CHAPTER 12: IN SILICO DOCKING STUDIES ON THE ISOLATED COMPOUNDS AGAINST DIABETIC TARGET PROTEINS

12.1. Introduction

The interactions between receptors and ligands are fundamental to drug discovery. There are many methods for predicting and analyzing the interactions between protein receptors and ligands. A common technique central to receptor-ligand interactions is molecular docking. Prediction of binding energies is possible via a series of scoring functions, hydrogen bonds and bumps, and high level physics-based scoring methods. There are six major classes of drugs to manage type 2 diabetes which work in different ways in the management of diabetes and help to maintain good blood glucose control. Molecular docking continues to hold great promise in the field to predict the site of actions of drugs with a suitable ligand. A number of reports are existing which cite successful relevance of Computer-assisted drug design (CADD) in developing computer based drug design. CADD screens small molecules by orienting and scoring them in the binding of specific drug targets and finds use in developing drugs that could be used in different therapeutic areas. Protein-ligand interactions are increasingly employed to derive three dimensional structures of protein complexes. Computational techniques have become important to understand the molecular mechanisms of biological systems, as well as in obtaining leads for novel drug molecules. Considering the wide ranging effects of transcription factors in beta cell physiology, and the diverse pharmacological ligands that are available to manage the metabolic disturbances in diabetes mellitus (DM), an exploratory in silico study using various receptors and enzymes involved in insulin secretion and glucose metabolism as potential docking partners was
performed. Metformin, a standard antidiabetic drug, is used as a reference standard to explore the possible mechanism of action of the isolated compounds.

### 12.2. Materials and methods

**Table 12.1. Proteins selected for molecular docking studies**

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Name of the Protein</th>
<th>PDB ID.No</th>
<th>Functions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>ATP sensitive K⁺ channel</td>
<td>2WLK</td>
<td>The ATP-sensitive K⁺ channel (KATP channel) activity plays a crucial role in glucose-stimulated insulin secretion by coupling β-cell metabolism to calcium entry.</td>
<td>303</td>
</tr>
<tr>
<td>2.</td>
<td>Insulin Receptor</td>
<td>2B4S</td>
<td>Insulin initiates its cellular responses by binding to its cellular receptor, a transmembrane, multi-subunit glycoprotein that contains insulin-stimulated tyrosine kinase activity.</td>
<td>304</td>
</tr>
<tr>
<td>3.</td>
<td>Phosphorylase kinase</td>
<td>2Y7J</td>
<td>Phosphorylase kinase plays an important role in stimulating glycogen breakdown into free glucose by phosphorylating glycogen phosphorylase and stabilizing its active conformation. This activity is particularly important in liver and muscle cells, While muscle cells generally break down glycogen to power their immediate activity, liver cells are responsible for maintaining glucose concentration in the bloodstream.</td>
<td>305</td>
</tr>
</tbody>
</table>
4. SGLT2 2XQ2

SGLT2 is a member of the SLC5 gene family and transports glucose across cells using the Na\(^+\) gradient established by Na\(^+\)-K\(^+\)-ATPases. SGLT2 is a low-affinity, high-capacity transporter expressed predominantly in the early proximal tubule of the kidney and accounts for about 90% of renal glucose reabsorption. Given that the kidney filters approximately 180 grams of glucose daily, SGLT2 inhibition may not just reduce hyperglycemia but may also promote negative energy balance and weight loss.

5. FOXO-1 3COX 6

FOXO-1 is essential for the maintenance of human ESC pluripotency. This function is probably mediated through direct control by FOXO-1 of OCT4 and SOX2 gene expression through occupation and activation of their respective promoters. In hepatic cells this transcription factor seems to increase the expression of PEPCK and glucose-6-phosphatase (the same enzymes that are blocked via the metformin /AMPK /SHP pathway). Blocking this transcription factor offers an opportunity for novel therapies for diabetes mellitus. In pancreatic alpha-cells FOXO-1 is important in regulating pro-glucagon expression. In
<p>| | | |</p>
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<tbody>
<tr>
<td>6.</td>
<td>Glycogen synthase</td>
<td>3ZRL</td>
</tr>
<tr>
<td>7.</td>
<td>Pyruvate kinase</td>
<td>4MP2</td>
</tr>
<tr>
<td>8.</td>
<td>Glycogen phosphorylase</td>
<td>3DDS</td>
</tr>
<tr>
<td>9.</td>
<td>Sulfonyleurea receptor</td>
<td>4AYT</td>
</tr>
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</table>
is the ATP-sensitive potassium (K$_{ATP}$) channel, which plays a major role in controlling the β-cell membrane potential. Inhibition of K$_{ATP}$ channels by glucose or sulfonylureas causes depolarization of the β-cell membrane; in turn, this triggers the opening of voltage-gated Ca$^{2+}$ channels, causing Ca$^{2+}$ influx and a rise in intracellular Ca$^{2+}$ which stimulates the exocytosis of insulin-containing secretory granules.

<table>
<thead>
<tr>
<th>10.</th>
<th>PPAR-γ</th>
<th>4EM9</th>
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<tbody>
<tr>
<td>PPAR-γ regulates fatty acid storage and glucose metabolism. The genes activated by PPAR-γ stimulate lipid uptake by fat cells and adipogenesis.</td>
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<thead>
<tr>
<th>11.</th>
<th>GLUT-4</th>
<th>Homology modelling</th>
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<tbody>
<tr>
<td>GLUT-4 is the insulin-regulated glucose transporter found in heart, skeletal muscle, and adipose tissues. It is responsible for insulin-regulated glucose disposal. Therefore, Its role becomes defective when insulin is dysfunctional.</td>
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Molecular docking

The structures considered for the study were obtained by isolating anti-diabetic fraction to chromatographic studies and spectral studies (Chapter 9). Purified compounds obtained from column chromatography were subjected to IR, NMR spectroscopic studies. Based on the spectral data the structure of the isolated compounds were deduced as 1,4a,5,7a-tetrahydro-5-hydroxy-7-(hydroxymethyl)-1-(tetrahydro-6-(hydroxymethyl)-3,4,5-trimethoxy-2H-pyran-2-yloxy)cyclopenta[c]pyran-4-carboxylic acid (C1) and 5,8-dihydro-7-isopentyl-2,3,5,8-tetramethoxynaphthalene-1,4,6-triol (C2). Metformin is used as a standard drug for docking. These compounds were used as ligands and their structures were drawn using CHEMDRAW (Version 11) (Cambridge soft, USA). The structures was obtained from the RCSB protein data bank (PDB) (http://www.rcsb.org/pdb). Protein Data Bank (PDB) is a freely accessible archive of the 3-D structural data of biological molecules. Structure based studies offers a unique vantage point in inferring the properties of a protein molecule from structural data. Hydrogen atoms were added to the protein solution to equalize to pH 7.0 using the protein preparation wizard in the Schrodinger suite. Water molecules and atoms of ligands are removed from the PDB file the protein preparation wizard module in the Maestro interface was used to assign bond orders and hydrogen bonding. Further, the proteins hydrogen bond network was also optimized using the wizard. The optimized preparation structure was then subjected to energy minimization and the termination condition. Termination condition for minimization was fixed as the step when the root mean square deviation of the heavy atoms in the structure relative to the starting structure exceeded 0.3 A. This process also ensures that the hydrogen atoms are placed in optimized geometries. The protein thus prepared was used for docking of the ligands.
Potential binding sites for the selected diabetic molecular target proteins (Table 12.2) were predicted using the SiteMap tool in the Schrodinger suite\textsuperscript{259, 260} and selected site had the highest score for molecular docking (Table 12.2). Receptor grid for the proteins were then generated for site that had higher score using Glide module (version 5.8) of the Schrodinger suite (Table 12.2). The grid box and center were set using site-map program of maestro interface. The Active/ Binding site are prepared for docking using glide module in maestro interface. Ligand Preparation module (version 2.5) of the Schrodinger suite was used to generate conformers of the ligands. The ligands were then docked using the extra precision mode in the Glide module\textsuperscript{261, 262, 263} of the Schrodinger suite. The docking results are compared with metformin a standard antidiabetic drug.

12.3. Results and Discussion

The ATP-sensitive potassium channel (K\textsubscript{ATP}) is a key component of stimulus-mediated secretion coupling to pancreatic β-cell. Interaction of the active molecules with K\textsubscript{ATP} was determined using in silico studies\textsuperscript{303}. The study results indicate that both compounds (C1 and C2) strongly interact with ATP sensitive K\textsuperscript{+} channel through different residues (Figures 12.1 and 12.2). The XP Glide score for both the compounds (-6.981 Kcal/mol for C1 and -9.425 Kcal/mol for C2) clearly suggests that both the compounds show better interaction than metformin XP Glide score -2.563 (Table 12.2 and Figure 12.3). The docking of C1 and C2 with ATP sensitive K channel resulted in the opening of Ca\textsuperscript{2+} channels in pancreatic β cells causing Ca\textsuperscript{2+} influx which might have facilitated the exocytosis of insulin from pancreatic β-cells, thus resulting in lowering of the blood glucose level.

Insulin receptor stimulates glucose uptake from the systemic circulation and suppress hepatic gluconeogenesis thereby serving a major role in glucose homeostasis.
and thus prevents the metabolic disorder diabetes mellitus. Interaction of Insulin receptor with the active molecules was studied using in silico methods. The study indicated that both compounds (C1 and C2) strongly interact with Insulin receptor through different residues (Figures 12.4 and 12.5). The XP Glide score for both the compounds were -7.882 Kcal/mol for C1 and -4.62 Kcal/mol for C2. This clearly suggests that both the compounds show better interaction than metformin (Table 12.2 and Figure 12.6). Thus, the compounds could, possibly, activate the insulin receptor which facilitate the active absorption of glucose in skeletal muscle through GLUT-4 leading to the lowering of blood glucose.

Phosphorylase kinase plays an important role in stimulating glycogen breakdown into free glucose by phosphorylating glycogen phosphorylase and stabilizing its active conformation. This activity is particularly important in liver and muscle cells, since both tissues generally breaks down glycogen in the liver cell and contribute to the increased blood glucose levels. The in silico study indicates that both compounds (C1 and C2) strongly interact with phosphorylase kinase through different residues (Figures 12.7 and 12.8). The XP Glide score for both the compounds includes -6.164 Kcal/mol for C1 and -5.253 Kcal/mol for C2. This clearly suggests that docking of both the compounds with phosphorylase kinase lead to the inhibition of glycogenolysis by deactivating glycogen phosphorylase and thus resulting in the lowering of blood glucose level (Table 12.2 and Figure 12.9).

SGLT2 is a sodium dependent glucose transporter protein primarily located in the kidney and plays a key role in glucose reabsorption. SGLT2, not just reduce hyperglycemia, but may also promote negative energy balance and weight loss and thus play an important role in energy metabolism in the body. The in silico study indicates that both compounds (C1 and C2) strongly interact with SGLT2 through
different residues (Figures 12.10 and 12.11). Among the two, C1 exhibited maximum numbers of hydrophobic interactions compared to C2. The XP Glide score for both the compounds includes -8.765 Kcal/mol for C1 and -7.056 Kcal/mol for C2. This clearly suggests that both the compounds inhibit SGLT2 which in turn prevents reabsorption of glucose in renal tubules and thus contributes to lowering of the blood glucose level (Table 12.2 and Figure 12.12).

FOXO-1 is the important transcription factor essential for the expression of PEPCK and glucose-6-phosphatase in liver cells. These enzymes play a key role in gluconeogenesis process and contribute to hyperglycemia\(^{308}\). The \textit{in silico} study indicates that both compounds (C1 and C2) strongly interact with FOXO-1 through different residues (Figures 12.13 and 12.14). Among the two compounds C1 exhibited maximum numbers of hydrophobic interactions compared to C2. The XP Glide score for both the compounds includes -4.377 Kcal/mol for C1 and -2.715 Kcal/mol for C2. This clearly suggests that both the compounds inhibits the activity of FOXO-1 and reduces the expression of these two gluconeogenic enzymes leading to the reduced blood glucose level. (Table 12.2 and Figure 12.15). Further FOXO-1 also increases the expression of glucagon and mediation of glucagon like peptide-1 action in pancreatic β cell. The docking of C1 and C2 with FOXO - 1 also reduces the glucagon and glucagon like peptide-1 expression thus leading to the lowering of blood glucose.

Glycogen synthase represents major pathway of glucose disposal in skeletal muscle after insulin stimulation. It plays an important role in the disposal of blood glucose by converting them in to glycogen. The glycogen synthase activity was found to be impaired in type 2 DM\(^{311}\). The \textit{in silico} study indicates that both compounds (C1 and C2) strongly interact with glycogen synthase through different residues
(Figures 12.16 and 12.17). The XP Glide score for both the compounds includes -5.42 Kcal/mol for C1 and -4.566 Kcal/mol for C2. This clearly suggests that both the compounds might have activated the target protein, facilitating the storage of excess glucose into glycogen and thus contribute in the lowering of blood glucose (Table 12.2 and Figure 12.18).

Pyruvate kinase is the key regulator of glycolytic pathway. Pyruvate kinase catalyzes the transphosphorylation from phosphoenolpyruvate (PEP) to ADP in the last step of glycolysis to generate ATP. The in silico study indicates that both compounds (C1 and C2) strongly interact with pyruvate kinase through different residues (Figures 12.19 and 12.20). The XP Glide score for both the compounds includes -6.746 Kcal/mol for C1 and -5.808 Kcal/mol for C2. This clearly suggests that both the compounds may activate this enzyme which might have resulted in the increased glucose oxidation leading to the reduction of blood glucose level (Table 12.2 and Figure 12.21).

Glycogen phosphorylase inhibitors (GPI) have been shown to be more potent in reducing hepatic glucose output in the presence of high glucose concentrations. Thus, as blood glucose concentration diminishes, GPI potency is decreased to provide better protection to patients from periods of hypoglycemia. The in silico study indicates that both compounds (C1 and C2) strongly interact with Glycogen phosphorylase through different residues (Figures 11.22 and 11.23). The XP Glide score for both the compounds is -7.558 Kcal/mol for C1 and -5.454 Kcal/mol for C2. This clearly suggests that both the compounds inhibit glycogen phosphorylase leading to the prevention of glycogen break down which in turn facilitate the lowering of blood glucose level. (Table 12.2 and Figure 12.24).
Sulfonylurea receptor activation stimulates insulin secretion from pancreatic β-cells. They primarily act by binding to the SUR subunit of the ATP-sensitive potassium (K\textsubscript{ATP}) channel and inducing channel closure. The in silico study indicates that both compounds (C1 and C2) strongly interact with Sulfonylurea receptor through different residues (Figures 12.25 and 12.26). The XP Glide score for both the compounds is -5.914 Kcal/mol for C1 and -5.951 Kcal/mol for C2. This clearly suggests that both the compounds might have inhibited the K\textsubscript{ATP} channel causing depolarization of β-cell membrane; this in turn might have triggered the opening of Ca\textsuperscript{2+} channel leading to increased Ca\textsuperscript{2+} influx resulting in the increased excocytosis of insulin and thus causing reduced blood glucose level. (Table 12.2 and Figure 12.27).

PPAR-γ plays a key role in the glucose and lipid metabolism. The activation of PPAR-γ increases insulin sensitization. This was experimentally proved by in vitro studies using cell lines \textsuperscript{318}. Compounds (C1 and C2) strongly interact with PPAR-γ through different residues (Figures 12.28 and 12.29). The XP Glide score for both the compounds are -4.7 Kcal/mol for C1 and -4.01 Kcal/mol for C2. This clearly suggests that both the compounds effectively activate the PPAR-γ which might have reversed insulin resistance or increased the insulin sensitization in muscle cells. Thus through increased uptake of glucose in muscle, the compounds might have reduced the blood glucose levels (Table 12.2 and Figure 12.30).

GLUT-4 is located in muscle cells and plays a key role in facilitating the entry of glucose to the hexose utilizing cells \textsuperscript{319}. Both compounds (C1 and C2) strongly interact with GLUT-4 receptor through different residues (Figures 12.31 and 12.32). The XP Glide score for both the compounds includes -5.28 Kcal/mol for C1 and -8.01 Kcal/mol for C2. These data suggest that both the compounds
might be activating GLUT-4 to facilitate the increased uptake of glucose in muscle cells and thus causing reduced blood glucose level (Table 12.2 and Figure 12.33).

Table 12.2. Glide scores for the isolated compounds and standard obtained through in silico docking.

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Docking studies</th>
<th>Name of the Protein</th>
<th>PDB ID No.</th>
<th>Site volume</th>
<th>Glide score</th>
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<td></td>
<td></td>
<td></td>
<td>C1</td>
<td>C2</td>
<td>C1</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>ATP sensitive K channel</td>
<td>2WLK</td>
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<td>3</td>
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<td>GLUT-4 Homology Modeling</td>
<td>446.929</td>
<td>446.929</td>
<td>-5.28</td>
<td>-8.01</td>
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MOLECULAR DOCKING STUDIES
Figure 12.1. Probable interaction of Compound 1 with ATP-sensitive potassium channel

A. Surface view, B. Ligand interaction diagram
Figure 12.2. Probable interaction of Compound 2 with ATP-sensitive potassium channel

A. Surface view, B. Ligand interaction diagram
Figure 12.3. Probable interaction of Metformin with ATP-sensitive potassium channel

A. Surface view, B. Ligand interaction diagram
Figure 12.4. Probable interaction of Compound 1 with Insulin receptor

A. Surface view, B. Ligand interaction diagram
Figure 12.5. Probable interaction of Compound 2 with Insulin receptor

A. Surface view, B. Ligand interaction diagram
Figure 12.6. Probable interaction of Metformin with Insulin receptor

A. Surface view, B. Ligand interaction diagram
Figure 12.7. Probable interaction of Compound 1 with Phosphorylase kinase

A. Surface view, B. Ligand interaction diagram
Figure 12.8. Probable interaction of Compound 2 with Phosphorylase kinase

A. Surface view, B. Ligand interaction diagram
Figure 12.9. Probable interaction of Metformin with Phosphorylase kinase

A. Surface view, B. Ligand interaction diagram
Figure 12.10. Probable interaction of Compound 1 with SGLT2

A. Surface view, B. Ligand interaction diagram
Figure 12.11. Probable interaction of Compound 2 with SGLT2

A. Surface view, B. Ligand interaction diagram
Figure 12.12. Probable interaction of Metformin with SGLT2

A. Surface view, B. Ligand interaction diagram
Figure 12.13. Probable interaction of Compound 1 with FOXO-1

A. Surface view, B. Ligand interaction diagram
Figure 12.14. Probable interaction of Compound 2 with FOXO-1

A. Surface view, B. Ligand interaction diagram
Figure 12.15. Probable interaction of Metformin with FOXO-1

A. Surface view, B. Ligand interaction diagram
Figure 12.16. Probable interaction of Compound 1 with Glycogen synthase

A. Surface view, B. Ligand interaction diagram
Figure 12.17. Probable interaction of Compound 2 with Glycogen synthase

A. Surface view, B. Ligand interaction diagram
Figure 12.18. Probable interaction of Metformin with Glycogen synthase

A. Surface view, B. Ligand interaction diagram
Figure 12.19. Probable interaction of Compound 1 with Pyruvate kinase

A. Surface view, B. Ligand interaction diagram
Figure 12.20. Probable interaction of Compound 2 with Pyruvate kinase

A. Surface view, B. Ligand interaction diagram
Figure 12.21. Probable interaction of Metformin with Pyruvate kinase

A. Surface view, B. Ligand interaction diagram
Figure 12.22. Probable interaction of Compound 1 with Glycogen phosphorylase

A. Surface view, B. Ligand interaction diagram
Figure 12.23. Probable interaction of Compound 2 with Glycogen phosphorylase

A. Surface view, B. Ligand interaction diagram
Figure 12.24. Probable interaction of Metformin with Glycogen phosphorylase

A. Surface view, B. Ligand interaction diagram
Figure 12.25. Probable interaction of Compound 1 with Sulfonylurea receptor

A. Surface view, B. Ligand interaction diagram
Figure 12.26. Probable interaction of Compound 2 with Sulfonylurea receptor

A. Surface view, B. Ligand interaction diagram
Figure 12.27. Probable interaction of Metformin with Sulfonylurea receptor

A. Surface view, B. Ligand interaction diagram
Figure 12.28. Probable interaction of Compound 1 with PPAR-γ
A. Surface view, B. Ligand interaction diagram
Figure 12.29. Probable interaction of Compound 2 with PPAR-γ

A. Surface view, B. Ligand interaction diagram
Figure 12.30. Probable interaction of Metformin with PPAR-γ

A. Surface view, B. Ligand interaction diagram
Figure 12.31. Probable interaction of Compound 1 with GLUT-4

A. Surface view, B. Ligand interaction diagram
Figure 12.3. Probable interaction of Compound 2 with GLUT-4

A. Surface view, B. Ligand interaction diagram
Figure 12.33. Probable interaction of Metformin with GLUT-4

A. Surface view, B. Ligand interaction diagram