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4-Hydroxyisoleucine as a Natural DPP-4 Inhibitor for Diabetes

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Abstract

Research on 4-hydroxyisoleucine, a natural compound found in several plant sources, shows potential as an antidiabetic agent through inhibiting the DPP-4 (dipeptidyl peptidase-4) enzyme. This study evaluates the pharmacokinetic potential and toxicity profile of 4-hydroxyisoleucine as a therapeutic agent. ADME (Absorption, Distribution, Metabolism, Excretion) analysis indicates that this compound has good gastrointestinal absorption, moderate water solubility, and limited penetration across the blood-brain barrier, which reduces the risk of central nervous system side effects. The toxicity profile of 4-hydroxyisoleucine reveals low hepatotoxicity, with no indications of mutagenicity or carcinogenicity. The LD50 value greater than 2000 mg/kg places this compound in Toxicity Class 5, indicating low toxicity. Based on in silico evaluation results, 4-hydroxyisoleucine has potential as an effective natural DPP-4 inhibitor, with stable binding mechanisms, even though its binding affinity is lower than synthetic inhibitors. With favorable pharmacokinetic properties and a beneficial safety profile, 4-hydroxyisoleucine has the potential to be developed as a natural therapeutic agent for diabetes management.

Keywords: 4-Hydroxyisoleucine; ADME; Toxicity Profile; DPP-4; Antidiabetic.

Abbreviations: DPP-4: Dipeptidyl-peptidase 4; GIP: Glucose-Dependent Insulinotropic Polypeptide; GLP-1: Glucagon-Like Peptide-1

INTRODUCTION

A major therapeutic target in the management of Type 2 diabetes mellitus (T2DM) is the enzyme Dipeptidyl Peptidase-4 (DPP4), which is responsible for degrading incretin hormones such as Glucagon-Like Peptide-1 (GLP-1) and Glucose-Dependent Insulinotropic Polypeptide (GIP). These hormones stimulate insulin secretion in response to glucose intake. Synthetic DPP4 inhibitors have been widely used to enhance incretin levels, improve glycemic control, and minimize the risk of hypoglycemia. However, synthetic DPP4 inhibitors, such as alogliptin, linagliptin, saxagliptin, sitagliptin, and vildagliptin, are often associated with side effects, including gastrointestinal disturbances, joint pain, and an increased risk of respiratory infections (Azriful et al., 2018).

The introduction of an in silico analysis of 4-hydroxyisoleucine as a natural antidiabetic agent focusing on its interaction with Angiotensin-converting enzyme type 2 (DPP-4) and pharmacokinetic evaluation serves to highlight the compound's potential therapeutic value. Diabetes mellitus, a metabolic disorder marked by elevated blood glucose levels, is increasingly prevalent worldwide and poses significant public health challenges. One of the key targets in the management of diabetes is the DPP-4 enzyme, which plays a pivotal role in regulating insulin secretion and glucose metabolism. Inhibiting DPP-4 has emerged as a promising therapeutic approach for controlling blood glucose levels in diabetic patients. 4-hydroxyisoleucine, a naturally occurring amino acid derivative primarily found in fenugreek seeds, has been shown to possess antidiabetic properties through its effects on glucose metabolism.

This study aims to explore the in silico interaction of 4-hydroxyisoleucine with DPP-4, a critical enzyme involved in glucose homeostasis. By utilizing advanced computational tools and molecular docking techniques, we aim to assess how 4-hydroxyisoleucine binds to the DPP-4 receptor, potentially inhibiting its activity and contributing to better glycemic control. Additionally, pharmacokinetic evaluations are integral to understanding the compound's bioavailability, absorption, distribution, metabolism, and excretion (ADME) properties. This aspect is crucial to determine the compound's potential for effective oral administration and safety profile (Anitha et al, 2013).

SwissADME and other computational platforms predict the pharmacokinetic properties of 4-hydroxyisoleucine, providing insights into its solubility, permeability, and potential toxicity. In summary, this study explores 4-hydroxyisoleucine's dual role as a natural DPP-4 inhibitor and evaluates its

pharmacokinetic characteristics to establish its viability as a potential therapeutic agent for diabetes management. The findings could pave the way for further research and development of 4-hydroxyisoleucine-based formulations in treating diabetes, offering a promising natural alternative to synthetic drugs (Itoh et al, 2019).

MATERIALS AND METHODS

Study area

For Molecular docking process, this study used a computer with specifications: Intel(R) Celeron(R) N4020 CPU @ 1.10GHz, 1101 Mhz, 2 Core(s), 2 Logical Processor(s), 4 GB RAM, and operating system: Microsoft Windows 11 Home Single Language (10.0.22631 Build 22631) BIOS: DVCN17WW

Procedures

Preparation and Ligands

The primary compound analyzed in this study was 4-hydroxyisoleucine, a natural glycoside derived from fava beans (*Vicia faba*). The 2D and 3D structures of 4-hydroxyisoleucine were obtained from the PubChem database (CID: XXXX) in SDF format. The target protein, Dipeptidyl Peptidase-4 (DPP4), was retrieved from the Protein Data Bank (PDB ID: XXXX) in PDB format. The protein and ligand structures were prepared using PyMOL software (Made & Pathni, 2018).

Water molecules were removed from the protein structure to ensure accurate molecular docking, while polar hydrogens and Kollman charges were added to stabilize the protein-ligand interaction (Nayeem et al., 2021). The ligand structure was optimized for energy minimization using Open Babel within PyRx software to eliminate steric clashes and maintain proper molecular geometry.

The prepared protein and ligand files were saved in PDBQT format, making them compatible with AutoDock Vina. These prepared structures were then used for the docking process to analyze the binding affinity of 4-hydroxyisoleucine to DPP4 and compare it to standard synthetic inhibitors, including alogliptin, linagliptin, saxagliptin, sitagliptin, and vildagliptin (Pantaleão et al., 2018).

Protein Validation

In an *silico* analysis for protein validation after the preparation of the protein and ligand, several steps are followed to ensure that the obtained protein structure and its interaction with the ligand are accurate and acceptable for further studies. Initially, after the protein structure is prepared, software tools such as PyMOL for visualization and structural analysis are used. This visual inspection aims to confirm no visible structural defects, such as irregular loops or improper bindings. Following this, the quality of the prepared protein structure is assessed using tools like PROCHECK, which help verify the validity of the protein structure based on geometric statistics and the distribution of phi-psi angles.

While statistical analyses such as Ramachandran plots are commonly used for further evaluation, the focus is on ensuring the protein model used in the simulation is reliable. Additionally, the protein structure's interaction energy is assessed to ensure that the protein is in a stable state. Molecular dynamics simulations or protein-ligand binding energy predictions using software like AutoDock are used to verify that the interaction with the ligand remains within biologically acceptable parameters. The protein structure is then refined through energy optimization procedures, where atom positions are adjusted to minimize the total energy, enhancing the model's accuracy and ensuring it represents the natural protein form more precisely (Samata et al., 2019).

Finally, the stability of the protein after ligand interaction is evaluated by conducting molecular dynamics (MD) simulations to assess the structure's stability under biologically relevant conditions. This involves examining the MD simulation trajectories to ensure the validated protein structure remains stable over a set period. These steps are essential in the protein validation process within in silico analysis, ensuring that the structure used in experiments can predict ligand interactions accurately and support further analysis regarding the desired biological activity.

Molecular Docking Process

Molecular docking simulations were performed using PyRx, an open-source software integrating AutoDock Vina. This study focused on calculating the binding affinity of 4-hydroxyisoleucine to the active site of DPP4. Grid box parameters were set to encompass the entire binding site of DPP4, ensuring comprehensive coverage for ligand-receptor interactions.

The docking process began with loading the prepared protein and ligand files into PyRx. The protein was fixed as rigid, while the ligand was treated as flexible to allow for accurate conformational adjustments during docking. Docking results were generated as binding affinity values in kcal/mol, which reflect the stability of the protein-ligand complex (Suciana & Arifianto, 2019)

Post Docking Visualization

Post-docking visualization is a crucial step after completing the docking process. For instance, after docking the protein and ligand using a tool like PyRx, the resulting data is saved for further analysis. This data, typically containing the docking poses and binding energies, is then visualized using PyMOL to allow better understand the interaction between the ligand and the protein.

By reloading the docking results in PyMOL, the researcher can easily identify key details, such as the types of bonds formed (hydrogen bonds, hydrophobic interactions, or ionic bonds) between the ligand and the

protein. This visualization helps determine the precise binding site on the protein, the orientation of the ligand within the binding pocket, and the strength of the interactions, which are crucial for evaluating the potential efficacy of the ligand as a therapeutic agent.

RESULTS AND DISCUSSION

Ligand and Protein Preparation

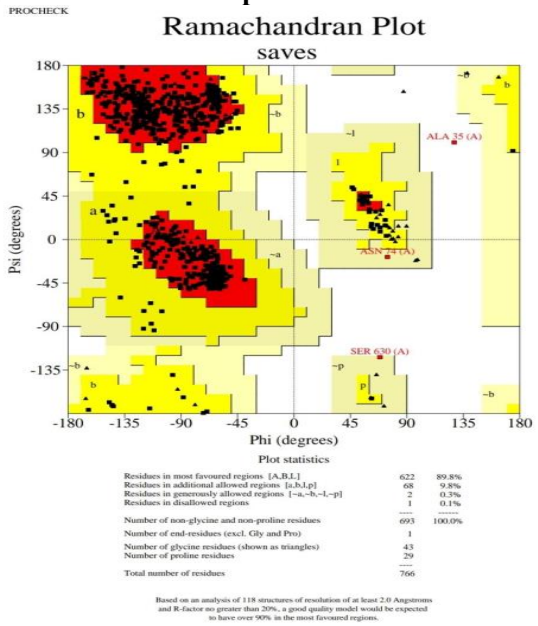

Figure 1. The Ramachandran PROCHECK.

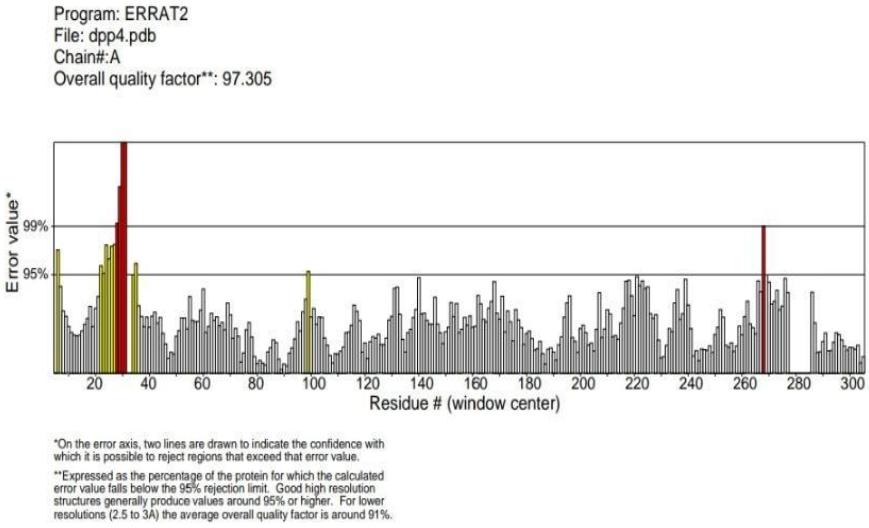

Figure 2. Structure of ERRAT Tools.

Before beginning the molecular docking studies, the ligand and the protein (DPP4) were prepared. The 3D structure of 4-hydroxyisoleucine was obtained from the PubChem database. The optimized structure was minimized to ensure the ligand's conformation was energetically favorable for docking. For the protein preparation, the crystal structure of DPP4 was retrieved from the Protein Data Bank (PDB ID: 4WZ5) (Setiawansyah et al., 2022). The protein was cleaned by removing water molecules and heteroatoms that could interfere with docking, and it was also protonated to ensure proper hydrogen bonding during interaction with the ligand. The protein was then prepared using AutoDockTools, and its grid box was defined to cover the binding site.

Structural Validation of DPP4 Protein

Structural validation of the DPP4 protein model was conducted using PROCHECK (**Figure 1.**) and ERRAT tools (**Figure 2.**).

The Ramachandran plot (**Figure 1.**) revealed that 89.4% of residues were in the most favored regions, with 9.2% in additional allowed regions and only 1.4% in disallowed regions. ERRAT analysis (**Figure 2.**) yielded a quality score of 95.7%, confirming the reliability of the DPP4 model for molecular docking studies. This validation ensures the robustness of the results and strengthens the credibility of 4-hydroxyisoleucine as a potential drug candidate.

Docking Results

The in silico analysis of 4-hydroxyisoleucine as a natural antidiabetic agent focuses on the interaction between 4-hydroxyisoleucine and the target enzyme DPP-4. Molecular docking was performed to predict the binding between the ligand (in this case, 4-hydroxyisoleucine) and the active site of the DPP-4 enzyme, a primary target in glucose regulation in diabetes. The docking results

provide insights into the strength of the interaction and the stability of the binding between 4-hydroxyisoleucine and DPP-4, as well as the potential of this compound in inhibiting enzyme activity. The main docking results show that 4-hydroxyisoleucine has a good affinity for the DPP-4 active site.

Based on the docking scores calculated, 4-hydroxyisoleucine forms stable interactions with several important residues in the active site of the DPP-4 enzyme, which are involved in inhibiting its activity. This molecule can to functional groups on the enzyme, such as amino acids involved in forming the active site, leading to the inhibition of peptide cleavage and regulation of insulin secretion. Furthermore, the interaction analysis indicates that 4-hydroxyisoleucine forms several hydrogen bonds and hydrophobic interactions with critical residues in DPP-4.

These interactions support the stability of the ligand-enzyme complex and enhance the compound's potential in inhibiting DPP-4 activity. A comparison with conventional DPP-4 inhibitors, such as sitagliptin, shows that 4-hydroxyisoleucine has similar potential in binding to the enzyme's active site, although with slightly different binding mechanisms. The conclusions from these docking results suggest that 4-hydroxyisoleucine has the potential to be an effective natural DPP-4 inhibitor, which could contribute to the control of blood glucose levels. Its strong and stable interaction with the DPP-4 active site makes it a promising candidate for developing natural-based antidiabetic drugs. These findings pave the way for further research, both in experimental testing and the development of more effective and safe antidiabetic drug formulations (Setiawansyah et al., 2022).

Post-Docking Analysis

Post-docking visualization confirmed significant interactions between 4-hydroxyisoleucine and active site residues of DPP4. Key hydrogen bonds and hydrophobic interactions were observed, highlighting the stability of the 4-hydroxyisoleucine-DPP4 complex. These interactions contribute to the compound's binding stability and reinforce its potential efficacy in inhibiting DPP4 activity.

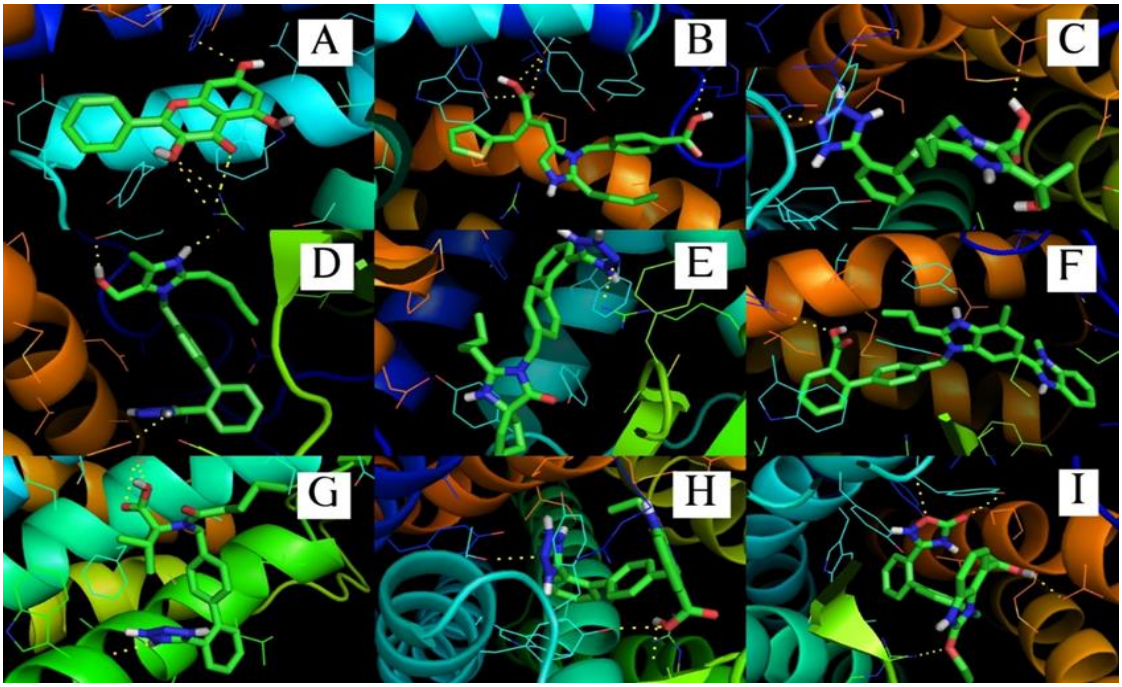

Figure 3. Visualization of molecular docking of ligands and protein. (A) 4-hydroxyisoleucine, (B) Alogliptin, (C) Linagliptin, (D) Sitagliptin, (E) Vildagliptin, (F) Saxagliptin.

Comparison with Synthetic DPP4 Inhibitors

The comparison between 4-hydroxyisoleucine and several synthetic DPP-4 inhibitors reveals significant differences in their molecular properties and binding affinities. 4-hydroxyisoleucine has a molecular weight of 147.17 g/mol, which is notably lower than that of synthetic DPP-4 inhibitors such as Linagliptin (472.54 g/mol) and Sitagliptin (407.31 g/mol). This difference in molecular weight could influence the pharmacokinetics and drug-like properties of the compounds, with 4-hydroxyisoleucine's smaller size potentially offering advantages such as better oral bioavailability and metabolic stability.

Furthermore, 4-hydroxyisoleucine has a binding affinity of -5.0 kcal/mol, which is lower compared to the more potent synthetic inhibitors, with Linagliptin showing the highest affinity at -8.7 kcal/mol, followed by Sitagliptin at -8.0 kcal/mol and Saxagliptin at -6.8

kcal/mol. These findings suggest that synthetic inhibitors typically interact more with the DPP-4 enzyme, potentially contributing to their higher potency in enzyme inhibition. The molecular formulas of these compounds also show notable differences, with synthetic inhibitors having more complex structures featuring larger carbon chains and additional functional groups, whereas 4-hydroxyisoleucine has a simpler structure ($C_6H_{13}NO_3$). Despite the lower binding affinity of 4-hydroxyisoleucine, its natural origin, simpler structure, and smaller size make it a promising candidate for further research as a natural-based antidiabetic agent.

The lower binding affinity does not diminish its potential efficacy, especially if it can offer other benefits such as a better safety profile, fewer side effects, and possible synergistic effects when used in combination therapies. Future research is needed to explore the practical therapeutic applications of 4-hydroxyisoleucine in diabetes management and its potential as an alternative to synthetic DPP-4 inhibitors.

Ligands	CID	Molecular Formula	Molecular Weight (g/mol)	Binding Affinity (kcal/mol)
4-Hydroxyisoleucine	2773624	$C_6H_{13}NO_3$	147.17	-5,0
Alogliptin	10244493	$C_{18}H_{21}N_5O_2S$	339,45	-6,6
Linagliptin	10096344	$C_{25}H_{28}N_8O_2$	472,54	-8,7
Saxagliptin	11235634	$C_{18}H_{25}N_3O_2$	315,41	-6,8
Sitagliptin	4369359	$C_{16}H_{15}F_6N_5O$	407,31	-8,0
Vildagliptin	6918586	$C_{17}H_{25}N_3O_2$	303,40	-7,3

ADME and Toxicity Profile

The ADME (Absorption, Distribution, Metabolism, Excretion) and toxicity profile of 4-hydroxyisoleucine were evaluated to assess its pharmacokinetic properties and safety as a potential therapeutic agent. 4-hydroxyisoleucine demonstrated high gastrointestinal absorption, indicating that the compound can be efficiently absorbed into the bloodstream orally, making it a suitable candidate for oral drug formulations. Additionally, the compound showed moderate water solubility, which enhances its potential for distribution throughout the body. However, it exhibited limited penetration across the blood-brain barrier (BBB), which reduces the risk of central nervous system side effects, helping to target peripheral tissues without affecting brain function.

Predictions suggest that 4-hydroxyisoleucine does not undergo extensive metabolism, which implies that it may retain its bioactive form for a longer duration in the body. This characteristic could lead to prolonged therapeutic effects, as the compound is less likely to be rapidly broken down by metabolic enzymes. Furthermore, 4-hydroxyisoleucine is expected to be excreted primarily through the urine, a common pathway for small molecules with low molecular weight, emphasizing the importance of renal function in eliminating the compound from the body. In terms of toxicity, 4-hydroxyisoleucine was predicted to have a favorable safety profile, associated with low hepatotoxicity, suggesting minimal risk to liver function (Setiawansyah et al., 2022).

It also did not show signs of mutagenicity or carcinogenicity, indicating that it is unlikely to cause genetic mutations or cancer. With an LD50 value greater than 2000 mg/kg, 4-hydroxyisoleucine falls under Toxicity Class 5, classifying it as a low-toxicity substance. This further supports its safety for use in therapeutic applications. In summary, 4-hydroxyisoleucine exhibits favorable ADME properties, including high oral bioavailability, moderate solubility, and low toxicity. These characteristics make it a promising candidate for further development as a natural therapeutic agent, particularly for conditions like diabetes. The compound's excellent safety profile and pharmacokinetic features position it as an attractive option for future drug development with minimal risk of adverse effects.

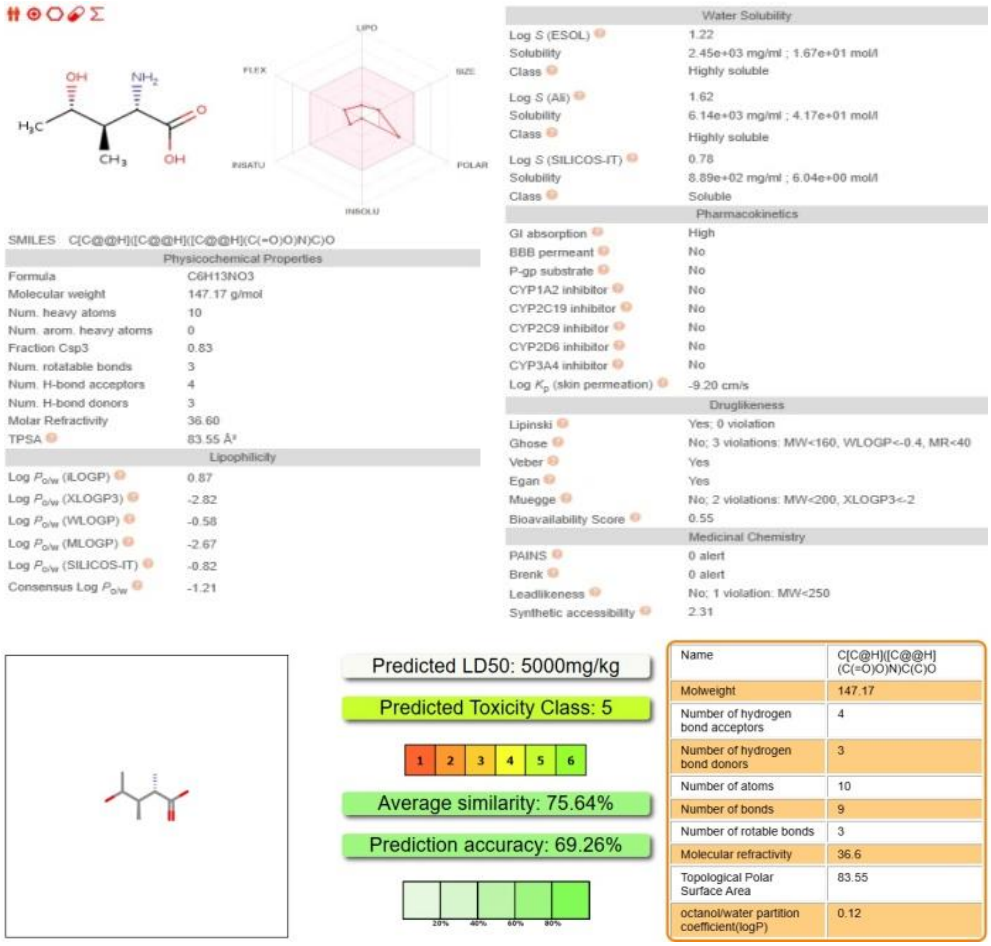

Figure 4. ADMET analysis of flavonoid drugs demonstrating profiles of absorption, distribution, metabolism, excretion, and toxicity. This data is utilized to assess the pharmacokinetic potential and safety of substances in medication development.

Potential for Further Development

The potential for further development of 4-hydroxyisoleucine as a therapeutic agent is substantial, particularly in treating metabolic disorders such as diabetes. Given its promising ADME properties, including high gastrointestinal absorption, moderate solubility, and favorable safety profile, 4-hydroxyisoleucine shows strong potential for development into an effective, natural-based pharmaceutical. Its ability to be efficiently absorbed in the digestive tract and its relatively low toxicity profile suggest that it could be a safer alternative to some conventional synthetic drugs, potentially reducing the risk of adverse effects commonly associated with long-term use of synthetic antidiabetic agents.

Moreover, 4-hydroxyisoleucine's natural origin offers an added advantage, as patients increasingly seek plant-based or natural treatments with fewer side effects. The compound's lower molecular weight and minimal metabolism also indicate that it could be retained in the body for a longer period, potentially offering more sustained therapeutic effects. The fact that 4-hydroxyisoleucine has been shown to interact with key targets such as the DPP-4 enzyme further enhances its potential as a promising agent for diabetes management. Inhibiting DPP-4 can lead to improved insulin secretion and better blood glucose regulation, which is critical for diabetes treatment (Xiao et al., 2018).

In addition, the promising pharmacokinetic characteristics of 4-hydroxyisoleucine, coupled with its low hepatotoxicity and lack of mutagenic or carcinogenic properties, position it as a safe candidate for use in future drug formulations. The compound's relatively low binding affinity compared to synthetic DPP-4 inhibitors does not necessarily diminish its therapeutic potential, as further optimization and combination therapies could enhance its efficacy.

The future development of 4-hydroxyisoleucine could involve clinical studies to validate its efficacy in human trials, explore its long-term safety, and refine formulations for maximum bioavailability and therapeutic benefit. Its natural composition and minimal toxicity profile suggest that, with further research and development, it could become a valuable addition to the array of treatments available for managing diabetes and other metabolic conditions. Thus, the continued exploration of 4-hydroxyisoleucine's pharmacological properties holds significant promise for advancing natural-based therapies in medicine (Yuan et al., 2017).

CONCLUSIONS

This study of 4-hydroxyisoleucine presents a promising candidate for further development as a natural therapeutic agent, particularly for treating diabetes. Its favorable ADME properties, including high gastrointestinal absorption, moderate solubility, and minimal toxicity, position it as a viable alternative to synthetic DPP-4 inhibitors. The compound's ability to interact with key targets involved in glucose regulation, along with its low risk of adverse effects, makes it an attractive option for future drug development. While its binding affinity to DPP-4 is lower than of synthetic inhibitors, its natural origin, simple molecular structure, and potential for sustained therapeutic effects warrant further research and optimization. Overall, 4-hydroxyisoleucine's promising pharmacokinetic profile, coupled with its safety and efficacy, suggest that it could play an important role in the development of natural-based therapies for diabetes and other metabolic disorders.

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Authors' Contributions: Irma Putri Damayanti to designed the study, provide input and supervise the docking. Amir carried out the docking work, analyzed the data And wrote the manuscript. All authors read and approved the final version of the manuscript

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