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Antidiabetic Activity of Allin Isolated from *Allium Sativum*: Role of P13K/AKT Signalling

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Abstract

This study presents a comprehensive molecular docking analysis aimed at elucidating the potential antidiabetic activity of allin, a compound isolated from Allium sativum (garlic). Through computational modelling, we investigated the binding interactions between allin and Rheb, revealing that allin exhibited the lowest binding affinities with a binding energy of -3.24 kcal/mol. The docking results unveiled a significant role of the P13K/AKT signalling pathway in mediating the antidiabetic effects of allin. The interaction between allin and Rheb was characterized by the establishment of a single hydrogen bond involving SER-16 and GDP-201. This interaction contributed to the formation of a binding pocket encompassing key residues such as PRO-37, ARG-15, SER-16, THR-88, LEU-123, GLU-126, and GDP-201. The molecular docking analysis sheds light on the intricate molecular mechanisms underlying the antidiabetic potential of allin, providing insights into its specific interactions with key signalling components. Furthermore, our findings suggest a potential modulation of the P13K/AKT signalling pathway by the allin, emphasizing its significance in the context of antidiabetic activity. The results of this study suggest contributes valuable information to the understanding of the molecular basis of allin's therapeutic potential and provides a foundation for further experimental validations and exploration of its application in diabetes management.

Keywords: *Allin, Allium sativum (garlic), Antidiabetic Activity, Diabetes Management, Health and Well-being, Novel Methods, Public Health, P13K/AKT Signalling Pathway, Rheb.*

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by a sustained elevation

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in blood glucose levels, ensuing from defective insulin production, resistance to insulin, or even both. The condition poses one of the essential global health challenges, while its increased prevalence contributes to a series of complications that include cardiovascular disease, neuropathy, nephropathy, and retinopathy. Treatment options are essentially based on insulin replacement, oral hypoglycemic agents, and changes in lifestyle. Although these approaches are effective, they are often associated with drawbacks such as side effects and potential hypoglycemia. Thus, there is an emerging demand for antidiabetic agents with less side effects, which presently results in increased research into natural products, of which allin, a compound extracted from *Allium sativum*, commonly referred to as garlic, is one. Traditionally, garlic was used for its different health benefit attributes [1]. It contains an array of bioactive compounds, including allicin, allin, ajoene, and allyl disulfide, which are being exhaustively analyzed for their therapeutic properties [2]. Allin, alias S-allyl cysteine sulfoxide, is attracting much attention due to its antidiabetic effects, caused by antioxidant, anti-inflammatory, and hypoglycemic activities [3]. Allin is likely to play a very key role in the overall health benefits from garlic consumption since it is the stable precursor of allicin. The role of garlic in diabetes has similarly been well-documented in various studies. For example, some research has established that garlic extracts have potentially huge reductions in serum glucose, cholesterol, and triglyceride levels in diabetic animal models [4]. In one study, it was observed that garlic extract administration exhibited a more potent dose-dependent reduction in blood glucose levels compared with the standard antidiabetic drugs, including glibenclamide [5]. The finding thus has the potential for assuming a complementary therapy in diabetes management, whereby therapy directed toward improving glycemic control could reduce the risk of diabetes complications. Of interest are the molecular mechanisms underlying the antidiabetic properties of allin, especially in relation to the phosphatidylinositol-3-kinase/Akt signaling pathway. This pathway is thus considered to be the major regulator of glucose homeostasis and insulin signaling and, therefore, a prime target for understanding how allin exerts its effects. Activation of the PI3K/Akt pathway has been linked to an improvement in insulin sensitivity associated with increased glucose uptake in peripheral tissues [6]. The interaction of allin with this signaling pathway, therefore, would be quite useful to be researched further for its potential as an antidiabetic agent. Apart from direct effects on glucose metabolism, garlic and its constituents have been found to exert antioxidant effects that can modulate oxidative stress, one of the causative factors for pathogenesis of diabetes. Oxidative stress leads to damage to the cell and subsequently causes inflammation, aggravating insulin resistance and impairing pancreatic function [7]. Allin could contribute to the preservation of pancreatic beta-cell function and improvement of insulin secretion by reducing oxidative stress. Pharmacological activities of garlic do not stop there at its antidiabetic effects. Literature available reports on its potential in managing hyperlipidemia, hypertension, and even certain types of cancer [8]. The diversity of biological activities attributed to garlic compounds underpins their therapeutic potential and points toward additional research required in this area. Considering the increasing prevalence of diabetes and limited efficacy of available therapies, investigating natural products such as allin isolated from *Allium sativum* could turn out to be a perspective direction in the search for new antidiabetic therapies. The targeting of the PI3K/Akt signaling pathway will give insight into the mechanisms via which allin exerts its activity, opening the scope for future studies that might result in

effective, safe, and more accessible treatments for diabetes. In a nutshell, the antidiabetic activity of the compound allin isolated from *Allium sativum* presents a very attractive field of study that needs further research [9]. Such potential of allin to improve glucose metabolism, reduce oxidative stress, and enhance insulin sensitivity through modulating the PI3K/Akt pathway makes it a very valuable candidate for the management of diabetes. In view of the ever-increasing burden of diabetes across the world, exploring the therapeutic potential of natural products like garlic might open up new opportunities for finding newer solutions to this important health challenge. Future studies should be directed toward the elaboration of the detailed mechanism of action of allin and its efficacy trials in a clinical setup while aiding in the development of some relatively safer and more effective antidiabetic drugs [10].

Materials and Methods

To investigate potential interactions between allin and key proteins in the PI3K/Akt pathway, we employed molecular docking analysis. We retrieved the 3D structures of allin and target proteins, including phosphoinositide 3-kinase (PI3K) and protein kinase B (Akt), from the Protein Data Bank (PDB). Autodock, a widely-used docking software, was used for the docking simulations. The Lamarckian Genetic Algorithm was applied to search for the best binding conformations and estimate binding affinities. Molecular docking is a computational technique that predicts the binding of one molecule to another, such as a ligand to a receptor, by simulating the physical forces between them. In this study, we focused on the PI3K/Akt pathway, which is a crucial signalling pathway involved in cell growth, survival, and proliferation. Identifying potential interactions between allin and key proteins this study aimed to contribute valuable insights into the potential modulatory roles of allin within the intricate molecular network of the PI3K/Akt signalling pathway.

Preparation of Ligand

Allin (CID 154496136) 3D chemical structure was obtained from the PubChem database. The chemical structure was downloaded in SDF file format and then converted to PDB file format using an online translator. Finally, using Auto Dock Tool for additional analysis resulted in a change to the ligand format.

Preparation of Receptor

In the preparation of receptor Phosphoinositide 3-kinase O15530, AKT (Protein kinase B) Q9Y243, Phosphatase P53041, Mtor (mammalian target of rapamycin) P42345 , GSK-3 (Glycogen synthase kinase 3) P49840, Phosphoinositide dependent kinase-2 Q14289, Rheb (Ras homolog enriched in brain)-Q15382 in this preparation of the receptors from uniport protein data bank and the allin 3D chemical structure were obtained from the Pubchem database the structure of the protein was downloaded in SDF format and they are converted into PDF formatted by the google translator.

Active Site Indicator

The binding site identification was carried out using the CSATp server, which is capable of recognizing the atoms that line pockets, pocket openings, and hidden cavities. This server also provides information on the quantity and location of pockets and cavities, as well as the placement and width of mouth openings.

Docking

Auto Dock Tools were used to dock the obtained molecules. The binding energies on the macromolecule coordinates were subsequently assessed through the generation of three-dimensional grid boxes using the Auto Grid technique. Auto Grid was used to create

grid maps that encompassed the complete ligand at the docking target site. The binding site was eventually encircled by cubic grids once the entire ligand was inserted into it. The 4.2.6 graphical user interface of Auto Dock, made available by MGL Tools, was used to design the Auto Dock atom kinds. It was decided to use the Lamarckian genetic algorithm [11], one of the most efficient docking methods that Auto Dock has on hand. Auto Dock was utilised to compute and evaluate the binding free energy and the optimal conformation fit of a ligand within the macromolecular structure. Understanding the nature of has advantages through technique.

Results

Interaction Between Akt and Allin

Our result highlighted that allin exhibited significant binding affinities between allin and AKT, with a substantial binding energy of -4.94 Kcal/Mol, as delineated in (Table 1). Notably, the interaction between allin and AKT forms a binding pocket, involving critical amino acids such as ILE-6, VAL-7, LYS-8, GLU-9, TYR-26, ARG-41, and GLU-98, as illustrated in (Figure 1). Furthermore, the involvement of superoxide dismutase in this interaction is characterized by the establishment of a three-hydrogen bond interaction, engaging residues GLU-98, LYS-8, and GLU-9. These molecular insights shed light on the intricate interplay between allin and AKT, providing valuable information for further exploration and understanding of their functional dynamics.

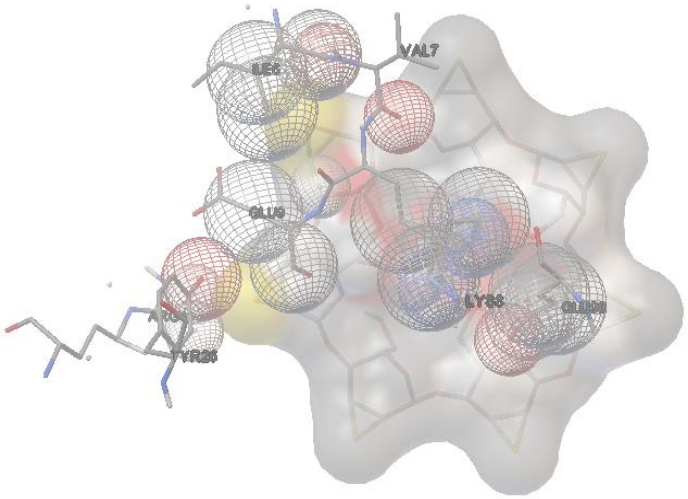

Figure 1. Schematic Representation of Akt Interaction with Allin

Interaction between mTOR and Allin

In our investigation, the findings revealed significant binding affinities between allin and mTOR, as evidenced by a notable binding energy of -6.16 Kcal/Mol (Table 1). The mTOR displayed a robust interaction through the establishment of a four-hydrogen bond network involving specific amino acid residues, namely GLU-2053, GLU-2060, GLN-2083, and ARG-2087. Further analysis indicated that mTOR exhibited the highest binding affinity towards allin, resulting in the creation of a distinct binding pocket. This pocket was characterized by the involvement of key residues, including GLU-2053, HIS-2056, ALA-2057, GLU-2060, MET-2080, GLN-2083, GLU-2084, and ARG-2087 (refer to Figure 2).

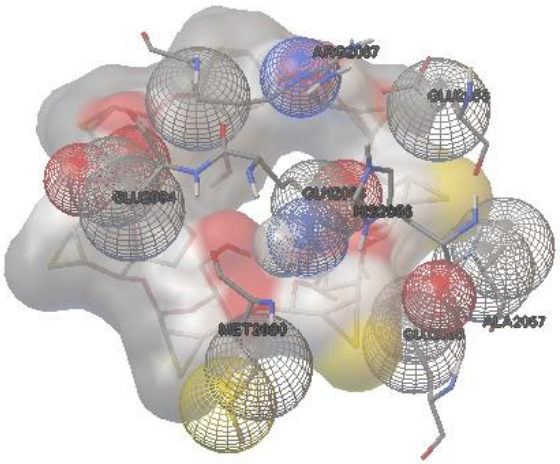

Figure 2. Schematic Representation of mTOR Interaction with Allin

Interaction between Phosphoinositide 3-Kinase and Allin

In our study, we found compelling evidence indicating that allin, a substance under investigation, demonstrated noteworthy binding affinities towards Phosphoinositide 3-kinase (PI3K). The calculated binding energy of -5.95 Kcal/Mol, as presented in (Table 1), underscores the strength of this interaction. Further analysis revealed a specific molecular mechanism involving the formation of a binding pocket. This pocket, crucial for the interaction with PI3K, is composed of key amino acids, namely GLN-73, LYS-77, ARG-106, SER-135, LEU-137, ASP-138, LYS-144, and TYR-146, as illustrated in (Figure 3). Notably, a singular hydrogen bond interaction was established between allin and PI3K, with GLN-73 playing a pivotal role. These findings contribute valuable insights into the molecular interactions underlying the observed binding affinities and shed light on the potential pharmacological implications of allin in modulating PI3K activity.

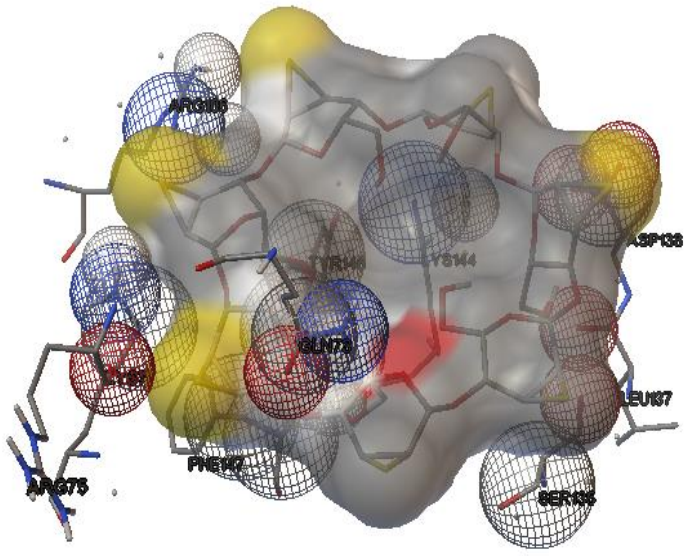

Figure 3. Schematic Representation of Phosphoinositide 3-kinase Interaction with Allin

Interaction between Phosphoinositide Dependent kinase-2 and Allin

The study revealed compelling findings regarding the interaction between Allin and Phosphoinositide dependent kinase-2 (PDK2). The results demonstrated significant binding affinities, as evidenced by a binding energy of -5.59 Kcal/Mol, as detailed in Table 1. Specifically, the interaction between Allin and PDK2 involved the establishment of a one-hydrogen bond interaction. This bonding event implicated the active participation of LYS-646, contributing to the formation of a distinctive binding pocket. The pocket, encompassing PRO-652, TYR-655, PRO-645, LYS-646, and ASP-648, was visualized in (Figure 4). These findings provide valuable insights into the molecular dynamics and binding characteristics of Allin with PDK2, shedding light on potential therapeutic implications.

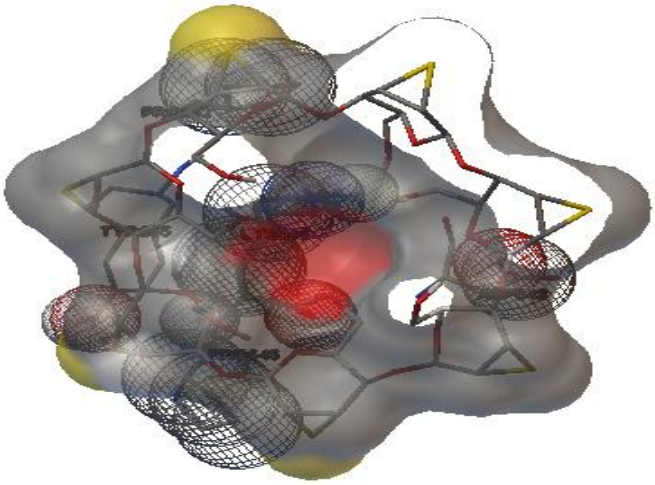

Figure 4. Schematic Representation of Phosphoinositide Dependent kinase-2 Interaction with Allin

Interaction between Phosphoinositide Dependent kinase-1 and Allin

The research findings indicate a noteworthy binding affinity of the compound allin towards Phosphoinositide dependent kinase-1, as evidenced by a significant binding energy of -6.05 Kcal/Mol, as detailed in (Table 1). The interaction with Phosphoinositide dependent kinase-1 involves the establishment of a Three-hydrogen bond interaction. This interaction is facilitated by TRP-20, GLY-101, and ASP-124, resulting in the creation of a binding pocket. The constituents of this binding pocket include ASP-124, CYS-123, TRP-20, ASP-16, ALA-70, CYS-100, GLY-101, VAL-102, and ARG-103, as depicted in (Figure 5). These findings shed light on the molecular mechanisms underlying the interaction between allin and Phosphoinositide dependent kinase-1, providing valuable insights for further study.

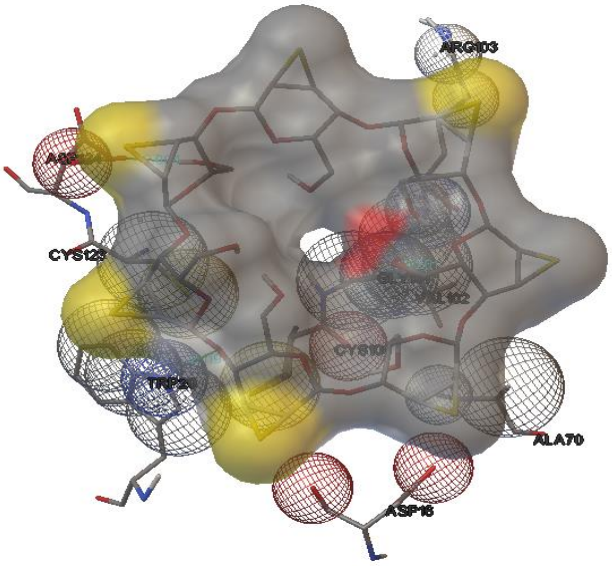

Figure 5. Schematic Representation of Phosphoinositide Dependent kinase-1 Interaction with Allin

Interaction between Phosphatase and Allin

In our study, the analysis revealed that allin demonstrated the lowest binding affinities towards Phosphatase, as evidenced by a binding energy of +5472.94 Kcal/Mol (Table 1). This interaction was characterized by the establishment of a one-hydrogen bond involving ASN-67. Consequently, a binding pocket was formed, encompassing key residues such as GLU-29, THR-33, ASN-36, ASP-37, LYS-40, ARG-74, ILE-63, ASN-67, LEU-70, TYR-95, LYS-97, and ARG-101 (illustrated in Figure 6). These findings shed light on the molecular intricacies of the interaction between allin and Phosphatase, providing valuable insights into the specific amino acid residues involved in the binding process.

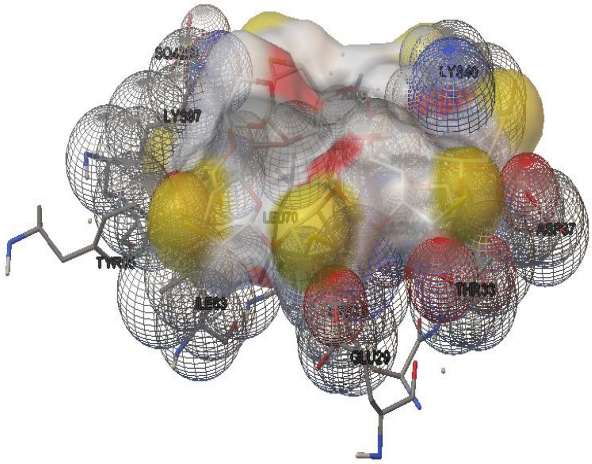

Figure 6. Schematic Representation of Phosphatase Interaction with Allin

Interaction between Rheb and Allin

The findings of our study revealed that allin demonstrated the least binding affinities towards Rheb, as evidenced by a binding energy of -3.24 kcal/mol, as outlined in (Table 1). The interaction between Rheb and allin was characterized by the establishment of a single hydrogen bond. This interaction was facilitated by the involvement of specific amino acid residues, namely SER-16, and GDP-201,

leading to the creation of a binding pocket. Noteworthy constituents of this pocket included PRO-37, ARG-15, SER-16, THR-88, LEU-123, GLU-126, and GDP-201, as illustrated in (Figure 7). The intricate interplay between these molecular components highlights the nuanced nature of the binding mechanism.

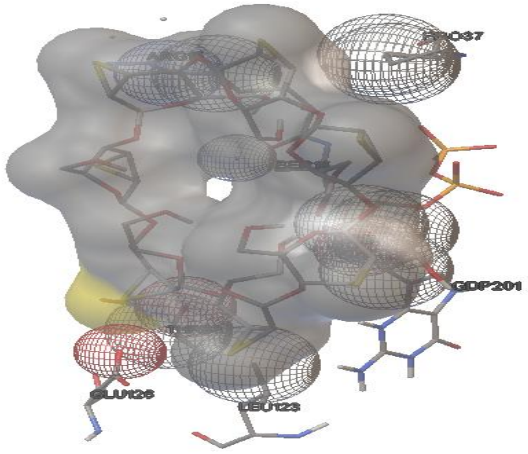

Figure 7. Schematic Representation of Rheb Interaction with Allin

Table 1. The Target Molecule and Compound Binding Energies and Amino Acid Interaction.

S.No.	Target Molecules	Compound	Binding Energy	Amino Acid Interacted	Bond Formed
01.	AKT	Allin	-4.94	ILE-6, VAL-7, LYS-8, GLU-9, TYR-26, ARG-41, GLU-98	3 H BOND GLU-98, LYS-8, GLU-9
02.	mTOR	Allin	-6.16	GLU-2053, HIS-2056, ALA-2057, GLU-2060, MET-2080, GLN-2083, GLU-2084, ARG-2087	4-H, GLU-2053, GLU-2060, GLN-2083, ARG-2087
03.	Phosphoinositide 3- kinase	Allin	-5.95	GLN-73, LYS-77, ARG-106, SER-135, LEU-137, ASP-138, LYS-144, TYR-146	1 H, GLN-73
04.	Phosphoinositide dependent kinase 2	Allin	-5.59	PRO-652, TYR-655, PRO-645, LYS-646, ASP-648	1-H, LYS-646
05.	Phosphoinositide dependent kinase 1	Allin	-6.05	ASP-124, CYS-123, TRP-20, ASP-16, ALA-70, CYS-100, GLY-101, VAL-102, ARG-103	3- H, TRP-20, GLY-101, ASP-124
06.	Phosphatase	Allin	+5472.94	GLU-29, THR-33, ASN-36, ASP-37, LYS-40, ARG-74, ILE-63, ASN-67, LEU-70, TYR-95, LYS-97, ARG-101	1-H, ASN-67

07.	Rheb	Allin	-3.24	ARG-15, SER-16, PRO-37, THR-88, LEU-123, GLU-126, GDP-201	2-H, SER-16, GDP- 201

Discussion

Molecular docking techniques were employed in this study, with Auto Dock version 4.2.6 software, to investigate potential in the field of drug discovery, molecular docking analysis is a useful tool to assist researchers predict small molecules will interact with target proteins. In the context of identifying the antidiabetic activity of Allin isolated from *Allium sativum*, molecular docking can be employed to understand the potential of Allin in modulating the P13K/Akt signalling pathway, which is known to play a crucial role in glucose metabolism and the development of diabetes. By utilizing in silico approaches, researchers can gain insights into the binding affinity and interactions of Allin with the P13K/Akt pathway components, providing a basis for further experimental validation.

The role of molecular docking in the identification of antidiabetic agents has been demonstrated in various studies. For instance, a study on the molecular docking and simulation of antidiabetic agents derived from Momordica charantia utilized in silico approaches to identify potential antihyperglycemic agents through the screening of peptides and assessment of their interactions with target proteins [12]. Similarly, in the investigation of the antidiabetic activity of the stem extract of Merremia tridentata, molecular docking was employed to identify desirable compounds for their hypoglycaemic effects, providing valuable insights for the development of potential antidiabetic agents [13]. These examples highlight the significance of molecular docking in the discovery of antidiabetic compounds, underscoring its relevance to the study of Allin's antidiabetic potential.

The PI3K/AKT pathway plays a crucial role in glucose homeostasis and is closely associated with diabetes. It is required for insulin-dependent regulation of systemic and cellular metabolism, and its imbalance can lead to the development of obesity and type 2 diabetes mellitus [14,15]. The pathway is a major effector of metabolic insulin action, and studies have shown that specific modulation of protein kinase signalling, such as AKT, can lead to insulin resistance and diabetes [16]. In the context of type 1 diabetes, the PI3K/Akt signalling pathway has been identified as a promising therapeutic target, as its upregulation can promote β-cell function, survival, and/or proliferation, thereby counteracting the large waves of apoptosis during pancreatic development [17]. The pathway is under strict control, and disturbances in its regulation, such as in insulin resistance, are associated with the development of various diseases, including diabetes [18]. Therefore, the PI3K/AKT pathway is a potential target for therapeutic interventions in diabetes.

In the specific context of the P13K/Akt signalling pathway, molecular docking can facilitate the understanding of how Allin may modulate this pathway to exert its antidiabetic effects. By virtually screening the interactions between Allin and the key proteins involved in the P13K/Akt pathway, such as P13K and Akt, researchers can assess the binding affinity, identify critical binding sites, and predict the conformation of the Allin-protein complexes. This information is valuable for elucidating the molecular mechanisms underlying Allin's potential antidiabetic activity and can guide further experimental studies [19]. The use of molecular docking in the context of antidiabetic research is not without its challenges and limitations. It is important to

acknowledge that molecular docking results are computational predictions and should be validated through experimental assays. Additionally, the accuracy of molecular docking outcomes is influenced by various factors, including the quality of the protein structure and the parameters used in the docking simulations [20]. Therefore, it is essential to interpret the docking results cautiously and complement them with experimental data to establish the antidiabetic potential of Allin and its role in modulating the P13K/Akt signalling pathway.

Conclusion

Molecular docking analysis is a valuable approach for identifying potential antidiabetic agents and understanding their interactions with target proteins, such as those involved in the P13K/Akt signalling pathway. By leveraging in silico methods, researchers can gain valuable insights into the binding mechanisms of Allin and its potential to modulate the P13K/Akt pathway, laying the groundwork for further experimental investigations. However, it is important to interpret the docking results in the context of their inherent limitations and to validate the findings through experimental studies to fully elucidate the antidiabetic activity of Allin and its implications for the P13K/Akt signalling pathway.

Conflict of Interest

The author hereby declares that there is no conflict of interest.

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