INVESTIGATING THE THERAPEUTIC POTENTIAL OF ORIENTIN IN DIABETES: MOLECULAR DOCKING ANALYSIS ON DIABETIC REGULATING PROTEINS

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Abstract: *Molecular docking analysis plays a crucial role in drug discovery and development by elucidating the interactions between drugs and target molecules. In this study, we employed molecular docking analysis to investigate the binding interactions between orientin, a well-known antidiabetic drug, and a set of genes involved in diabetes regulation. Three-dimensional models of the target proteins, including insulin receptor (IR), glycogen synthase kinase, and glucokinase, were generated using computational tools and available crystal structures. Rigorous refinement and validation techniques were applied to ensure model accuracy.Using Auto dock for molecular docking and Discovery Studio for 3D structure visualization, we performed docking of orientin, a known antidiabetic agent, into the active sites of the target proteins. The resulting binding modes and interactions were extensively analysed to uncover the molecular mechanisms underlying orientin's potential therapeutic effects in diabetes regulation. Our findings demonstrated favourable binding affinities and strong interactions between orientin and the target proteins. Notably, orientin exhibited robust binding to specific nuclear receptors, indicating its potential in activating these receptors and influencing gene expression related to glucose and lipid metabolism. Critical binding sites and residues involved in the drug-target interactions were also identified, providing valuable information for future structure-based drug design and optimization.Overall, our study highlights the potential mechanisms of action of orientin and paves the way for further experimental investigations to validate its therapeutic efficacy in managing diabetes. By utilizing molecular docking analysis, we have gained valuable insights into the interactions between orientin and key proteins involved in diabetes regulation. This contributes to a broader understanding of drug-target interactions and facilitates the development of novel treatments for diabetes.*

Keywords: *Diabetes, Orientin, molecular docking, Insulin receptor substrate-1, glucose synthase, therapeutic target.*

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INTRODUCTION

Diabetes, a chronic metabolic disorder, is characterized by elevated blood glucose levels resulting from either insufficient insulin production (Type 1 diabetes) or impaired insulin action (Type 2 diabetes) [1]. It poses a significant global health challenge, affecting millions of individuals worldwide. While conventional treatments, such as lifestyle modifications, oral antidiabetic drugs, and insulin therapy, have been the mainstay for diabetes management, novel therapeutic approaches are emerging and showing promise in improving treatment outcomes [2].Type 1 diabetes is characterized by the immune system

mistakenly attacking and destroying the insulin-producing beta cells in the pancreas, leading to a complete absence of insulin production. As a result, individuals with Type 1 diabetes require lifelong insulin therapy to regulate their blood glucose levels [3]. On the other hand, Type 2 diabetes typically arises from a combination of genetic and lifestyle factors. It involves insulin resistance, where the body's cells become less responsive to insulin, combined with inadequate insulin secretion from the pancreas. Managing Type 2 diabetes involves lifestyle modifications, oral antidiabetic medications, and in some cases, insulin therapy [4].

In the field of diabetes treatment, emerging therapies are being developed to target the underlying mechanisms of the disease and improve glucose regulation. These innovative approaches encompass a range of strategies, including medications that specifically act on pathways involved in insulin production, insulin signaling, glucose metabolism, or modulation of the immune system [5]. Additionally, advancements in gene therapy and regenerative medicine offer promising avenues for future diabetes treatments.For example, GLP-1 receptor agonists are a class of injectable medications that mimic the action of GLP-1, a hormone that enhances insulin secretion, suppresses glucagon release, slows gastric emptying, and promotes feelings of satiety. Beyond their ability to improve blood glucose control, these agents also contribute to weight loss and offer cardiovascular benefits [6].The field of insulin therapy has also seen significant progress, resulting in the development of insulin analogues with enhanced pharmacokinetic and pharmacodynamic properties. Rapid-acting, long-acting, and ultra-long-acting insulin analogues provide more precise control over blood glucose levels and minimize the risk of hypoglycaemia [7].Furthermore, ongoing research is exploring the potential of gene and cell therapies for diabetes. These approaches aim to restore normal insulin production and function by introducing functional genes or cells into the body. While still in the early stages of development, these therapies hold the potential for long-term diabetes management and even potential cures [8].

Orientin, a natural flavonoid compound, has demonstrated potential in providing beneficial effects in the context of diabetes [9]. Its strong antioxidant and anti-inflammatory properties are particularly advantageous in mitigating the oxidative stress and chronic inflammation commonly associated with diabetes. These factors play significant roles in the development and progression of diabetes and its complications [10].In terms of glucose metabolism, orientin has been found to modulate this process and improve insulin sensitivity. By enhancing glucose uptake by cells, it facilitates better glucose utilization and regulation. Studies have indicated that orientin can activate glucose transporters, including GLUT4, which are vital for facilitating glucose uptake in various tissues such as skeletal muscle and adipose tissue [11].Furthermore, orientin exhibits potential in regulating lipid metabolism, which is often dysregulated in diabetes. It helps to regulate lipid synthesis, inhibits adipogenesis (the formation of fat cells), and promotes lipid breakdown. These actions contribute to the management of lipid profiles, which can be beneficial in the context of diabetes [12].

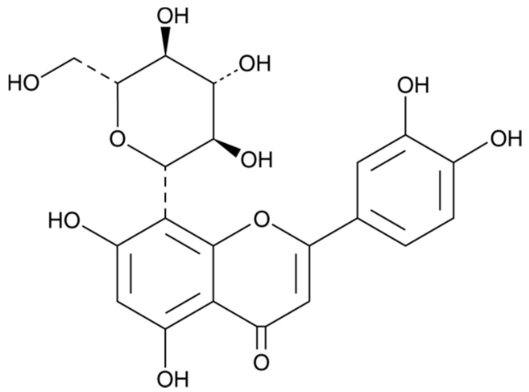

Fig 1. Structure of Orientin

Molecular docking is a computational technique that allows the analysis of how a smaller molecule docks or fits into a larger macromolecule, evaluating the complementary fit at specific binding sites [13]. This approach is widely used in structure-based drug design. In our study, we employed molecular docking to investigate the molecular mechanisms of orientin, a compound of interest, in relation to diabetic regulators such as insulin receptor (IR), glycogen synthase kinase, and glucokinase. Through our analysis, we found that Orientinexhibited a significant binding affinity with these diabetic regulators. This suggests that orientin may hold potential as a therapeutic option for the treatment of diabetes.

MATERIALS AND METHODS

Protein preparation

To conduct our molecular docking studies, we accessed the crystal structures of our target proteins involved in diabetes regulation, namely insulin receptor (IR), glycogen synthase kinase, and glucokinase, from the Protein Data Bank (PDB). The specific PDB IDs we utilized were 1IR3, 3F7Z, and 4IXC. For our analysis, we focused on Chain A of each protein structure, which represents the primary protein of interest.To prepare these protein structures for molecular docking experiments, we employed a Python molecule viewer. This tool enabled us to remove water ions and ligands, ensuring that only the protein component remained for further analysis [14]. By eliminating non-protein elements, we obtained refined structures ready for subsequent docking simulations.Next, to identify potential drug-binding sites within the proteins, we utilized the PockDrug-server.

Ligand preparation

In this study, we selected Orientin (CID ID: 5281675) as our compound of interest. The three-dimensional (3D) structure of Orientin was obtained from PubChem, which is a comprehensive database of chemical molecules. To assess the drug-like properties of Orientin, we employed the SWISS-ADME prediction tools. These tools provided valuable information regarding various drug-related properties, including solubility, lipophilicity, and drug-likeness [15].To optimize the geometry and minimize the energy of the synthetic compounds, we utilized the Avogadro server. This process involved refining the 3D coordinates of the compounds. Additionally, the geometry of the synthetic drug, Orientin, was improved, and partial charges were calculated. These refined structures and partial charges were saved as mol2 files, which were then used in AutoDock Tools (ADT) for the preparation of pdbqt files. Pdbqt files are a required format for performing molecular docking studies using AutoDock, a widely used software for molecular docking simulations.

Molecular docking procedure

To identify the active site residues located within the substrate-binding domain (SBD) of insulin receptor (IR), glycogen synthase kinase, and glucokinase, relevant information was gathered from the literature. This information guided the subsequent docking analysis. AutoDock, a widely utilized docking software, was employed to facilitate the docking simulations. Grids were generated using AutoDock to define the active site regions within the target proteins.For the generation of grid maps, a box size of 90 × 90 × 90 xyz points was set, centered on the active site residues of insulin receptor (IR), glycogen synthase kinase, and glucokinase. AutoGrid, a component of AutoDock, was utilized to create these grid maps. These maps provide a spatial representation of the active site regions, allowing for more precise docking simulations.

RESULTS

In our studies, we utilized AutoDock 4.2, a well-established and widely used docking technique, to determine the binding free energy (ΔG) between molecules [15]. Our docking approach involved keeping the target protein rigid while allowing the ligand to explore a range of torsional degrees of freedom, in addition to the six spatial degrees of freedom governed by translational and rotational constraints.

AutoDock employed the Lamarckian Genetic Algorithm (LGA) to search for the best conformers, considering various conformational possibilities.For the docking simulations, we set the grid spacing at 0.45 Å and performed a maximum of 10 docking runs for each molecule. The energy evaluation was set to 2.5×105, with a maximum of 27,000 generations. The mutation rate and crossover were set to 0.05 and 0.9, respectively. These parameters were chosen to efficiently explore the conformational space and identify the most favourable binding poses of the ligand within the active sites of the target proteins.To analyse the resulting docking conformers, we utilized the LIGPLOT system, which provides insights into the prevalence of intermolecular interactions within the complex structures. This analysis considered various types of interactions, including van der Waals interactions, hydrophobic effects, and hydrogen bonds formed between the ligand and the protein. Understanding these interactions is crucial, as they play significant roles in the deformation and translational and rotational entropy loss during the binding process.

In this study, the crystal structures of the targeted diabetic regulatory proteins, namely the insulin receptor (IR), glycogen synthase kinase, and glucokinase, were utilized to explore compounds that could strongly bind to their DNA binding domains. Prior to the molecular docking simulations, an analysis of the binding sites within these proteins was conducted. The receptor grid generation panel was employed to generate a grid map for each receptor, with a scaling factor of 1.0 applied.For the docking simulations, the ligands were docked using a docking protocol. This protocol allowed for the exploration of all reasonable orientations of each low-energy conformer within the specified binding site. During the docking process, the torsional degrees of freedom of the ligand were relaxed, allowing for flexibility, while the conformation of the protein remained fixed.

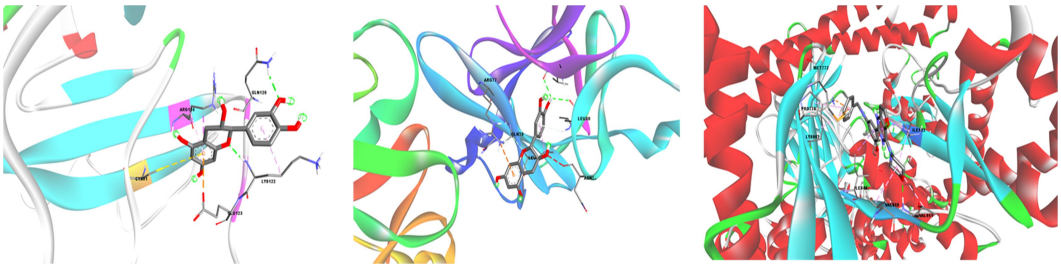

Fig 2. Molecular docking analysis of Orientin with diabetic regulating targets (IR, GSK3β, and GCK)

The molecular docking analysis yielded notable results, as summarized in Table 1. orientin demonstrated higher binding affinities with the diabetic regulating targets, with values of -6.6 kcal/mol for insulin receptor (IR), -7.2 kcal/mol for glycogen synthase kinase 3β (GSK3β), and -8.9 kcal/mol for glucokinase (GCK). These binding energy values indicate strong interactions between orientin and the target proteins.The 3D and 2D structural representations of the docking analysis, depicted in Figure 2, provided visual insights into the binding interactions. The analysis revealed the formation of hydrogen bonds between orientin and specific active site residues of the diabetic regulating targets. Notably, interactions occurred at residues such as LYS122 and GLN10 for IR, LEU30 for GSK3β, and ILE932 for GCK. These interactions play a crucial role in the binding process and contribute to the stability and specificity of the drug-target interactions.The significant binding affinities observed suggest that orientin has the potential to inhibit inflammatory activity through its interactions with the diabetic regulating targets. These findings indicate that orientin could serve as a lead compound for targeting the signaling pathways associated with diabetes, potentially offering improved therapeutic outcomes in the treatment of diabetes.

Table 1. Molecular docking analysis

S. no	Drug	Protein	Binding energy (kcal/mol)	No. of H bonds involved	Amino acid residues

1.	Orientin (5281675)	1IR3	-6.6	2	LYS122, GLN120
2.		3F7Z	-7.2	1	LEU30
3.		4IXC	-8.9	1	ILE932

CONCLUSION

In this study, we employed molecular docking analysis to investigate the interaction between the antidiabetic drug orientin and key diabetic regulatory proteins, including insulin receptor (IR), glycogen synthase kinase, and glucokinase. Based on the findings of this molecular docking study, we can conclude that targeting the diabetic regulatory proteins with orientin holds promise as a potential therapeutic strategy for managing diabetes. The strong binding affinities observed suggest that orientin may have the ability to modulate the function of these proteins and potentially influence the underlying molecular mechanisms of diabetes. Further experimental investigations are warranted to validate the therapeutic efficacy of orientin and explore its potential application in the management of diabetes.

CONFLICT OF INTERESTS

No conflict of interest from any of the authors.

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