RESEARCH ARTICLE

In-silico Study of Epicathecin from *Cinnamomum verum* Against Insulin Receptor for Diabetes Mellitus

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ABSTRACT

Obesity has become more common in recent years. Fatalism can raise the chance of fatal metabolic and cardiovascular diseases. Type 2 diabetes is one of the metabolic illnesses brought on by fat. Insulin receptor resistance, also known as non-insulin dependent type 2 diabetes, is a consequence of type 2 diabetes. Reduced insulin sensitivity and the inability of pancreatic beta cells to produce enough insulin to offset the development of resistance are the main causes of type 2 diabetes mellitus. Insulin receptor problems that decrease INSR expression on the cell surface and follow receptor abnormalities are the common mechanisms behind insulin receptor resistance. The single-nucleotide found in INSR. Increasing the expression of 4IBM is one strategy to combat its effects on insulin receptor function and increased risk of INSR-mediated illnesses. Based on earlier studies, cinnamon (*Cinnamomum verum*), a traditional plant, has been shown to have potential as a treatment for a number of illnesses, including diabetes mellitus. Using Autodock 4 and the Lamarckian genetic algorithm as a basis, this *in-silico* study intends to investigate the potential of active compounds found in cinnamon and their role as 4IBM protein inhibitors for therapy in type 2 diabetes mellitus. The binding energy ranged from -7.95 to -6.15 kcal/mol, according to docking data, with the compound epicatechin having the lowest binding energy and an inhibitory constant of 7.80. Further investigation into the active ingredients in cinnamon and their potential use in the treatment of diabetes mellitus can be based on the findings of this study.

Keywords: Epicatechin, *Cinnamomum verum*, Diabetes mellitus, 4IBM, Autodock 4.

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INTRODUCTION

The prevalence and severity of obesity increased to 42.4 and 9.2%, respectively, in 2017–2018 compared to 1999–2000.[1,2] A complex interaction of cultural, social, and genetic factors influences the condition of obesity. Obesity can increase one's chance of dying from metabolic and cardiovascular disorders.[3,4] Obesity is associated with the following conditions: dyslipidemia, ischemic heart disease, hypertension, non-alcoholic fatty liver disease (NAFLD), and type 2 diabetes mellitus (DM). Insulin deficit is often connected with insulin resistance, which is a subtype of type 2 diabetes.[5] Insulin resistance can also be referred to as not being insulin dependent. Type 2 diabetes is primarily caused by insulin resistance, which is typified by reduced insulin sensitivity

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and inadequate insulin production by the pancreatic beta cells. Increasing insulin production is required in order to bring blood sugar levels down.[6,7]

Blood glucose normalization, concurrent disease management, and lifestyle modification are the current methods of treating type 2 diabetes. The first line of treatment for insulin resistance in the liver and peripheral tissues is metformin. Another drug that reduces insulin resistance is called glitazone, which works as a PPAR-c agonist. On the other hand, glitazone treatment regimen adverse effects include subcutaneous fat accumulation.[8,9]

Flaws in the insulin receptor that reduce INSR expression on the cell surface and flaw in the post-receptor that lead to inadequate signal transduction are often the cause of resistance to the insulin receptor. These abnormalities in the receptor lead to insulin receptor resistance. The single nucleotide 4IBM in the INSR affects insulin receptor activation and increases the likelihood of INSR-mediated disease. One way to go around this is to up the expression of 4IBM.[10,11]

Cinnamomum verum, the traditional plant used as cinnamon, is widely utilized as a treatment to treat a variety of ailments. Cinnamon may be utilized to treat diabetic mellitus, according to earlier research. By stimulating insulin absorption through the activation of insulin receptor kinase, autophosphorylation of insulin receptors, and the production of glycogen, the active compounds in cinnamon have the power to regulate blood sugar levels.[12] One of the active components of cinnamon is epicathecin. Epicathecin helps people with diabetes mellitus reduce their blood glucose levels. This study aims to evaluate cinnamon's epicathecin content and potential as a 4IBM protein inhibitor for type 2 diabetes *in-vitro* therapy.[13,14]

By simulating the atomic-level interaction between a small molecule and a protein using the molecular docking technique, we can gain a deeper understanding of basic biological processes and clarify small molecule behavior at target protein binding sites. Predicting the ligand structure, as well as its orientation and location inside these sites (also known as pose), and determining the binding affinity are the two main steps in the docking process. The theoretical portion will address the sample methods and scoring schemes that are the focus of these two procedures.[15,16]

Knowing where the binding site is before docking procedures start considerably improves docking efficiency. It is true that before ligands are docked into a binding site, information about it is often available. Comparing the target protein to other proteins in the same family or to proteins that have co-crystallized with other ligands can also reveal details about the locations. Docking without assuming anything about the binding position is known as blind docking.[17]

Fischer's lock-and-key theory, which postulates that the ligand fits into the receptor like a lock and key, offers an early explanation for the process of ligand-receptor interaction. This concept served as the foundation for the earliest known docking techniques, which viewed the ligand and receptor as solid entities. The lock-and-key theory is then expanded upon by Koshland's "induced-fit" theory, which contends that interactions between ligands and proteins continuously modify the active region of the protein. This idea states that the ligand and receptor should be seen as flexible during docking. As such, compared to the rigorous treatment, it may offer a more precise description of the binding events.[15] Due of the constraints of computer resources, docking which involves a flexible ligand and a rigid receptor remains the most extensively utilized technique. Despite the abundance of recent initiatives to address the flexibility of the receptor, flexible receptor docking, and more specifically the flexibility of the backbone in receptors, continues to be a major difficulty for the docking techniques that are now in use. The local move Monte Carlo (LMMC) approach is one that we, the authors of this work, offer as a possible answer to challenges involving flexible receptor docking.[12-18]

MATERIALS AND METHODS

Software

The software for simulating molecular modeling is called AutoDock. It works particularly well for protein-ligand docking. You can download AutoDock 4 under the GNU General Public License. Among docking software programs, AutoDock is frequently cited in the scientific literature.[12-15]

Protein Selection

Using PDB ID: 4IBM, the protein structure (Figure 1) of the insulin receptor was retrieved from the protein data bank (PDB). Protein and nucleic acid experimental structures can be found in the PDB database. There are two chains in the structure of the 4IBM protein: chain A and chain B. Only chain A was employed for docking in this experiment. The free program Autodock 4 is used to compute protein molecule binding affinities in order to conduct molecular research.[18]

Insulin receptor (IR) and insulin-like growth factor 1 receptor (IGF-1R) dual inhibitors are closely related receptor tyrosine kinases that are being studied as potentially useful therapeutic drugs for the treatment of cancer. In this article, we discuss a category of dual inhibitors that are extremely selective with regard to IGF-1R and IR.[18] These inhibitors were identified as possible targets by concurrently screening a panel of 300 human protein kinases with known kinase inhibitors. Based on biochemical and structural analyses, it is believed that this class's remarkable selectivity stems from its capacity to attach

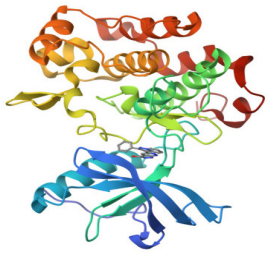

Figure 1: Structure of protein

to the ATP-binding pocket of an inactive and unphosphorylated IGF-1R/IR while simultaneously maintaining the activation loop in an inactive conformation resembling that of a native protein.[26] The research is based on this hypothesis. In contrast to IR/IGF-1R, which has a different structure when it is unphosphorylated or inactive, one of the drugs in this class was initially discovered to be an inhibitor of the serine/threonine kinase ERK. The reason this drug works so well against these two physically different kinase families is that it surprisingly interacts with the ATP-binding pocket of ERK in a completely different conformation than that of IGF-1R/IR. These results point to a unique direction for polypharmacology, wherein a single medication that targets distinct conformations of each target kinase can block two or more unrelated kinases.[17,18]

Ligand Selection

The active compounds found in cinnamon were the ligands that were searched with Pubchem (https://Phytochem.nal.usda.gov/Phytochem/search). Epicathecin, the active ingredient found in considerable amounts in cinnamon, was consumed. Epicathecin's molecular structure (Figure 2) was obtained from Pubchem (Pubchem CID: 72276).

Molecular Docking

Swiss ADME was used to examine the ligands to be employed for their potential as oral medicines based on Lipinski's rule of five. A website called Swiss ADME can be used to evaluate a compound's pharmacokinetics and drug-likeness. Four solubility and permeability-related characteristics are included in Lipinski's rule: molecular weight, log P, number of hydrogen bond donors, and number of acceptors. Ligands exhibiting transgressions of two or more Lipinski rules were excluded from this investigation.[14-17]

Identification of Active Site

With the use of the computed atlas for surface topography of proteins (CASTp), amino acids implicated in the creation of active sites were identified. On protein structures obtained from PDB, the location and orientation of active sites can be ascertained using the CASTp website. Before docking, the grid box's position is ascertained using this active site determination method.

Ligand Preparation

The 3D conformation of the ligand structure was obtained and saved in SDF format. The Avogadro program was then used to optimize the geometry. The ligand was then transformed into PDBQT format so that Autodock 4 program could process it further. Both non-polar hydrogen incorporation and polar hydrogen addition were done.

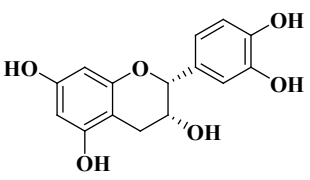

Figure 2: Structure of epicatechin

Protein Preparation

Using Autodock 4, extraneous water molecules and other chains are eliminated to prepare the macromolecules that are obtained from PDB. The protein molecule then receives the addition of Kollman charge and polar hydrogen.

Validation of Target in Protein

Re-molecular tethering of the natural ligand into the active side of its receptor served as validation for the molecular tethering technique. The dimensions of the grid box are 24 x 22 x 30, and its coordinates are 3.517 Å, -10.108 Å, and 6.836 Å, in that order. The root means square deviation, or RMSD, value was used to assess the validity of the parameters used in the molecular tethering approach. If the RMSD result is less than 2.0 Å10, the molecular tethering method's validation is deemed valid.

Molecular Target Simulation

To validate the molecular tethering method's parameters, the test chemical is tethered to the receptor's active side using the same grid box and coordinates. Using the *. pdbqt file format and the Autodock 4 application, molecular docking was carried out. The Lamarckian genetic algorithm was used to carry out the docking process. The analysis's output takes the shape of binding patterns with additional amino acid residues on the receptor's active side, hydrogen bonding, and bond-free energy. The protein-ligand complex shape that has been chosen for additional examination is the one with the lowest binding free energy value. The strongest bond between the ligand and protein is indicated by the data with the highest negative ΔG. The gathered data on the test ligand is contrasted with that of the natural ligand. PyMol software was used to view the docking results in ".pdbqt" format, which were subsequently converted to ".pdb" using BIOVIA Discovery Studio 2019 software.

RESULTS AND DISCUSSIONS

This study uses proteins in the insulin receptor, which plays a role in the regulation of glucose levels in the blood. The structure of the protein used was taken from the PDB. The protein has two monomeric chain arrangements, namely the A and B chains. Only the A chain of the insulin receptor (PDB-ID: 4IBM) and the active ingredients in cinnamon which were sourced from the Pubchem website—were evaluated in this investigation. (-) epicatechin was the active component found in the cinnamon bark under investigation. Lipinski's rule of five was used to evaluate this compound's viability as a treatment even more. Among the metrics evaluated are molecular weight, logP, the number of donors and acceptors of hydrogen bonds, and the quantity of donors of hydrogen bonds. During the investigation, no violations of Lipinski's rule of five were found (Table 1).

The next step is identifying the insulin receptor protein's active site, which is done with the use of the CASTp website (Figure 3). The location of the active site occupied by the INSR amino acid is obtained from CASTp and will be utilized as a guide for the placement of grid boxes in Autodock 4.

Table 1: Drug likeness of study epicatechin

Chemical constituent	*Molecular weight (MW)*	*Hydrogen bond acceptor (HBA)*	*Hydrogen bond donor (HBD)*	*Log P (MLOGP)*
(-) Epicathecin $C_{15}H_{14}O_6$	290.30 g/mol	6	5	0.240

Table 2: Molecular docking of ligands with INSR protein

Compound	Binding Energy (kcal/mol)	Inhibitory Constant (μM)	Van der Waals Bond	Cluster RMSD
(-) Epicathecin	-7.95	7.80	-	0.00

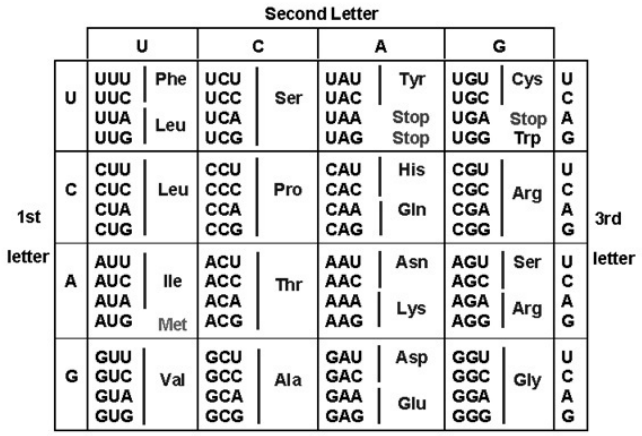

Figure 3: Amino acids involved in the active site of the INSR gene

According to the docking outcome between the protein and ligand (Table 2) using Autodock 4, the binding energy varied between -7.95 kcal/mol and 7.80 μM for the inhibitory constant (Figure 3). Research has shown that epicathecin may have a number of health benefits, including lowering blood glucose levels in diabetics and acting as an antioxidant, antiangiogenic, and antiproliferative effect on cancer cells. Pancreatic β islet cells' capacity can be increased and the degradation process can be minimized by material containing epicathecin.

CONCLUSION

Along with the rise in other metabolic disorders, the number of patients with diabetes mellitus is rising. Normal glucose levels have not yet been achieved with type 2 diabetes treatments. Thus, the creation of novel treatments is required. Inducing INSR to become more sensitive is one such approach that may lower blood glucose levels. A plant with many active compounds and therapeutic qualities, cinnamon is used in traditional medicine. Compound (-) epicatechin was discovered to have a good potential to elicit INSR activation in this study. More *in-vitro* and *in-vivo* research is anticipated to better investigate how (-) epicathecin affects type 2 diabetes.

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