SGLT2 protein. The three-dimensional structures of the protein and ligands were retrieved from the Protein Data Bank (PDB) and PubChem databases, respectively. Ligand structures were optimized using the Avogadro software. Molecular docking simulations were subsequently performed using AutoDock Tools and the Vina algorithm. Binding affinities and interacting amino acid residues were then analyzed.
An inverse correlation was observed between binding energy and structural variation, indicating that the introduced variants negatively impacted drug performance, diminishing the efficacy of empagliflozin and sotagliflozin. Specifically, the F453A variant, characterized by a mutation in Phe453- a critical residue for ligand binding- presented the largest structural variation and lowest binding energies to the drugs (-10.1 kcal/mol for empagliflozin and -9.4 kcal/mol for sotagliflozin). This reduction in binding affinity would impede the drugs' capacity to lower blood glucose levels, thus underscoring the significance of Phe453.

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Introduction

Type 2 diabetes mellitus (T2DM), one of the most common metabolic disorders, is characterized by two main factors: decreased insulin secretion by pancreatic β-cells and insulin resistance in insulin-sensitive tissues. Insulin release and activity are tightly regulated due to their essential role in glucose homeostasis. Defects in the mechanisms involved in insulin synthesis, release, or detection are reported to contribute to metabolic disorders and disease development (Galicia-Garcia *et al.*, 2020). Type 2 diabetes can occur at any age, but it is more prevalent in individuals over 45 years old. Factors such as genetic predisposition, overweight, and physical inactivity have been identified as key contributors to the prevalence of this disease (Mordarska and Godziejewska-Zawada, 2017). SGLT2 inhibitors, the newest class of oral anti-hyperglycemic drugs, have been approved for managing this condition.

These inhibitors help lower blood sugar by reducing renal glucose reabsorption without stimulating insulin secretion (Hsia *et al.*, 2017).

SGLT2 is a membrane protein responsible for transporting substances across cell membranes. It is located in the proximal convoluted tubule of the kidney and functions as a sodium-glucose cotransporter in the apical membrane. There are two main types of transporters responsible for glucose absorption. The first is the Slc2 family, which moves sugars independently of ions. The second is the Slc5 family, which facilitates sodium-dependent sugar transport. Slc5 transporters utilize the sodium gradient generated by Na^+/K^+ ATPase in the basolateral membrane to transport glucose into the cell. These transporters reabsorb glucose and sodium ions in a 1:1 ratio in the S1 and S2 segments of the proximal tubule. SGLT2 inhibitors block the reabsorption of glucose and sodium ions, increasing their concentration in the lumen of the proximal tubule. Proximal tubule cells cannot metabolize glucose through glycolysis because they lack the rate-limiting enzyme hexokinase. As a result, glucose that enters the cells via SGLT2 is transported out and returned to the peritubular capillaries. Other glucose transporters and sodium channels, located away from the SGLT2 binding site, may partially counteract the effects of SGLT2 inhibitors. Approximately 90% of the glucose filtered by the glomerulus is reabsorbed through SGLT2 in the S1 and S2 segments of the proximal tubule, while the remaining 10% is reabsorbed by SGLT1 in the S3 segment of the proximal tubule (Hotait *et al.*, 2022; Nishiyama and Kitada, 2023).

SGLT2 inhibitors are a new class of blood glucose-lowering agents that effectively reduce hyperglycemia. These inhibitors block the SGLT2 protein, lowering the renal glucose threshold to approximately ~100 mg/dL and increasing glucose excretion through urine without raising the risk of hypoglycemia. This mechanism results in lower blood glucose levels and a reduced urine volume load (Cefalo *et al.*, 2019; Mahaffey *et al.*, 2018). Importantly, the mechanism of action of SGLT2 inhibitors is independent of insulin regulation. These drugs do not rely on insulin secretion by pancreatic β-cells or the presence of insulin resistance. Instead of stimulating insulin release, they improve β-cell function by alleviating glucotoxicity (Mahaffey *et al.*, 2018). SGLT2 inhibitor-induced glucosuria improves β-cell function and insulin sensitivity, while reducing tissue glucose uptake and increasing endogenous glucose production (EGP). These combined effects result in lower fasting and postprandial blood glucose levels (Xu *et al.*, 2022). Several selective SGLT2 inhibitors are available on the market, including empagliflozin, dapagliflozin, and luseogliflozin, which specifically target SGLT2. Additionally, dual SGLT1/SGLT2 inhibitors, such as sotagliflozin and canagliflozin, can inhibit both transporters (Dai *et al.*, 2023).

Empagliflozin can be used either as a monotherapy or in combination with other anti-diabetic agents, such as metformin, to lower blood sugar levels. In general, empagliflozin is effective in reducing cardiovascular mortality, lowering the risk of heart failure, improving blood sugar control, reducing blood pressure, enhancing lipid profiles, promoting weight loss, and improving metabolic parameters (Fitchett *et al.*, 2019; Home, 2019; Hsia *et al.*, 2017). Empagliflozin effectively lowers blood glucose with minimal side effects; however, its efficacy varies among individuals due to genetic and epigenetic factors, particularly variations in the SLC5A2 gene (Kaur *et al.*, 2021).

Sotagliflozin, also known as LX4211, is a small-molecule inhibitor of both SGLT1 and SGLT2. Its effectiveness in inhibiting SGLT2 is comparable to that of selective inhibitors such as dapagliflozin and canagliflozin. However, it is more than 10 times potent than these drugs in inhibiting SGLT1. Despite this, its full effects on SGLT2 have yet to be fully elucidated (Cefalo *et al.*, 2019; Nuffer *et al.*, 2019).

Molecular docking is a key tool in computer-aided drug design that predicts the interaction between small molecules and proteins at the atomic level. This technique allows researchers to study how small molecules interact with a target protein's binding site and understand the underlying biochemical processes. Molecular docking relies on the structural information of the target protein, typically obtained through X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, or cryo-electron microscopy. By predicting ligand binding, this method determines the optimal position of the ligand within the protein. Key advantages of

molecular docking include its high accuracy and speed (Agu *et al.*, 2023).

In this study, molecular docking was used to evaluate the effects of two inhibitors, empagliflozin and sotagliflozin, on the SGLT2 protein. Variants of the SGLT2 protein having mutations located within the binding site region (A73, I76, A389, S392, S393) were extracted from UniProt (Table 1). V95, located in transmembrane helix 2 of hSGLT2, plays a role in forming the hydrophobic pocket that interacts with the distal aromatic ring of gliflozin inhibitors. V157, situated in transmembrane helix 4, influences the size and shape of the hydrophobic pocket and is crucial for the binding of gliflozins. L283 is located in transmembrane helix 7 and forms part of the sugar-binding pocket in the outward-facing conformation of hSGLT2. This residue plays a role in the selectivity between SGLT2 and SGLT1. F453, a residue in transmembrane helix 10 that forms T-shaped π–π stacking interactions with F98 of TM2 in the inward-open conformation. This interaction is critical for the external gate function, preventing leakage from outside the cell when the transporter is in the inward conformation (Hiraizumi *et al.*, 2024). These SGLT2 variants were analyzed to investigate how these mutations influence SGLT2's interaction with the inhibitors.

Despite significant advances in the development of SGLT2 inhibitors, previous studies have certain limitations. For instance, (Hiraizumi *et al.*, 2024) used cryo-electron microscopy to determine the high-resolution structure of the hSGLT2–MAP17 complex bound to five distinct inhibitors, revealing detailed binding site interactions, protein conformational dynamics, and the mechanistic role of sodium ions in transport. However, their study is primarily structure-focused and lacks comprehensive analyses of structure-activity relationships (SAR) and the therapeutic potential of these inhibitors. In contrast, (Maccari and Ottanà, 2022) explore the pharmaceutical aspects, examining the design, optimization, and development of SGLT2 inhibitors through SAR and clinical features. However, their study lacks experimental data and detailed insights into the binding interactions between the drugs and their target protein. In this study, the selected ligand was structurally optimized, and molecular docking was performed to calculate the binding energies of the ligand-SGLT2 complex for both the native protein and its genetic variants. Additionally, critical amino acid residues involved in ligand interactions were identified, along with the types of bonds formed between the binding site and the inhibitors empagliflozin and sotagliflozin.

Materials and Methods

Utilized data

For molecular docking, the structure of the SGLT2 protein (PDB ID 7VSI) with a molecular weight of 79.85 kDa was obtained from the Protein Data Bank (PDB) in PDB format (Fig. 1A). The structure of empagliflozin (PubChem CID 11949646) and sotagliflozin (PubChem CID 24831714) were retrieved from the PubChem database in SDF 3D format (Fig. 1B-C). The PyMOL tool (version 3.0.3) was used to convert this format to PDB format. To optimize the structures of the drugs used in this study, the AVOGADRO software (version 1.2.0) was utilized, and the optimized structures were saved in PDB format for use in subsequent steps.

Targeted mutations in protein structure

Variants of the SGLT2 protein were selected based on their proximity to the enzyme's active site from the UniProt database. The "Wizard" option in the Protein Mutagenesis tool of PyMOL software was used to create mutations and generate the structures of these variants, starting from the 7VSI structure (Table 1). Additionally, to achieve a stable structure and alleviate potential structural stresses after introducing the desired mutations, the "Sculpting" option in PyMOL, which is based on energy minimization, was employed. Protein structure energy minimization was performed using the MMFF94s force field. The minimization protocol involved an initial 5000 steps of steepest descent, followed by conjugate gradient optimization until a convergence criterion of 0.1 kcal/(mol·Å) was achieved. The structure of the natural protein and its generated variants were compared using the "Alignment" option in PyMOL, and the extent of structural differences was reported as RMSD.

Molecular docking analysis

Molecular docking analysis was performed using AutoDock Tool 1.5.7 with the Vina algorithm.

The required format for both the protein and ligand to enter the docking process is pdbqt, which is generated using the PMV software (version 1.5.7). To prepare the protein structure in this format, the ligand was removed from the protein-ligand complex, and the protein was saved in PDBQT format after adding charges and hydrogen atoms. Similarly, after adding charges and hydrogen atoms to the optimized ligands and defining the torsional angles to ensure flexible ligands (The number of rotatable bonds was determined to be 10 for empagliflozin and 9 for sotagliflozin), the ligands were also saved in pdbqt format.

The next step involved determining the Grid Box coordinates. The key parameters for defining the Grid Box included the placement of critical amino acids within the binding site, identified using the UniProt database. Another important parameter was the Grid Box size, ensuring sufficient space for the selected ligands. Based on these considerations, the Grid Box coordinates were set with a search space of 0.375 and box dimensions of X: 40 Å, Y: 40 Å, and Z: 40 Å, with the center coordinates set to X: 39.377, Y: 51.962, and Z: 45.106. After preparing the input files, including the wild-type protein, variants, target ligands, and Grid Box coordinates, Molecular docking was performed using the Vina algorithm with an exhaustiveness parameter of 64 to enhance conformational.

For statistical analysis, each docking simulation was replicated ten times (n= 10), and the average binding energy from these replicates was reported. To identify statistically significant differences in the binding energies between each ligand and various protein variants, an Analysis of Variance (ANOVA) followed by Tukey's post hoc test was applied. A separate ANOVA was performed for each of the two ligands. Furthermore, a t-test was employed to compare the binding energies of a specific protein across the two investigated ligands. All statistical analyses were conducted using the R programming language (version 4.4.1).

The outputs obtained from Vina were prepared using the Open Babel software (version 3.1.1) and then analyzed and visualized with BIOVIA Discovery Studio (version 24.1.0.23298) to identify the amino acids and interactions involved in the docking process.

Validation

The molecular re-docking process was performed using AutoDock Tool 1.5.7 to confirm the ligand's binding pose within the SGLT2 protein and to ensure consistency with the Vina output.

Results and Discussion

The variants were selected and extracted based on their location within the enzyme's binding site and the functional changes they induce in the protein.

Table 1. SGLT2 variants in this study.

Type	Description
V95I	Strong reduction in D-glucose transporter activity. Impairs inhibition by empagliflozin on glucose uptake.
V157A	Decreases D-glucose transporter activity. Impairs inhibition by empagliflozin on glucose uptake.
L283M	Strong reduction in D-glucose transporter activity. Impairs inhibition by empagliflozin on glucose uptake.
F453A	Slightly decreases D-glucose transporter activity. Impairs empagliflozin binding and its inhibition of glucose uptake.

SGLT2 is a human protein (*Homo sapiens*) classified as a membrane transport protein and consists of a single A chain comprising 672 amino acids. Its structure was determined using electron microscopy with a resolution of 2.95 Å. The SGLT2 protein interacts with the PDZK1 protein, also known as MAP17 (Fig. 1A). PDZK1 has a molecular weight of 17 kDa and consists of 56 amino acids. This interaction enhances the activity of the SLC5A2 transporter (García-Heredia *et al.*, 2020; Niu *et al.*, 2022).

To validate the molecular docking process, re-docking was performed. The Vina output and the original ligand were compared to assess the similarity and overlap between the ligands under study. The results showed a high degree of alignment between the optimized original ligand and the Vina output ligand, which confirms the consistency of the docking simulation process (Fig. 2).

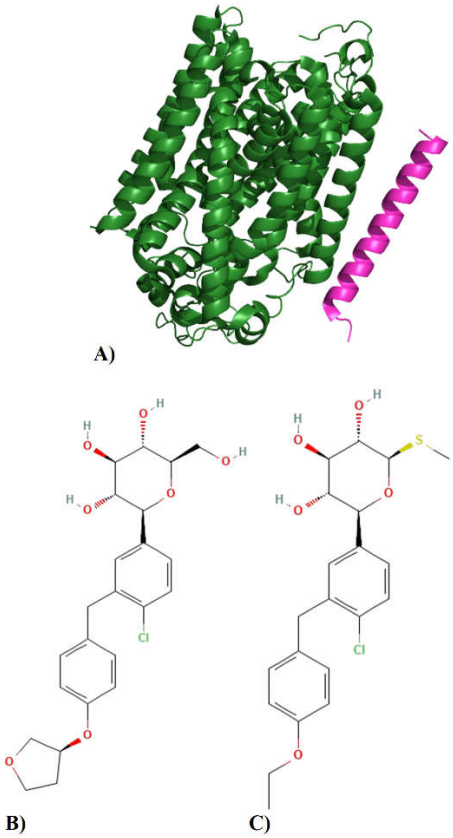

Fig. 1. A) The structure of SGLT2-MAP17, with SGLT2 shown in green and MAP17 in red; B) The 2D structure of Empagliflozin; C) The 2D structure of sotagliflozin.

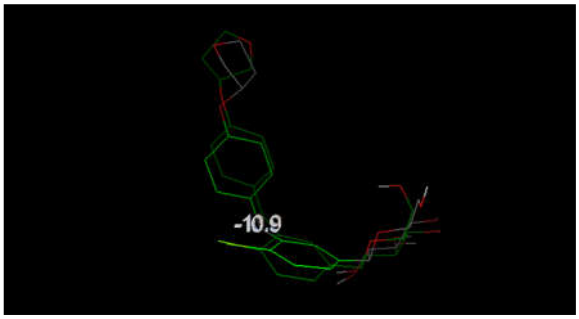

Fig. 2. The results of re-docking indicate the similarity between the two ligands

After performing the molecular docking process, the final results are quantitative and indicate the best binding energy in terms of the ligand's position within the predetermined coordinates. Docking results demonstrate optimal binding energies of -10.9 and -10.5 kcal/mol for empagliflozin and sotagliflozin, respectively, interacting with the native protein. Mutations introduced into the SGLT2 protein diminished the binding energies of empagliflozin and sotagliflozin, consequently impairing the protein's performance in interacting with these drugs. The F453A variant exhibited the most severe reduction in binding affinity (-10.1 kcal/mol for empagliflozin; -9.4 kcal/mol for sotagliflozin), likely due to disruption of π-stacking with Phe453 (Table 2).

As presented in Table 2, Several amino acids were consistently involved in the binding of empagliflozin and sotagliflozin to SGLT2, in both native and variant forms, highlighting their critical role in the binding mechanism. His80, Phe98, Leu84, and Phe453 are consistently observed in the ligand binding process with SGLT2 proteins across almost all forms. Asparagine 75 participates in the binding of empagliflozin to SGLT2 in the Val95I, V157A, L283M, and F453A variants. Additionally, asparagine 75 is involved in sotagliflozin binding to the L283M and F453A variants. Serine 287 contributes to empagliflozin binding in the V157A, L283M, and F453A variants, and to sotagliflozin binding in the L283M and F453A variants. Glutamine 457 is involved in empagliflozin binding to native SGLT2 and the L283M variant, and in sotagliflozin binding to the V95I variant. Glutamic acid 99 participates in sotagliflozin binding to native SGLT2 and the V95I variant. Threonine 87 influences empagliflozin binding in the V95I and V157A variants (Table 2).

Statistical analysis of docking results revealed significant differences in average binding energies among protein variants within each ligand type. An Analysis of Variance (ANOVA) indicated a statistically significant effect of protein variant on binding energy for both empagliflozin ($F= 21.84$, $p= 4.55e^{-10}$) and sotagliflozin ($F= 31.61$, $p= 1.5e^{-12}$). A Tukey post-hoc test, performed for empagliflozin binding energies, revealed significant pairwise differences ($p< 0.05$) among protein variants SGLT2-V95I, SGLT2-F453A, V95I-F453A3, V157A-F453A, and L283M-F453A. For sotagliflozin binding energies with different SGLT2 variants, the Tukey post-hoc test indicated significant pairwise differences ($p<0.05$) among all protein variants, with the

exceptions of the SGLT2-V95I and V157A-L283M pairs ($p>0.05$), as shown in Table 3.

Furthermore, a t-test analysis was conducted to assess the statistical significance of binding energies for each protein with empagliflozin versus sotagliflozin. The results indicated that proteins, with the exception of V95I, exhibited a significantly higher affinity for empagliflozin compared to sotagliflozin ($p<0.05$). For the V95I variant, no significant difference in binding energies between the two ligands was observed ($p> 0.05$).

Table 2. The obtained results for binding energy, dissociation constant, and interacting amino acids (22).

Drugs	Protein	BA	BC	Interacting amino acids
Empagliflozin	SGLT2	-10.9	-1.00×10^{-8}	His80, Val95, Phe98, LYS321, Ser287, Trp291, Tyr290, Leu274, Phe453, Asp454, GLN457, Tyr526
	V95I	-10.5	-1.97×10^{-8}	Asn75, His80, Leu84, Tyr290, Phe453, Asp454, Gln457, Thr87, Phe98
	V157A	-10.7	-1.41×10^{-8}	Asn75, His80, Leu84, Thr87, Val95, Leu274, Tyr290, Phe453, Gln457, Phe98, Ser287
	L283M	-10.7	-1.41×10^{-8}	Asn75, His80, Leu84, Phe98, Val157, Ser287, Lys321, Phe453, Gln457
	F453A	-10.1	-3.88×10^{-8}	Asn75, His80, Leu84, Thr87, Phe98, Leu274, Yur290, Ala453, Gln457, Ser287
Sotagliflozin	SGLT2	-10.5	-1.97×10^{-8}	Asn75, His80, Leu84, Val95, Phe98, GLU99, ser287, Phe453, Gln457, LYS321
	V95I	-10.4	-2.33×10^{-8}	Asn75, His80, Leu84, Val95, Phe98, Glu99, Leu274, Leu283, Val286, Ser287, Tyr290, Trp291, Phe453, GLN457
	V157A	-9.9	-5.43×10^{-8}	His80, Phe98, Glu99, Trp291, Lys321, Phe453
	L283M	-9.9	-5.43×10^{-8}	Asn75, His80, Phe98, Val157, Leu274, Val286, Tyr290, Trp291, Ser287, Lys321, Phe453
	F453A	-9.4	-1.27×10^{-8}	Asn75, His80, Leu84, Val95, Phe98, Glu99, Leu274, Ser287, Val286, Tyr290, Trp291, Lys321, Ala453

BA= Binding affinity (kcal/mol); BC= Binding constant (mol/L).

Table 3. ANOVA test analysis results.

Drugs	Comparison	Binding energy difference (kcal/mol)	Adjusted p-value
Empagliflozin	SGLT2-V95I	0.39	0.001119
	SGLT2-V157A	0.20	0.215571
	SGLT2-L283M	0.20	0.215571
	SGLT2-F453A	0.81	0
	V95I-V157A	-0.19	0.2608853
	V95I-L283M	-0.19	0.2608853
	V95I-F453A	0.42	0.0004052
	V157A-L283M	0.00	1
	V157A-F453A	0.61	0.0000004
	L283M-F453A	0.61	0.0000004
Sotagliflozin	SGLT2-V95I	0.12	0.8175513
	SGLT2-V157A	0.60	0.0000244
	SGLT2-L283M	0.60	0.0000244
	SGLT2-F453A	1.11	0
	V95I-V157A	0.48	0.0008106
	V95I-L283M	0.48	0.0008106
	V95I-F453A	0.99	0
	V157A-L283M	0.00	1
	V157A-F453A	0.51	0.0003459
	L283M-F453A	0.51	0.0003459

Figure 3 illustrates the two-dimensional interactions between the SGLT2 protein and empagliflozin and sotagliflozin. The amino acid residues Asn75, Phe98, Lys321, Ser287, and Trp291 participate in ligand binding by forming hydrogen bonds with the hydroxyl groups of the ligands. Additionally, Phe98, His80, and Phe453 interact with the benzene rings of the ligands, forming Pi interactions. Figure 4 illustrates the critical amino acids involved in the interactions between the native and variant forms of SGLT2 with empagliflozin and sotagliflozin.

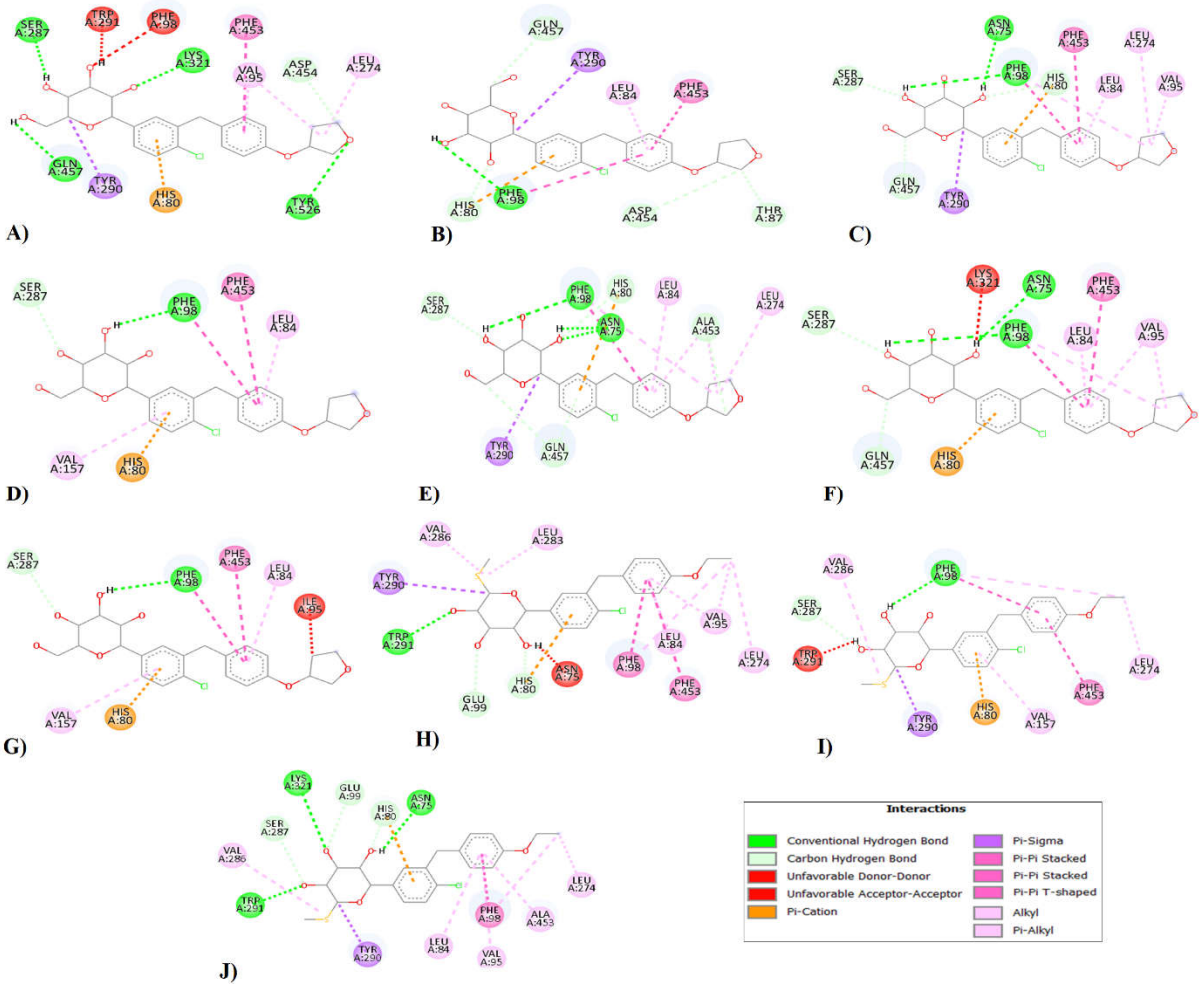

Fig. 3. The interaction between the native and variants of SGLT2 with empagliflozin and sotagliflozin: A) Interaction between the native SGLT2 protein and empagliflozin; B) Interaction between the SGLT2 protein with the V95I variant and empagliflozin; C) Interaction between the SGLT2 protein with the V157A variant and empagliflozin; D) Interaction between the SGLT2 protein with the L283M variant and empagliflozin; E) Interaction between the SGLT2 protein with the F453A variant and empagliflozin; F) Interaction between the native SGLT2 protein and sotagliflozin; G) Interaction between the SGLT2 protein with the V95I variant and sotagliflozin; H) Interaction between the SGLT2 protein with the V157A variant and sotagliflozin; I) Interaction between the SGLT2 protein with the L283M variant and sotagliflozin; J) Interaction between the SGLT2 protein with the F453A variant and sotagliflozin.

SGLT2, a renal membrane protein responsible for glucose reabsorption, possesses a binding pocket accommodating both glucose and glucose-like inhibitors. Previous studies have established that residues Phe98, Asn75, Ser287, Glu99, Trp291, Gln457, Arg267, and Tyr290 form crucial hydrogen bonds with the hydroxyl groups of the inhibitor's glucose moiety, stabilizing the ligand within the binding pocket and driving the binding mechanism (Bhattacharya *et al.*, 2021; Chang *et al.*, 2019; Ganwir *et al.*, 2024; Prasetiyo *et al.*, 2025). Additionally, Phe98 is integral to a hydrophobic cage, comprised of residues His80, Phe98, Phe453, and His268, which encapsulates the aglycone portion of the inhibitors. This hydrophobic environment, facilitated by π–π stacking interactions between the aromatic moieties of the inhibitors and these residues, significantly enhances binding stability (Hiraizumi *et al.*, 2024; Maccari and Ottanà, 2022; Mashraqi *et al.*, 2021). Our molecular docking analyses validate these findings, demonstrating the consistent involvement of Phe98, Ser287, Asn75, His80, Gln457, and Phe453 in the binding of empagliflozin and sotagliflozin to SGLT2.

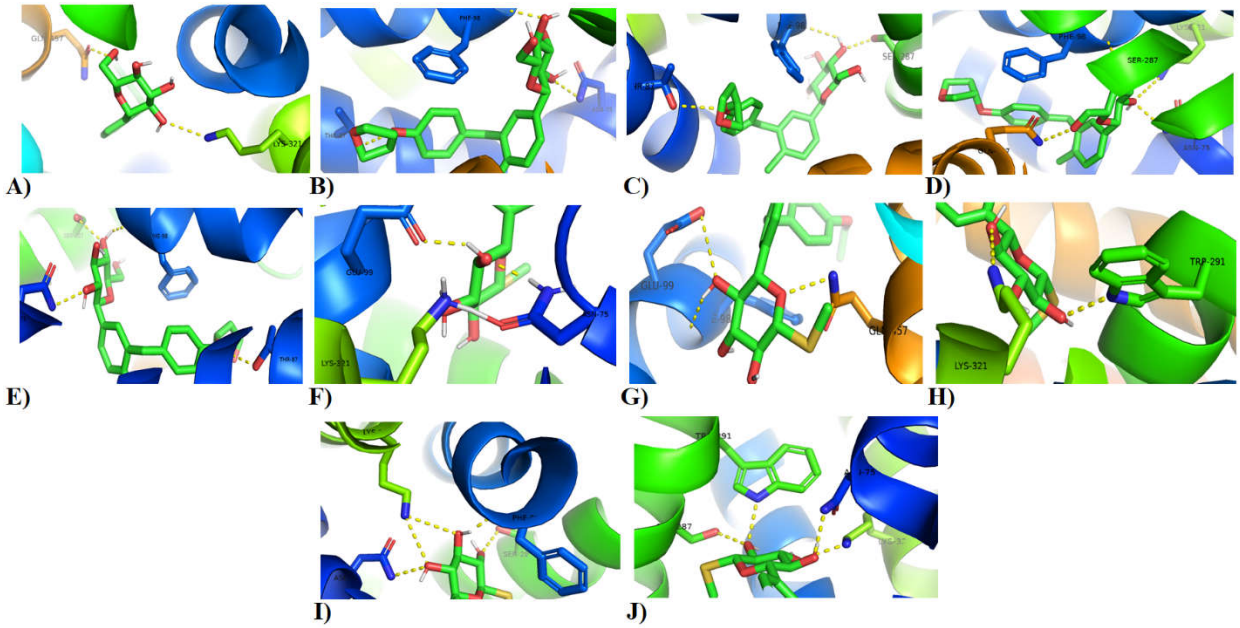

Fig. 4. The three-dimensional interactions between the native and variant forms of SGLT2 with empagliflozin and sotagliflozin, highlighting the critical amino acids involved: A) Interaction between the native SGLT2 protein and empagliflozin; B) Interaction between the SGLT2 protein with the V95I variant and empagliflozin; C) Interaction between the SGLT2 protein with the V157A variant and empagliflozin; D) Interaction between the SGLT2 protein with the L283M variant and empagliflozin; E) Interaction between the SGLT2 protein with the F453A variant and empagliflozin; F) Interaction between the native SGLT2 protein and sotagliflozin; G) Interaction between the SGLT2 protein with the V95I variant and sotagliflozin; H) Interaction between the SGLT2 protein with the V157A variant and sotagliflozin; I) Interaction between the SGLT2 protein with the L283M variant and sotagliflozin; J) Interaction between the SGLT2 protein with the F453A variant and sotagliflozin.

The F453A variant exhibited the most pronounced structural deviations, correlating with the lowest binding affinities for empagliflozin (-10.1 kcal/mol) and sotagliflozin (-9.4 kcal/mol). As demonstrated in Tables 2 and 3, there is an inverse correlation between structural variation and the binding efficacy of empagliflozin and sotagliflozin: increased variation diminishes binding. This phenomenon is likely attributable to steric clashes or disruptions of critical binding site interactions induced by the variant. Notably, as described before, Phe453 is pivotal for protein-ligand complex stabilization via π–π stacking interactions with the ligands' aromatic moieties. The F453A variant, wherein Phe453 is replaced with alanine, induces substantial conformational alterations and disrupts stabilizing interactions, resulting in a marked reduction in the binding affinities of both empagliflozin and sotagliflozin. Consequently, this mutation may compromise the therapeutic efficacy of empagliflozin and sotagliflozin in lowering blood glucose levels in individuals with type 2 diabetes bearing this SGLT2 variant (Table 4).

Table 4. Protein structural alignment and comparison with SGLT2 Native.

Protein	RMSD (Å)
Native	0.00
V95I	0.175
V157A	0.203
L283M	0.211
F453A	0.233

Protein mutations play a significant role in reducing drug efficacy by altering protein–drug binding interactions. In breast cancer patients, ESR1 mutations (e.g., L384V, R548P) reduce the binding affinity of drugs such as tamoxifen and raloxifene, thereby compromising treatment effectiveness (Wan *et al.*, 2021). Similarly, computational studies have shown that mutations like S904F in RET kinase confer resistance to vandetanib by inducing structural and dynamic changes. Mutations at Asp168 in the NS3/4A protease of the hepatitis C virus also enhance

resistance to inhibitors by disrupting the hydrogen-bonding network (Friedman, 2022). Additionally, the T790M mutation in the epidermal growth factor receptor (EGFR)- often occurring alongside the L858R mutation- contributes to resistance against gefitinib and erlotinib in approximately half of clinical cases. This resistance arises not from steric hindrance, but from increased ATP binding affinity, a mechanism confirmed through crystal structure analysis (Lahti *et al.*, 2012).

Molecular docking provides valuable insights into ligand–target interactions; its computational results require validation through molecular dynamics (MD) simulations to assess binding stability, as well as experimental assays to confirm pharmacological efficacy. These limitations are inherent to in silico approaches and should be acknowledged.

Conclusion

Postprandial hyperglycemia, a hallmark of type 2 diabetes mellitus, is often mitigated through the use of SGLT2 inhibitors, which function to lower blood glucose levels. This study aimed to elucidate the effects of empagliflozin and sotagliflozin on both native and variant forms of the SGLT2 protein. Molecular docking analyses were conducted to determine optimal binding energies, serving as a measure of drug efficacy on SGLT2 function. Binding affinity decreased with increasing structural deviation (RMSD), suggesting variant-induced impairment of drug efficacy, indicating that the introduced variants negatively impacted drug performance, thereby diminishing the efficacy of empagliflozin and sotagliflozin. Specifically, the F453A variant, characterized by a mutation in Phe453- a critical residue for ligand binding- exhibited binding energies of -10.1 kcal/mol for empagliflozin and -9.4 kcal/mol for sotagliflozin. This variant, demonstrating the greatest structural variation compared to the native protein, would likely experience attenuated inhibitor binding to SGLT2. This reduction in binding affinity would impede the drugs' capacity to lower blood glucose levels, thus underscoring the significance of Phe453. Our study identified several key residues consistently observed in docking analyses, including Phe98, Asn75, His80, Ser287, Leu84, Gln457, and Phe453, which demonstrate their pivotal role in the ligand binding mechanism to the SGLT2 protein through the formation of stabilizing hydrogen bonds and π-π stacking interactions. These findings may contribute to a more comprehensive understanding of drug binding to SGLT2 and facilitate the development of enhanced therapeutic agents targeting this protein for the treatment of type 2 diabetes mellitus. However, it should be noted that these findings require experimental validation, and future studies are recommended to confirm these results. Genetic screening for SGLT2 variants (e.g., F453A) could enable personalized therapy in T2DM by identifying patients likely to exhibit reduced empagliflozin /sotagliflozin efficacy. This approach would optimize drug selection, prioritizing alternative SGLT2 inhibitors or adjunct therapies in carriers of resistance-linked mutations, thereby improving glycemic control.

Conflict of interests

There is no conflict of interest between authors.

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