© 2024 Journal of Pharmacy & Pharmacognosy Research, 12 (5), 929-942, 2024
ISSN 0719-4250
https://jppres.com

DOI: https://doi.org/10.56499/jppres23.1936_12.5.929

Original Article

Molecular docking and dynamics studies of 8,9-dimethoxy ellagic acid contained in *Peperomia pellucida* (L.) Kunth against various diabetes mellitus receptors

[Estudios de acoplamiento y dinámica molecular del ácido 8,9-dimetoxielágico contenido en *Peperomia pellucida* (L.) Kunth frente a varios receptores de diabetes mellitus]

Yasmiwar Susilawati[1,2], Raden Bayu Indradi[2], Aiyi Asnawi[3], Ellin Febrina[4*]

[1]Herbal Study Center, Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang km. 21, Jatinangor, 45363, Indonesia.
[2]Department of Biology Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jatinangor 45363, Indonesia.
[3]Department of Pharmacochemistry, Faculty of Pharmacy, Universitas Bhakti Kencana, Jl. Soekarno-Hatta No. 754, Bandung 40617, Indonesia.
[4]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang km. 21, Jatinangor 45363, Indonesia.
*E-mail: ellin.febrina@unpad.ac.id

Abstract

Context: The search for antidiabetic drugs that target the receptors involved in diabetes has received significant attention in recent years. *Peperomia pellucida* (L.) Kunth's ethanol extract and ethyl acetate fraction have antihyperglycemic activity. 8,9-dimethoxy ellagic acid (DEA) has shown significant diabetes mellitus activity in mice, but its interaction with diabetes receptors remains unknown.

Aims: To perform molecular docking and molecular dynamics simulations to explore the binding interactions and stability of DEA within the binding sites of enzymes involved in diabetes.

Methods: At the outset, the utilization of molecular docking was limited to forecasting the DEA's binding orientations and affinities within the active sites of the enzymes implicated in diabetes. Following this, molecular dynamics simulation was employed to investigate the interactions, stability, and dynamic behavior of these complexes over a period of 100 nanoseconds.

Results: Molecular docking results revealed that DEA interacts with all selected receptors involved in diabetes and interacts more strongly with the aldose reductase receptor (PDB ID 3S3G) than the native ligand, with a binding energy of -10.3 kcal/mol. However, further molecular dynamics simulations confirmed the stability of the receptor complex with DEA over 100 ns, which is less potent than that of the native ligand. This is probably due to the rigidity of the DEA molecular structure.

Conclusions: This study highlights the potential of DEA derived from *P. pellucida* as an inhibitor of various receptors involved in diabetes.

Keywords: 8,9-dimethoxy ellagic acid; antidiabetic; *in silico*; *Peperomia pellucida*.

Resumen

Contexto: La búsqueda de fármacos antidiabéticos que se dirijan a los receptores implicados en la diabetes ha recibido mucha atención en los últimos años. El extracto etanólico de *Peperomia pellucida* (L.) Kunth y la fracción de acetato de etilo tienen actividad antihiperglucemiante. El ácido 8,9-dimetoxielágico (DEA) ha mostrado una actividad significativa en la diabetes mellitus en ratones, pero su interacción con los receptores de la diabetes sigue siendo desconocida.

Objetivos: Realizar simulaciones de dinámica molecular y acoplamiento molecular para explorar las interacciones de unión y la estabilidad de la DEA dentro de los sitios de unión de las enzimas involucradas en la diabetes.

Métodos: Al principio, la utilización del acoplamiento molecular se limitaba a pronosticar las orientaciones y afinidades de unión de la DEA dentro de los sitios activos de las enzimas implicadas en la diabetes. A continuación, se empleó simulación de dinámica molecular para investigar las interacciones, la estabilidad y el comportamiento dinámico de estos complejos durante un período de 100 nanosegundos.

Resultados: Los resultados del acoplamiento molecular revelaron que la DEA interactúa con todos los receptores seleccionados implicados en la diabetes e interactúa más fuertemente con el receptor de aldosa reductasa (PDB ID 3S3G) que el ligando nativo, con una energía de unión de -10,3 kcal/mol. Sin embargo, otras simulaciones de dinámica molecular confirmaron la estabilidad del complejo del receptor con DEA durante 100 ns, que es menos potente que el del ligando nativo. Probablemente esto se deba a la rigidez de la estructura molecular de la DEA.

Conclusiones: Este estudio destaca el potencial de la DEA derivada de *P. pellucida* como inhibidor de diversos receptores implicados en la diabetes.

Palabras Clave: ácido 8,9-dimetoxi elágico; antidiabético; *in silico*; *Peperomia pelucida*.

ARTICLE INFO
Received: December 31, 2023.
Accepted: March 17, 2024.
Available Online: April 21, 2024.

AUTHOR INFO
ORCID:
0000-0003-1907-3405 (YS)
0000-0002-2223-6925 (RBI)
0000-0002-8179-0520 (AA)
0000-0003-3004-5069 (EF)

INTRODUCTION

Diabetes mellitus is a group of diseases that is characterized by metabolic hyperglycemia, also known as high blood sugar levels. This condition occurs as a direct result of the body's inability to produce or make effective use of insulin. Insulin is an essential hormone that helps move glucose from the bloodstream into the cells of the body, where it can be used as a source of energy (American Diabetes Association, 2013). Both type 1 and type 2 diabetes are considered to be the most common forms of the disease. Diabetes type 1, also known as insulin-dependent diabetes, is a condition that typically affects children and adolescents; however, adults can develop the condition as well. People who have type 1 diabetes have damage to the cells of the pancreas that produce insulin, which leads to a lack of insulin throughout the body (Atkinson et al., 2014). Insulin resistance is a condition that can lead to type 2 diabetes, which is a more general form of the disease. Insulin resistance occurs when the body is unable to use the insulin that it produces effectively. This condition is more common in adults and has been linked to being overweight, leading a life devoid of style, and having genetic predispositions (DeFronzo et al., 2015). Over several decades, there has been a worldwide upward trend in diabetes-related death rates, which is finally in line with the rising incidence of the disease. According to the International Diabetes Federation (IDF), the number of adults with diabetes was estimated to be 463 million in the year 2019, which is anticipated to increase to 700 million in the year 2045 (Ogurtsova et al., 2022). It is anticipated that the number of deaths caused by diabetes will increase in tandem with the rising prevalence of the disease, particularly in low- and middle-income countries (Zimmet et al., 2001).

Treatment for diabetes entails the utilization of a variety of strategies for regulating blood sugar levels, minimizing the risk of developing complications and maximizing one's quality of life. Insulin therapy, PPAR-γ receptor agonists, DPP-4 inhibitors, SGLT-2 inhibitors, and lifestyle changes are all essential components of an effective diabetes management plan. Insulin therapy is not without its risks, some of which include hypoglycemia, an increase in weight, and insulin allergies. PPAR-γ agonists have been shown to increase insulin sensitivity in tissue (DeFronzo et al., 2015), while DPP-4 inhibitors have been shown to decrease incretin levels (Karagiannis et al., 2012). SGLT-2 inhibitors work by lowering the amount of glucose that is absorbed by the kidneys and raising the amount of glucose that is excreted in the urine (Zimmet et al., 2001). On the other hand, existing anti-diabetic drugs still have weaknesses both in terms of efficacy and side effects. Thus, the search for new anti-diabetic drugs is a very interesting challenge.

One interesting source of drug compounds is natural products. Traditional medicine has made use of natural products for decades or even centuries; some of these products have demonstrated hypoglycemic benefits and have the potential to be used as treatments for diabetes. It has been discovered that medicinal plants (Kusuma et al., 2017), polyphenols (Aryaeian et al., 2017), amino acids, and peptides (Elam et al., 2021) can all treat diabetes and have hypoglycemic effects. Natural products are more effective in the treatment of diabetes because they have better biocompatibility, diversity of chemical structures, and multi-receptor targeting (Cragg and Newman, 2013).

Peperomia pellucida (L.) Kunth (family *Piperaceae*) is a plant medicine that is utilized to treat a variety of conditions. It has shown promise as a potential source of new drugs for the treatment of a wide range of diseases, including anti-inflammatory and analgesic effects (de Fátima Arrigoni-Blank et al., 2004), antimicrobial, antioxidant, and anticancer (Wei et al., 2011). In a study that was carried out by Susilawati et al. (2015), researchers were able to successfully isolate and identify the compound known as (*S*)-2-methyl-2-(4-methylpent-3-enyl)-6-(propan-2-ylidene)-3,4,6,7-tetrahydropyrano[4,3-*g*]chromen-9(2H)-one from *P. pellucida* (Susilawati et al., 2015) as well as *in silico* study (Susilawati et al., 2022). In other studies, compound 8,9-dimethoxy elagate (DEA) was successfully isolated and identified from *P. pellucida*. The method of inhibition of the α-glucosidase enzyme (Susilawati et al., 2017) was used to evaluate the DEA for its potential anti-diabetic activity. However, the mechanism of action of DEA on receptors involved in the treatment of diabetes has yet to be disclosed in this work.

Researching the ligand 8,9-dimethoxy ellagic acid (DEA) from *P. pellucida* is crucial for diabetes studies (Susilawati et al., 2017). DEA is an organic chemical extracted from *P. pellucida*, a medicinal plant known for its healing effects and historical use in several cultures (Clemen-Pascual et al., 2022). Exploring the potential of DEA as a therapeutic agent for diabetes could use the advantages of natural products in contemporary medicine. Initial research indicates that DEA may have antidiabetic effects. Exploring how DEA influences diabetes could help create innovative treatment approaches for controlling the condition. Natural compounds such as DEA are important resources for inspiring medication research and advancement. Researchers can enhance the therapeutic potential of DEA and create derivatives with better

efficacy and safety profiles by understanding its structure-activity correlations and pharmacological features. Examining the safety and potential toxicity of DEA is essential for its clinical advancement as a medicinal drug. Thorough toxicity studies can offer important insights into DEA's tolerance, side effects, and potential drug interactions, guaranteeing its safety for human consumption.

The connection between the ethnomedical applications of *P. pellucida* (family *Piperaceae*) and its intended activity is rooted in the ancestral wisdom transmitted across generations concerning the plant's healing attributes and its impact on human well-being. *P. pellucida* has a rich traditional usage in different civilizations globally, especially in tropical areas where it is plentiful (Boy et al., 2018). *P. pellucida* is used in ethnomedicine for several medicinal purposes, such as treating diabetes, hypertension, inflammation, wound healing, and gastrointestinal issues (Alves et al., 2019). These conventional practices are frequently derived from empirical observations and experiences gathered over time within specific localities. *P. pellucida* 's putative antidiabetic action is generally based on traditional knowledge of its therapeutic characteristics. Researchers seek to confirm and clarify the pharmacological foundation of these traditional practices through scientific research, which involves analyzing plant chemicals, conducting pharmacological tests, and carrying out clinical trials. Researchers can identify bioactive components in *P. pellucida* by analyzing its ethnomedical usage and linking them with specific pharmacological activity. This information could be used to create novel pharmaceuticals or herbal treatments based on *P. pellucida* for managing different illnesses.

In silico studies have been used to identify potential receptor targets for diabetes treatment. Aldose reductase inhibitors can prevent the accumulation of sorbitol, which is associated with diabetic complications such as nephropathy, cataracts, and neuropathy (Yayla and Binnetoğlu, 2022). Aldose reductase inhibitors can also prevent oxidative stress and inflammation, which are involved in the pathogenesis of diabetic complications. Alpha-amylase inhibitors can delay the digestion and absorption of carbohydrates, which can reduce postprandial glucose and insulin peaks. Alpha-glucosidase inhibitors can delay the absorption of ingested carbohydrates, which can reduce postprandial glucose and insulin peaks (Behl et al., 2022). Dipeptidyl peptidase IV inhibitors can increase the levels of endogenous GLP-1, which is involved in glucose-dependent insulin secretion (Yayla and Binnetoğlu, 2022). Modulation of insulin receptor function can improve insulin sensitivity and glucose uptake, which can improve glycemic control in diabetes (Yan et al., 2021). It is important to note that further research is needed to develop more effective and less toxic treatment options for diabetes.

In silico methods have been used in the development of medicinal compounds, both molecular docking (Asnawi et al., 2022; Febrina et al., 2021), pre-ADME, and molecular dynamic (Asnawi et al., 2023; Febrina et al., 2022). Molecular docking and molecular dynamics are two common examples of *in silico* methods utilized in the study of developing new medicines. Molecular docking is a technique that can be used to predict the orientation of molecules within deep protein-target protein-ligand complexes as well as their interactions with one another. This method helps determine the most stable conformation and calculates the binding energy associated with that conformation (Torres et al., 2019). Whereas, the simulation of the motion of atoms and molecules inside biological systems and molecules over a given time can be done through a technique known as molecular dynamics. This method provides a perspective on the mechanisms and kinetics of biological processes at the atomic level, such as changes in protein conformation, protein-ligand interactions, and the stability of protein-ligand complexes. For example, changes in protein conformation can be used to study the stability of protein-ligand complexes (Ischak et al., 2023).

This study evaluated the potential of DEA, a molecule isolated from *P. pellucida*, as an inhibitor of certain diabetes receptors. Specifically, we were interested in whether or not this chemical could prevent diabetes. Within the binding sites of the receptors, molecular docking and molecular dynamics simulations were used to investigate the binding interactions and stability of DEA.

MATERIAL AND METHODS

Molecular docking modelling

Molecular modeling simulation was run on a personal computer equipped with an Intel (R) Core (TM) i7-4600U CPU running at 2.10 GHz and 2.70 GHz, 8.00 GB of RAM, 512 GB of SSD, with a dual operating system of Linux Ubuntu 22.04 and Windows 10 Pro 64-bit, x64-based processor. Target proteins like aldose reductase (PDB ID 3S3G), alpha-amylase (PDB ID 1B2Y), alpha-glucosidase (PDB ID 2QMJ), dipeptidyl peptidase IV (PDB ID 3F8S), and insulin receptor (PDB ID 1IR3) were obtained from the RCSB Protein Data Bank (http://www.rcsb.org/, accessed on 03 May 2023). The NCBI PubChem database (https://pubchem.nlm.nih.gov/, accessed on 03 May 2023) was used to derive the three-dimensional structure of DEA, its native ligand (NL), and acarbose (reference drug). AutoDock Vina under PyRx 8.0 (Morris

et al., 2009) was used to interact DEA, its native ligand (NL), and acarbose (reference drug) with the proteins. The DEA, its native ligand (NL), and acarbose (reference drug) were converted to PDBQT (Asnawi et al., 2023) and then performed a docking study (Febrina et al., 2022). The BIOVIA Discovery Studio 2017 program was used to visualize the molecular docking data (Ischak et al., 2023). The presented technique was used to explore molecular docking (Asnawi et al., 2023; Febrina et al., 2021).

Molecular dynamics modeling

Molecular dynamics simulations were conducted using the GROMACS 2022.4 program. The test ligand file selected from the optimal conformation obtained during molecular docking prior to initiating the molecular dynamics simulations was prepare. The molecular dynamics simulation process involves assembling enzymes and ligands, creating topology and coordinates, minimizing complexes, heating, equilibrating complexes, and generating and analyzing molecular dynamics data. After creating the test ligand and protein files, they were topologically processed and combined into a unified complex. The complex was enclosed in a grid box, and aqueous solvents, along with various ions, were introduced to attain a neutral state. The ions included Na^+ or Cl^-. The intricate system underwent three rounds of testing. The initial phase of energy minimization involved reducing water molecules through 1000 steps. The subsequent phase involved minimizing the overall ligand-enzyme system and water molecules through 1000 steps. The final phase encompassed the entire system architecture. Heating was conducted in a gradient from 0-100 K, then from 100-200 K, and eventually reaching 200-310 K. After the system reached equilibrium, the final stages involved a production process lasting 100 nanoseconds. The root mean square deviation (RMSD), root mean square fluctuation (RSMF), molecular mechanics generalized born surface area (MMGBSA), and decomposition were used to examine the findings of the molecular dynamics simulation. Molecular dynamics was explored with the help of the provided method (Febrina et al., 2022; Ischak et al., 2023).

ADME and toxicity

The method employed for the predictive study of the metabolic and toxicological profiles of 8,9-dimethoxy ellagic acid (DEA) from *P. pellucida* using SwissADME comprised multiple steps. The chemical structure of DEA (SMILES: COc1cc2c(cc1OC)COc1c2cccc1) was initially entered into the SwissADME online portal. The tool was used to forecast several pharmacokinetic features, such as absorption, distribution, metabolism, and excretion (ADME). The features included measures like gastrointestinal absorption, blood-brain barrier permeability, cytochrome P450-mediated metabolism, and renal excretion.

In silico data analysis

The study utilized different methods to validate the docking method, evaluate predicted absorption and distribution properties, and assess potential toxicity. Analyzed chemical interactions between ligands and target proteins using molecular docking and molecular dynamics simulations. The free energy of each ligand was determined using techniques such as MMGBSA. The study examined individual species or compounds in comparison to native ligands on target proteins to assess their potential as therapeutic agents. Information was analyzed and presented through the use of tables, figures, and charts. Reference substances were utilized as benchmarks for comparison and validation to contextualize and evaluate efficacy.

RESULTS AND DISCUSSION

Molecular docking and molecular dynamics simulations are used to predict the binding affinity and orientation of a ligand molecule to a receptor protein and simulate the behavior of the ligand-receptor complex over time (Rahman et al., 2023; Yuliantini et al., 2024).

Binding mode interaction

Molecular docking is a computational method that enables the exploration of enzyme binding sites, substrate interactions, and their preferred orientations (Asnawi et al., 2022). It can be used to study the interaction between a protein and a ligand in the active site, which is the region of the protein where the ligand binds (Stanzione et al., 2021). It can provide insights into the binding mechanism and optimize ligand design and can be integrated with other computational methods for a more comprehensive understanding of the interaction.

The DEA ligand was able to interact with the binding site of the selected receptors (Fig. 1). The interaction of the native ligand in the active site of aldose reductase (PDB ID: 3S3G) has a free energy of binding (ΔG) of -7.9 kcal/mol. DEA shows a stronger binding affinity with a lower ΔG of -10.3 kcal/mol. DEA exhibits a more favorable binding energy compared to the native ligand, indicating a potentially stronger interaction with aldose reductase. In the active site of alpha-amylase (PDB ID: 1B2Y), the native ligand has a ΔG of -9.7 kcal/mol, and DEA has a lower ΔG of -7.8 kcal/mol. In this case, the native ligand shows more

favorable binding energy compared to DEA, suggesting that the native ligand has a stronger affinity for alpha-amylase.

The interaction of the native ligand in the active site of alpha-glucosidase (PDB ID: 2QMJ) has a ΔG of -7.8 kcal/mol, and DEA has a slightly lower ΔG of -7.1 kcal/mol. DEA demonstrates a slightly stronger binding affinity compared to the native ligand, indicating a potential improvement in the interaction with alpha-glucosidase. In the active site of dipeptidyl peptidase IV (PDB ID: 3F8S), the native ligand has a ΔG of -8.7 kcal/mol, and DEA exhibits a lower ΔG of -6.9 kcal/mol. DEA shows more favorable binding energy than the native ligand, suggesting a potentially stronger interaction with dipeptidyl peptidase IV. In the active site of insulin receptor (PDB ID: 1IR3, the native ligand and DEA have an ΔG of -8.6 and -7.4 kcal/mol, respectively. DEA demonstrates a slightly stronger binding affinity compared to the native ligand, indicating a potential improvement in the interaction with the insulin receptor.

Overall, the comparison of energies in various macromolecules reveals that DEA shows enhanced binding affinities compared to the respective native ligands in aldose reductase, alpha-glucosidase, dipeptidyl peptidase IV, and the insulin receptor. However, in the case of alpha-amylase, the native ligand exhibits a stronger binding affinity than DEA. These findings suggest that DEA has the potential to serve as a potent ligand for the studied macromolecules, but its effectiveness may vary depending on the specific target protein.

Hydrogen bonds are important for stabilizing ligand-receptor interactions and can play a crucial role in ligand recognition and binding affinity. On the other hand, hydrophobic interactions, characterized by non-polar interactions between hydrophobic regions of the ligand and receptor, contribute to the overall stability of the ligand-receptor complex (Fig. 2). In the active site of aldose reductase (PDB ID: 3S3G), while NL forms hydrogen bonds with Tyr49 and His111, DEA forms hydrogen bonds with Ala300, Leu301, Leu302, and Ser303. The DEA ligand forms a greater number of hydrogen bonds, indicating a potentially stronger and more extensive interaction network with the binding site residues. Both NL and DEA form hydrophobic interactions with Trp21, suggesting a common binding site residue that contributes to ligand stabilization.

However, NL forms an additional hydrophobic interaction with Phe123, while DEA does not exhibit such interaction. Overall, the NL and DEA ligands exhibit distinct interaction patterns within the binding site of 3S3G. NL primarily forms hydrogen bonds and hydrophobic interactions with specific residues, whereas DEA forms a larger number of hydrogen bonds and a similar hydrophobic interaction.

In the active site of α-amylase (PDB ID: 1B2Y), NL forms a larger number of hydrogen bonds (six) compared to DEA (three). NL interacts with a diverse set of residues, including Trp59, Gln63, Arg195, Glu233, Asp300, and His305. On the other hand, DEA primarily interacts with Gln63 and His305. The greater number of hydrogen bonds in NL suggests a more extensive interaction network and potentially stronger binding affinity. NL does not form any hydrophobic interactions, whereas DEA forms three hydrophobic interactions with Trp58, Trp59, and Tyr62. These hydrophobic interactions contribute to the stabilization of the ligand in the binding site. Overall, the NL and DEA ligands exhibit different interaction patterns within the binding site of 1B2Y. NL primarily forms hydrogen bonds with multiple residues, while DEA forms a smaller number of hydrogen bonds but also engages in hydrophobic interactions.

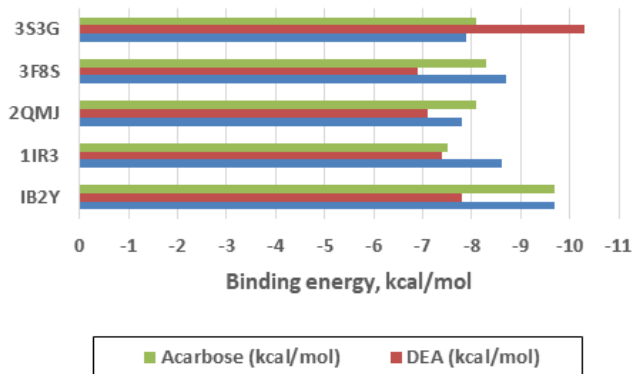

Figure 1. The free energy of binding of the NL, DEA, and acarbose (reference compound) on the active site of aldose reductase (PDB ID 3S3G), alpha-amylase (PDB ID 1B2Y), alpha-glucosidase (PDB ID 2QMJ), dipeptidyl peptidase IV (PDB ID 3F8S), and insulin receptor (PDB ID 1IR3).

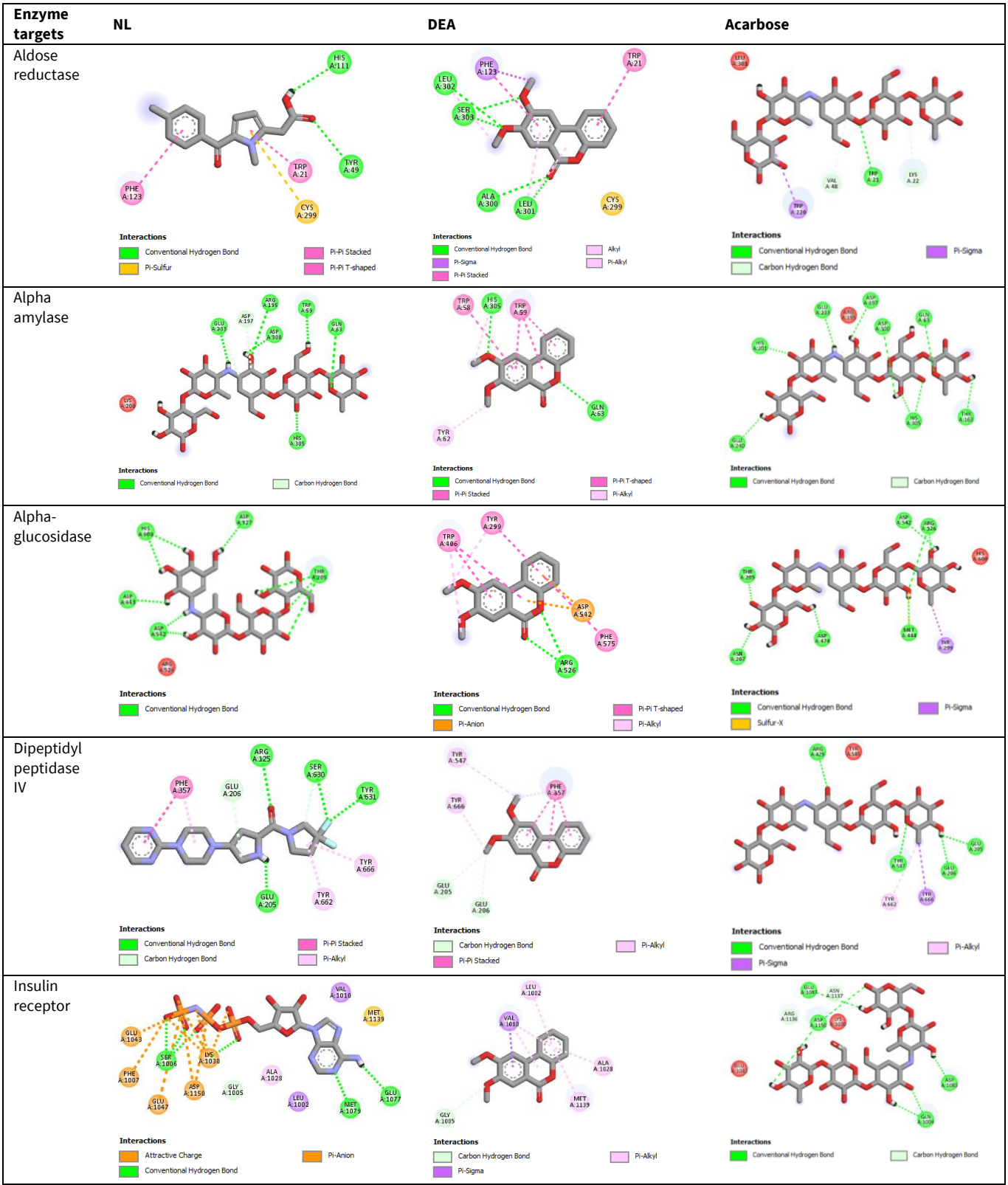

Figure 2. 2D visualization for the interaction of the NL, DEA, and acarbose (reference compound) on the active site of aldose reductase (PDB ID 3S3G), alpha-amylase (PDB ID 1B2Y), alpha-glucosidase (PDB ID 2QMJ), dipeptidyl peptidase IV (PDB ID 3F8S), and insulin receptor (PDB ID 1IR3).

In the active site of α-glucosidase (PDB ID: 2QMJ) NL forms a greater number of hydrogen bonds (five) compared to DEA (two). NL interacts with a diverse set of residues, including Thr205, Asp327, Asp443, Asp542, and His600. In contrast, DEA primarily interacts with Arg526. The larger number of hydrogen bonds in NL suggests a more extensive interaction network and potentially stronger binding affinity. NL does not form any hydrophobic interactions, while DEA forms three hydrophobic interactions with

Tyr299, Trp406, and Phe575. Overall, NL and DEA ligands exhibit different interaction patterns within the binding site of 2QMJ. NL primarily forms hydrogen bonds with multiple residues, indicating a potentially strong binding affinity. On the other hand, DEA forms a smaller number of hydrogen bonds but engages in hydrophobic interactions, which can also contribute to the stability of the ligand-receptor complex. These differences in interaction patterns suggest variations in binding affinity, selectivity, and potential functional roles of the ligands within the receptor.

In the active site of dipeptidyl peptidase IV (PDB ID: 3F8S), NL forms five hydrogen bonds, whereas DEA does not form any hydrogen bonds. This difference suggests that NL may have a stronger and more specific interaction with the receptor compared to DEA. Both NL and DEA engage in hydrophobic interactions. NL forms four hydrophobic interactions, while DEA forms six hydrophobic interactions. Overall, NL and DEA exhibit distinct interaction patterns within the binding site of 3F8S. NL forms hydrogen bonds with specific residues, indicating strong and specific interactions, and also engages in hydrophobic interactions. DEA, on the other hand, relies primarily on hydrophobic interactions for binding, as it does not form any hydrogen bonds. In the active site of the insulin receptor (PDB ID: 1IR3, NL forms six hydrogen bonds, while DEA does not form any hydrogen bonds. This indicates that NL has a stronger propensity for specific interactions mediated by hydrogen bonding in the binding site of 1IR3 compared to DEA. NL does not form any hydrophobic interactions, whereas DEA forms six hydrophobic interactions. Overall, the interaction patterns between NL and DEA in the binding site of 1IR3 highlight their distinct binding modes. NL forms multiple hydrogen bonds, indicating strong and specific interactions, while DEA predominantly relies on hydrophobic interactions.

Stability interaction of the complex

To confirm the DEA ligand's interaction stability with the binding site of aldose reductase (PDB ID 3S3G), a molecular dynamic simulation for 10 ns was used. The observed parameters include RMSD, RMSF, SASA, gyration, and energy components.

Root-mean-square deviation (RMSD)

RMSD is a measure of the average distance between the atoms of a superimposed structure, indicating how much the structure deviates from a reference structure. A higher RMSD value indicates a greater deviation or instability in the structure. For the protein, we can see that at the beginning (time 0 ns), the RMSD was relatively low (0.0836656 Å). As time progresses, the RMSD values increase, reaching a maximum value of 1.5218257 Å at 45 ns, and then gradually decrease again towards the end of the simulation, with a value of 0.9838794 Å at 99.99 ns. For the NL, the initial RMSD value was very low (0.0004906 Å). It then increases over time, with fluctuations, reaching a maximum value of 0.5661862 Å at 30 ns. After that, the RMSD decreases and stabilizes around 0.3-0.4 Å for the rest of the simulation, with some minor fluctuations (Fig. 3A).

RMSD is a measure of the average distance between the atoms of a superimposed structure, indicating how much the structure deviates from a reference structure. A higher RMSD value indicates a greater deviation or instability in the structure. For the protein, we can see that at the beginning (time 0 ns), the RMSD was relatively low (0.0836656 Å). As time progresses, the RMSD values increase, reaching a maximum value of 1.5218257 Å at 45 ns, and then gradually decrease again towards the end of the simulation, with a value of 0.9838794 Å at 99.99 ns. The initial RMSD value for the NL was very low (0.0004906 Å). It then increases over time, with fluctuations, reaching a maximum value of 0.5661862 Å at 30 ns. After that, the RMSD decreases and stabilizes around 0.3-0.4 Å for the rest of the simulation, with some minor fluctuations (Fig. 3A).

While for the protein in Fig. 3B, we can see that at the beginning (time 0 ns), the RMSD was relatively low (0.0874144 Å). The RMSD values fluctuate as time progresses but generally remain within a moderate range. The maximum RMSD value observed was 1.0589728 Å at 70 ns, indicating a relatively higher deviation from the reference structure. Towards the end of the simulation, the RMSD decreases and stabilizes around 0.5-0.8 Å for the protein. For the ligand, the initial RMSD value was also very low (0.0005031 Å). The RMSD values increase slightly over time, with some fluctuations. The maximum RMSD value observed was 0.3305948 Å at 95 ns. However, the RMSD generally remains below 0.3 for the ligand throughout the simulation.

Analyzed the stability of the complex by measuring the RMSD of the protein and acarbose (reference chemical) over time to study the dynamic behavior of the system (Fig. 3C). The RMSD values for the protein remained rather stable around an average of 0.5 Å during the 100 ns simulation, suggesting consistent structural changes. Minor oscillations were noticed, particularly at the beginning of the simulation, but the RMSD stayed within a tight range, indicating that the protein structure sustained its stability throughout the simulation. The RMSD readings for acarbose showed greater variations than those for the protein. At first, the RMSD was modest, suggesting a stable conformation. As the simulation continued, the RMSD

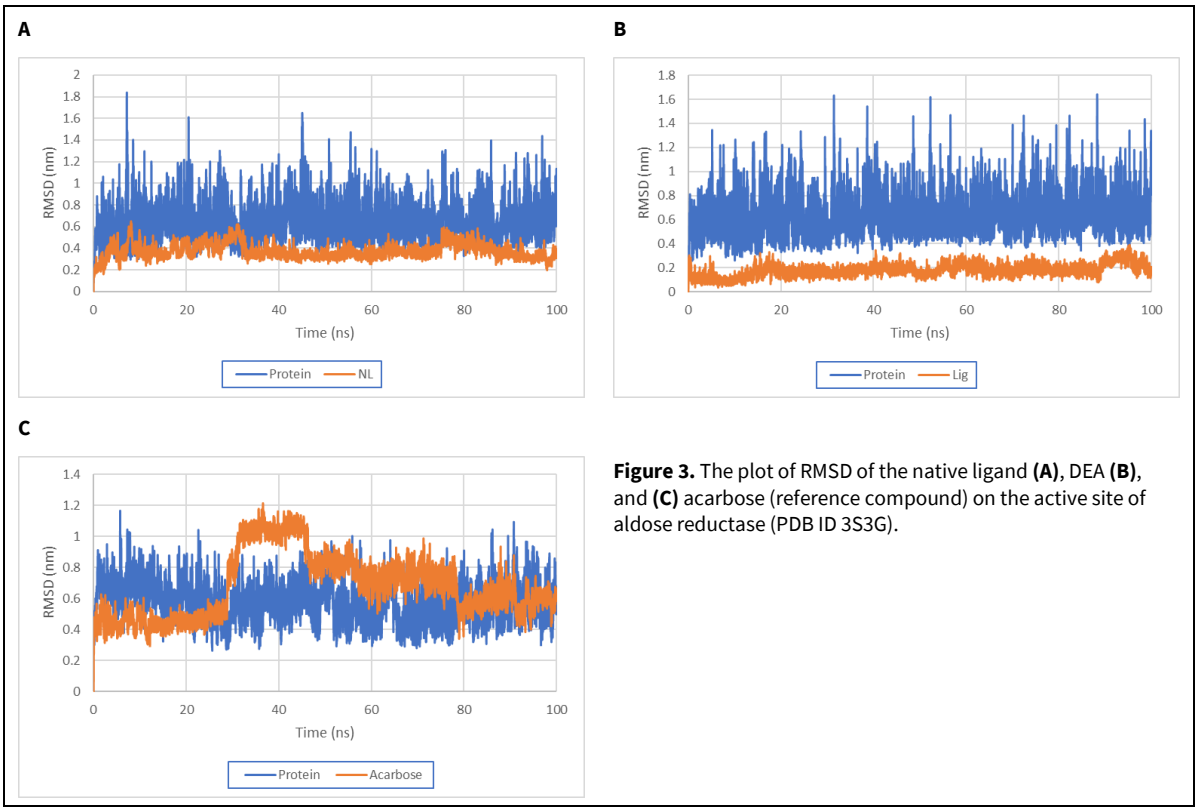

Figure 3. The plot of RMSD of the native ligand **(A)**, DEA **(B)**, and **(C)** acarbose (reference compound) on the active site of aldose reductase (PDB ID 3S3G).

steadily grew, suggesting departures from the original shape. This indicates that acarbose may have experienced dynamic structural alterations or assumed several conformations over the duration of the simulation. The RMSD analysis shows that the protein maintained a stable shape during the simulation, while acarbose had more dynamic activity, possibly exploring several binding modes or conformations inside the binding region. These findings provide vital insights into the stability and dynamic behavior of the protein-ligand complex and can inspire additional studies targeted at understanding the molecular mechanisms of ligand binding and therapeutic efficacy.

RMSD of the NL and DEA over time were compared. The RMSD values for NL exhibit some fluctuations but generally remain relatively low throughout the simulation. The maximum NL RMSD observed was 0.5661862 Å at 30 ns. Towards the end of the simulation, the NL RMSD decreases and stabilizes, with a value of 0.3266089 Å at 99.99 ns. The RMSD values for DEA also show fluctuations but generally remain relatively low compared to NL. The maximum RMSD observed was 0.3305948 Å at 95 ns. Similar to NL, the RMSD decreases towards the end of the simulation, with a value of 0.1568925 Å at 99.99 ns. The complex has reasonable stability throughout the simulation time, as indicated by the RMSD values. Fluctuations in the RMSD values for NL and DEA suggest structural changes or dynamics within the complex. Acarbose, serving as the reference compound, demonstrates consistent RMSD values in comparison to NL and DEA, indicating its crucial role in preserving the stability of the complex.

Root-mean-square fluctuation (RMSF)

The RMSF provides insight into the residual fluctuations of each residue in the complex. Higher RMSF values indicate greater flexibility and fluctuations, while lower RMSF values indicate more stable residues. The NL residues display varying degrees of fluctuation. The variations in the RMSF of amino acid residues at the active site of aldose reductase (PDB ID 3S3G) offer important insights into the enzyme's dynamic behavior when interacting with various ligands such as NL, DEA, and acarbose. Analysis of the data shows that some residues display significant variations in RMSF under the three different ligand situations. Residues 199 to 206 exhibit greater RMSF values in the presence of DEA compared to NL and acarbose (Fig. 4). These residues may exhibit increased flexibility or structural alterations when interacting with DEA, suggesting a potential unique binding mechanism or affinity for this ligand.

Residues 225 to 237 show significant variations in RMSF under all three ligand conditions. These residues are found inside the active site area of aldose reductase, where ligand binding and enzymatic action occur. The RMSF variations of these residues

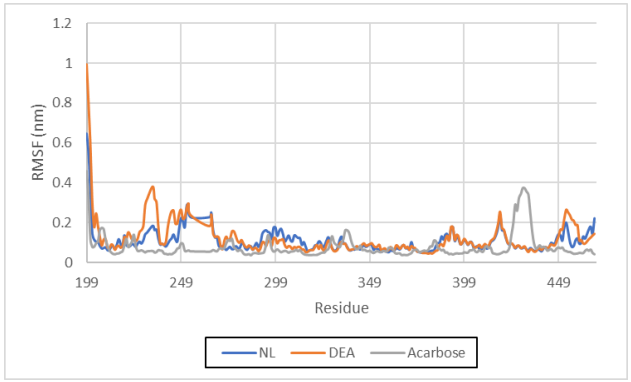

Figure 4. Plot of RMSF of the native ligand, DEA, and acarbose (reference compound) on the active site of aldose reductase (PDB ID 3S3G).

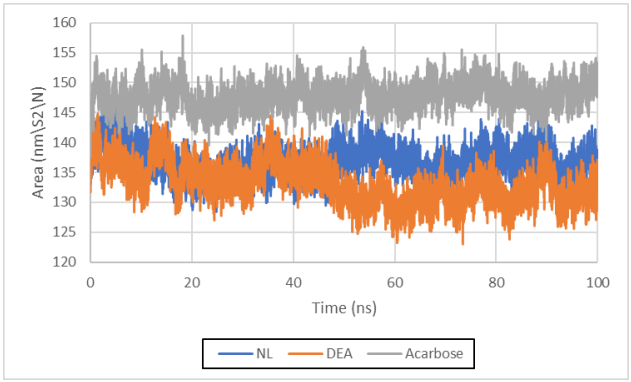

Figure 5. Plot of the SASA of the native ligand (blue), DEA (orange), and acarbose as the reference compound (grey) on the active site of aldose reductase (PDB ID 3S3G).

may indicate their role in ligand recognition, binding, or catalytic activity. Residues 264 to 268 show lower RMSF values, suggesting greater stability or less mobility in this enzyme region, likely because of their crucial function in upholding the active site's structural integrity.

Solvent-accessible surface area (SASA)

SASA provides information about the solvent-accessible surface area of molecules, which can be indicative of their exposure to the solvent and potential interactions with other molecules. SASA of the active site residues in aldose reductase (PDB ID 3S3G) offers important information on the exposure and accessibility of critical residues involved in ligand binding and catalysis. The SASA values, measured in nanoseconds (ns) at various time intervals, provide a dynamic view of the interactions between the enzyme and its ligands NL, DEA, and acarbose (Fig. 5). The data shows that the SASA values vary with time for all three ligand situations. At time point zero, the SASA values for NL, DEA, and acarbose are 133.042, 131.686, and 137.56 Å², respectively, suggesting comparable solvent exposure levels for the active site residues in each ligand-bound state.

Variations in SASA values are noticed throughout the simulation at different time points and under various ligand circumstances. Changes in SASA values can indicate alterations in the conformational dynamics of active site residues caused by ligand binding or solvent interactions. At later time intervals, such as 95 ns and 100 ns, small variations in SASA values are noticed between the ligand-bound states, indicating varying solvent accessibility and possible changes in the active site environment caused by each ligand. The observations emphasize the changing nature of interactions between ligands and proteins, stressing the significance of taking into account temporal variations in SASA to fully comprehend the dynamics of enzyme-ligand binding.

Comparing SASA values under various ligand settings shows different trends in solvent exposure for the active site residues. Acarbose had somewhat greater solvent-accessible surface area (SASA) values compared to NL and DEA at most time points, suggesting a possibly more open or solvent-accessible active site environment in the presence of acarbose. On the other hand, NL and DEA have lower SASA values, indicating a more compact or constrained active site conformation. The discrepancies in SASA values could indicate variances in ligand binding modalities, affinity, or the degree of induced conformational changes in the enzyme's structure.

Gyration

Gyration, a metric indicating the density or size of a protein's structure, provides important information about the flexibility and movement of the active site residues in aldose reductase (PDB ID 3S3G) while interacting with ligands and catalyzing reactions (Fig. 6). Studying gyration values over time offers insight into how the active site conformation changes in reaction to various ligand conditions such as NL, DEA, and acarbose. At time point zero, the gyration values for the NL, DEA, and acarbose ligand conditions are generally similar, suggesting similar degrees of compactness and structural stability in the active site.

Throughout the simulation, variations in gyration values occur at various time points and under varied ligand circumstances. The variations may indicate changes in the conformational dynamics of the active site residues caused by ligand binding or solvent interactions. Subtle discrepancies in gyration values between the ligand-bound states become noticeable after 55 ns and beyond. Acarbose demonstrates somewhat higher gyration values than NL and DEA

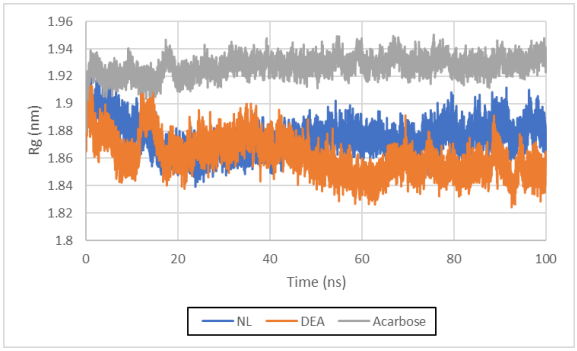

Figure 6. Plot of gyration of the native ligand (color in blue), DEA (color in orange), and acarbose as the reference compound (color in grey) on the active site of aldose reductase (PDB ID 3S3G).

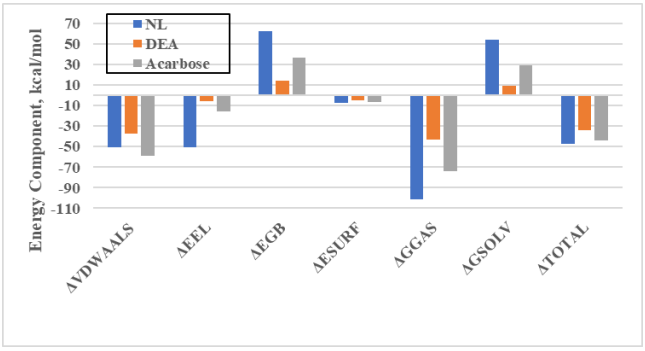

Figure 7. The energy component (ΔTotal) of the native ligand, DEA, and acarbose (reference compound) in complexes with aldose reductase (PDB ID 3S3G).

at most time intervals, indicating a potentially more prolonged or flexible active site conformation in the presence of acarbose.

Analyzing gyration values under various ligand circumstances shows diverse patterns in the structural compactness of the active site residues. Although NL and DEA often have comparable gyration profiles, acarbose regularly displays higher gyration values, suggesting a potentially more extended or solvent-accessible active site structure. The variances in gyration values may indicate variations in ligand-driven conformational changes, binding modalities, or the degree of induced flexibility within the active site of aldose reductase.

Energy component

The MMGBSA method is used to estimate the binding free energy of a protein-ligand complex. The energy components of NL and DEA ligands from the MMGBSA calculations (Fig. 7).

The energy factors related to ligand binding to the active site of aldose reductase (PDB ID 3S3G) offer vital insights into the energetics of ligand recognition and binding. An examination of the energy components, such as ΔVDWAALS, ΔEEL, ΔEGB, ΔESURF, ΔGGAS, ΔGSOLV, and ΔTOTAL, for NL, DEA, and acarbose shows unique trends in the energetics of ligand attachment. ΔVDWAALS and ΔEEL denote the binding free energy's van der Waals and electrostatic energy components, respectively. Acarbose has strong and complementary interactions with the active site residues, particularly in terms of van der Waals interactions (ΔVDWAALS = -58.63 kcal/mol) and electrostatic energy (ΔEEL = -15.25 kcal/mol).

ΔEGB represents the polar solvation energy, while ΔESURF represents the nonpolar solvation energy when the ligand binds. Acarbose has a more advantageous solvation energy profile, showing lower ΔEGB (-36.5 kcal/mol) and ΔESURF (-6.84 kcal/mol) values compared to NL and DEA. The results indicate that acarbose may have reduced negative solvation effects when binding to the active site of aldose reductase, which enhances its overall binding affinity.

The energy components ΔGGAS and ΔGSOLV indicate the changes in gas-phase and solvation-free energy when the ligand binds. Acarbose demonstrates a more favorable gas-phase energy change (ΔGGAS = -73.88 kcal/mol) than NL and DEA, suggesting stronger binding interactions with the active site residues. The solvation-free energy change (ΔGSOLV) for acarbose (-29.66 kcal/mol) is more favorable than that of NL and DEA, indicating that acarbose is less destabilized by solvent interactions during binding.

An in-depth examination of energy components offers useful insights into the energetics of ligand binding to the active site of aldose reductase. Acarbose is the most energetically favorable ligand due to its stronger van der Waals and electrostatic contacts, as well as more favorable solvation and gas-phase energy changes compared to NL and DEA. These results highlight the need to take into account various energy components when studying ligand-binding energetics and creating stronger inhibitors that target aldose reductase for treating diseases like diabetes and its consequences. Additional computational and experimental research is needed to confirm these findings and provide guidance for strategic medication development aimed at aldose reductase.

ADME prediction

SwissADME was used to estimate the metabolic and toxicity profiles of 8,9-dimethoxy ellagic acid (DEA) from *P. pellucida*, which offers useful information on its pharmacokinetic features and probable toxicity. The analysis includes multiple parameters

like molecular weight (MW), heavy atom count, sp3 carbon fraction, rotatable bond count, hydrogen bond acceptors and donors, topological polar surface area (TPSA), molecular polarizability (MR), and partition coefficients (log P values) determined through various methods.

DEA has a molecular weight of 242.27 g/mol, including 18 heavy atoms and a considerable amount of aromatic heavy atoms, suggesting a complex and potentially bioactive structure. The molecule shows moderate lipophilicity, as indicated by its logP values determined by several techniques. DEA is expected to have good solubility based on the ESOL and Ali techniques, indicating favorable aqueous solubility, which is important for medication absorption and bioavailability. DEA is expected to have significant gastrointestinal absorption in terms of pharmacokinetics, indicating good oral bioavailability. It is not expected to cross the blood-brain barrier (BBB), suggesting minimal activation in the central nervous system. DEA is not anticipated to function as a substrate or inhibitor of P-glycoprotein (Pgp), a crucial efflux transporter that plays a role in drug distribution.

DEA has a positive toxicity profile and does not show any alarms for pan-assay interference substances (PAINS) or Brenk compounds. Furthermore, it shows minimal violation scores for Lipinski, Ghose, Veber, Egan, and Muegge rules, suggesting favorable drug-like properties and potential for bioavailability. The molecule exhibits poor synthetic accessibility, indicating potential for production and development as a therapeutic candidate. SwissADME predictive study indicates that DEA has promising pharmacokinetic features and low toxicity, suggesting it could be a viable therapeutic candidate. Additional experimental validation is needed to verify these predictions and investigate the therapeutic possibilities of DEA in treating different disorders, such as diabetes.

Molecular docking was performed by placing rigid molecules or fragments into the protein's active site using clique-searching approaches (Stanzione et al., 2021). Docking can be achieved by sampling conformations of the ligand in the protein's active site and then ranking these conformations based on their binding energy (Meng et al., 2011). Post-docking interactions between active site residues of a protein and a ligand can be analyzed to understand the binding mechanism and optimize ligand design (Febrina et al., 2021). Overall, molecular docking can provide insights into the binding mechanism and optimize ligand design and can be integrated with other computational methods for a more comprehensive understanding of the interaction.

Aldose reductase is an NADPH-dependent oxidoreductase that catalyzes the reduction of a broad range of aldehydes, including glucose. Since aldose reductase has been strongly implicated in the development of the chronic complications of diabetes mellitus, much effort has been devoted to understanding the structure and mechanism of this enzyme, and many aldose reductase inhibitors have been developed as potential drugs for the treatment of these complications (Borhani et al., 1992). The crystal structure of human aldose reductase (PDB id 3S3G) complexed with tolmetin has been determined at 1.5 Å resolution (Zheng et al., 2012). This structure has been used in molecular docking studies to identify potential aldose reductase inhibitors for treating diabetes (Zhang et al., 2022). Some efforts to identify treatments for chronic diabetic complications have resulted in the discovery of a novel series of highly potent and selective aldose reductase inhibitors. These inhibitors have shown clear beneficial clinical effects on type 2 diabetes (Yang et al., 2015).

Alpha-amylase is an enzyme that catalyzes the hydrolysis of internal alpha-1,4-glycosidic linkages in starch into glucose, maltose, and maltotriose units (Srishti et al., 2022). Alpha amylase inhibitors can delay the digestion and absorption of carbohydrates, which can reduce postprandial glucose and insulin peaks (Ćorković et al., 2022). Inhibition of alpha-amylase is important for decreasing postprandial blood glucose, making it a good target for the management of diabetes (Magaji et al., 2020). Molecular docking studies have been used to identify potential inhibitors of alpha-amylase for the treatment of diabetes (Susilawati et al., 2022). *Moringa oleifera* extracts have been found to have alpha-amylase inhibitory activity, which may be useful for the management of diabetes.

Alpha-glucosidase is an enzyme that catalyzes the hydrolysis of α-glucosidic linkages of carbohydrates into monosaccharides that can be absorbed. Alpha-glucosidase inhibitors can delay the absorption of ingested carbohydrates, which can reduce postprandial glucose and insulin peaks (Behl et al., 2022). The crystal structure of alpha-glucosidase (PDB id 2QMJ) has been used in molecular docking studies to identify potential inhibitors of alpha-glucosidase for the treatment of diabetes (Siregar et al., 2020). Alpha-glucosidase inhibitors from plants have been identified as potential candidates for the treatment of type-2 diabetes (Dirir et al., 2022).

Dipeptidyl peptidase IV (DPP-IV) is an enzyme that cleaves incretin hormones, which regulate insulin secretion and glucose homeostasis, and is a potential target for the treatment of type 2 diabetes mellitus (Guasch et al., 2012). DPP-IV inhibitors are a class of

oral hypoglycemics that work by inhibiting the action of DPP-IV, thereby prolonging the incretin effect *in vivo* (Singh et al., 2021). *In silico* prediction has been used to identify novel DPP-IV inhibitors of natural origin for the treatment of type 2 diabetes mellitus.

The insulin receptor (IR) is a transmembrane receptor that is activated by insulin, IGF-I, and IGF-II and belongs to the large class of receptor tyrosine kinase (Escribano et al., 2017). The insulin receptor plays a key role in the regulation of glucose homeostasis, and its dysregulation can cause metabolic diseases such as diabetes (Hall et al., 2020). The crystal structure of the insulin receptor tyrosine kinase domain (PDB id 1IR3) has been determined and used in molecular docking studies to identify potential inhibitors of the insulin receptor for the treatment of diabetes (Li et al., 2014). The insulin receptor activates a complex intracellular signaling network through IRS proteins and the canonical PI3K and ERK cascades, which play a role in glucose homeostasis and insulin resistance in diabetes (De Meyts, 2016). The insulin receptor gene undergoes differential splicing that generates two IR isoforms, IR-A and IR-B, which have different physiological roles in glucose homeostasis and insulin resistance in diabetes (Belfiore et al., 2017). Insulin receptor endocytosis is a key mechanism that regulates the intensity and duration of insulin signaling, and its dysregulation can contribute to insulin resistance and diabetes (Hall et al., 2020).

CONCLUSION

DEA ligands have been presented and analyzed for their interactions in inhibiting several antidiabetic enzymes, both in terms of molecular docking and molecular dynamics. The molecular docking interaction analysis revealed that the ligand DEA interacted better in the active pocket of aldose reductase, with a binding free energy of -10.3 kcal/mol. However, molecular dynamics studies demonstrate that the ligand DEA's ability to stabilize the complex was still weaker than that of the native ligand and acarbose (reference compound).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

The authors thank Universitas Padjadjaran Grants (HIU), which funded this research through the Riset Data Pustaka dan Daring (RDPD) scheme from the Directorate of Research and Community Services, Universitas Padjadjaran No.1549/UN6.3.1/PT.00/2023.

REFERENCES

Alves NSF, Setzer WN, da Silva JKR (2019) The chemistry and biological activities of *Peperomia pellucida* (Piperaceae): A critical review. J Ethnopharmacol 232: 90–102. https://doi.org/10.1016/j.jep.2018.12.021

American Diabetes Association (2013) Diagnosis and classification of diabetes mellitus. Diabetes Care 37: S81–S90. https://doi.org/10.2337/dc14-S081

Aryaeian N, Sedehi SK, Arablou T (2017) Polyphenols and their effects on diabetes management: A review. Med J Islam Repub Iran 31: 134. https://doi.org/10.14196/mjiri.31.134

Asnawi A, Aman LO, Nursamsiar, Yuliantini A, Febrina E (2022) Molecular docking and molecular dynamic studies: Screening phytochemicals of *Acalypha indica* against Braf kinase receptors for potential use in melanocytic tumours. Rasayan J Chem 15: 1352–1361. https://doi.org/10.31788/RJC.2022.1526769

Asnawi A, Nedja M, Febrina E, Purwaniati P (2023) Prediction of a stable complex of compounds in the ethanol extract of celery leaves (*Apium graveolens* L.) function as a VKORC1 antagonist. Trop J Nat Prod Res 7: 2362–2370. http://www.doi.org/10.26538/tjnpr/v7i2.10

Atkinson MA, Eisenbarth GS, Michels AW (2014) Type 1 diabetes. Lancet 383: 69–82. https://doi.org/10.1016/S0140-6736(13)60591-7

Behl T, Gupta A, Albratty M, Najmi A, Meraya AM, Alhazmi HA, Anwer MK, Bhatia S, Bungau SG (2022) Alkaloidal phytoconstituents for diabetes management: Exploring the unrevealed potential. Molecules 27: 5851. https://doi.org/10.3390/molecules27185851

Belfiore A, Malaguarnera R, Vella V, Lawrence MC, Sciacca L, Frasca F, Morrione A, Vigneri R (2017) Insulin receptor isoforms in physiology and disease: An updated view. Endocrine Rev 38: 379–431. https://doi.org/10.1210/er.2017-00073

Borhani DW, Harter TM, Petrash JM (1992) The crystal structure of the aldose reductase.NADPH binary complex. J Biol Chem 267: 24841–24847. https://doi.org/10.2210/pdb1abn/pdb

Boy HIA, Rutilla AJH, Santos KA, Ty AMT, Yu AI, Mahboob T, Tangpoong J, Nissapatorn V (2018) Recommended medicinal plants as source of natural products: A review. Digit Chin Med 1: 131–142. https://doi.org/10.1016/S2589-3777(19)30018-7

Clemen-Pascual LM, Macahig RAS, Rojas NRL (2022) Comparative toxicity, phytochemistry, and use of 53 Philippine medicinal plants. Toxicol Rep 9: 22–35. https://doi.org/10.1016/j.toxrep.2021.12.002

Ćorković I, Gašo-Sokač D, Pichler A, Šimunović J, Kopjar M (2022) Dietary polyphenols as natural inhibitors of α-amylase and α-glucosidase. Life (Basel) 12: 1692. https://doi.org/10.3390/life12111692

Cragg GM, Newman DJ (2013) Natural products: A continuing source of novel drug leads. Biochim Biophys Acta 1830: 3670–3695. https://doi.org/10.1016/j.bbagen.2013.02.008

de Fátima Arrigoni-Blank M, Dmitrieva EG, Franzotti EM, Antoniolli AR, Andrade MR, Marchioro M (2004) Anti-inflammatory and analgesic activity of *Peperomia pellucida* (L.) HBK (Piperaceae). J Ethnopharmacol 91: 215–218. https://doi.org/10.1016/j.jep.2003.12.030

De Meyts P (2016) The insulin receptor and its signal transduction network. Endotext [Internet]. Feingold KR, Anawalt B, Blackman MR, et al., editors. South Dartmouth (MA): MDText.com, Inc.; 2000. https://www.ncbi.nlm.nih.gov/books/NBK378978/

DeFronzo RA, Ferrannini E, Groop L, Henry RR, Herman WH, Holst JJ, Hu FB, Kahn CR, Raz I, Shulman GI, Simonson DC,

Testa MA, Weiss R (2015) Type 2 diabetes mellitus. Nat Rev Dis Primers 1: 15019. https://doi.org/10.1038/nrdp.2015.19

Dirir AM, Daou M, Yousef AF, Yousef LF (2022) A review of alpha-glucosidase inhibitors from plants as potential candidates for the treatment of type-2 diabetes. Phytochem Rev 21: 1049–1079. https://doi.org/10.1007/s11101-021-09773-1

Elam E, Feng J, Lv Y-M, Ni Z-J, Sun P, Thakur K, Zhang J-G, Ma Y-L, Wei Z-J (2021) Recent advances on bioactive food derived anti-diabetic hydrolysates and peptides from natural resources. J Funct Foods 86: 104674. https://doi.org/10.1016/j.jff.2021.104674

Escribano O, Beneit N, Rubio-Longás C, López-Pastor AR, Gómez-Hernández A (2017) The role of insulin receptor isoforms in diabetes and its metabolic and vascular complications. J Diabetes Res 2017: 1403206. https://doi.org/10.1155/2017/1403206

Febrina E, Alamhari RK, Asnawi A, Abdulah R, Lestari K, Levita J, Supratman U (2021) Molecular docking and molecular dynamics studies of *Acalypha indica* L. phytochemical constituents with caspase-3. Int J App Pharm 13: 210–215. https://doi.org/10.22159/ijap.2021.v13s4.43861

Febrina E, Asnawi A, Abdulah R, Lestari K, Supratman U (2022) Identification of flavonoids from *Acalypha indica* L. (Euphorbiaceae) as caspase-3 activators using molecular docking and molecular dynamics. International J App Pharm 14: 162–166. https://doi.org/10.22159/ijap.2022.v14s5.34

Guasch L, Sala E, Ojeda MJ, Sala E, Cereto-Massagué A, Mulero M, Valls C, Pinent M, Ardévol A, Garcia-Vallvé S, Pujadas G (2012) Identification of novel human dipeptidyl peptidase-IV inhibitors of natural origin (Part II): *In silico* prediction in antidiabetic extracts. PLoS ONE 7(9): e44971. https://doi.org/10.1371/journal.pone.0044971

Hall C, Yu H, Choi E (2020) Insulin receptor endocytosis in the pathophysiology of insulin resistance. Exp Mol Med 52: 911–920. https://doi.org/10.1038/s12276-020-0456-3

Ischak NI, Aman LO, Hasan H, Kilo AL, Asnawi A (2023) *In silico* screening of *Andrographis paniculata* secondary metabolites as anti-diabetes mellitus through PDE9 inhibition. Res Pharm Sci 18: 100–111. https://doi.org/10.4103/1735-5362.363616

Karagiannis T, Paschos P, Paletas K, Matthews DR, Tsapas A (2012) Dipeptidyl peptidase-4 inhibitors for treatment of type 2 diabetes mellitus in the clinical setting: Systematic review and meta-analysis. BMJ 344: e1369. https://doi.org/10.1136/bmj.e1369

Kusuma SAF, Wardhani P, Febrina E (2017) Stool form scale as an indicator of klutuk banana (*Musa balbisiana* Colla) fruit extracts inhibition effect against *Shigella dysenteriae* Atcc 13313 *in vivo*. Asian J Pharm Clin Res 10: 266–268. https://doi.org/10.22159/ajpcr.2017.v10i12.21592

Li Q, Wong YL, Kang C (2014) Solution structure of the transmembrane domain of the insulin receptor in detergent micelles. Bioch Biophys Acta 1838: 1313–1321. https://doi.org/10.1016/j.bbamem.2014.01.005

Magaji U, Sacan O, Yanardag R (2020) Alpha amylase, alpha glucosidase and glycation inhibitory activity of *Moringa oleifera* extracts. S Afr J Bot 128: 225–230. https://doi.org/10.1016/j.sajb.2019.11.024

Meng X-Y, Zhang H-X, Mezei M, Cui M (2011) Molecular docking: A powerful approach for structure-based drug discovery. Curr Comput Aided Drug Des 7: 146–157. https://doi.org/10.2174/157340911795677602

Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ (2009) AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem 30: 2785–2791. https://doi.org/10.1002/jcc.21256

Ogurtsova K, Guariguata L, Barengo NC, Ruiz PL-DR, Sacre JW, Karuranga S, Sun H, Boyko EJ, Magliano DJ (2022) IDF diabetes Atlas: Global estimates of undiagnosed diabetes in adults for 2021. Diabetes Res Clin Pract 183: 109118. https://doi.org/10.1016/j.diabres.2021.109118

Rahman H, Bintang MI, Asnawi A, Febrina E (2023) Exploring the molecular interactions between volatile compounds in coconut shell liquid smoke and human bitter taste TAS2R46 based on the molecular docking and molecular dynamics. Trop J Nat Prod Res 7: 5587–5594 http://www.doi.org/10.26538/tjnpr/v7i12.31

Singh A-K, Yadav D, Sharma N, Jin J-O (2021) Dipeptidyl peptidase (DPP)-IV inhibitors with antioxidant potential isolated from natural sources: A novel approach for the management of diabetes. Pharmaceuticals 14: 586. https://doi.org/10.3390/ph14060586

Siregar M, Awaluddin A, Nurnahari N, Nur S, Febrina E, Asnawi A (2020) Molecular docking and molecular dynamic simulation of the aglycone of curculigoside a and its derivatives as alpha glucosidase inhibitors. Rasayan J Chem 13: 690–698. http://dx.doi.org/10.31788/RJC.2020.1315577

Srishti K, Rohit R, Rashmi K (2022) Microbial production of amylase using lignocellulosic biomass: Recent developments and prospects. Res J Biotechnol 17: 192–199. http://dx.doi.org/10.25303/1705rjbt192199

Stanzione F, Giangreco I, Cole JC (2021) Use of molecular docking computational tools in drug discovery. Prog Med Chem 60: 273–343. https://doi.org/10.1016/bs.pmch.2021.01.004

Susilawati Y, Megantara S, Levita J (2022) Antidiabetic activity of novel chromene compound isolated from *Peperomia pellucida* L. Kunth and *in silico* study against DPP-IV, alpha-glucosidase, alpha-amylase, and aldose reductase for blood glucose homeostasis. Int J App Pharm 14: 110–116. https://doi.org/10.22159/ijap.2022.v14s5.22

Susilawati Y, Nugraha R, Krishnan J, Muhtadi A, Sutardjo S, Supratman U (2017) A new antidiabetic compound 8, 9-dimethoxy ellagic acid from sasaladaan (*Peperomia pellucida* L. Kunth). Res J Pharm Biol Chem Sci 8: 269–274.

Susilawati Y, Nugraha R, Muhtadi A, Soetardjo S, Supratman U (2015) (S)-2-Methyl-2-(4-methylpent-3-enyl)-6-(propan-2-ylidene)-3,4,6,7-tetrahydropyrano[4,3-g]chromen-9(2H)-one. Molbank 2015: M855. https://doi.org/10.3390/M855

Torres PHM, Sodero ACR, Jofily P, Silva-Jr FP (2019) Key topics in molecular docking for drug design. Int J Mol Sci 20: 4574. https://doi.org/10.3390/ijms20184574

Wei LS, Wee W, Siong JYF, Syamsumir DF (2011) Characterization of anticancer, antimicrobial, antioxidant properties and chemical compositions of *Peperomia pellucida* leaf extract. Acta Med Iran 49: 670–674. https://pubmed.ncbi.nlm.nih.gov/22071643/

Yan L-S, Cheng BC-Y, Zhang S-F, Luo G, Zhang C, Wang Q-G, Fu X-Q, Wang Y-W, Zhang Y (2021) Tibetan medicine for diabetes mellitus: Overview of pharmacological perspectives. Front Pharmacol 12: 748500. https://doi.org/10.3389/fphar.2021.748500

Yang S, Qin X, Luo T, Hao X, Zhu C (2015) Novel Nitro Derivatives of Benzothiadiazine 1, 1-Dioxide as Aldose Reductase Inhibitors. Proceedings of the 2015 International Conference on Industrial Technology and Management Science. Atlantis Press, pp. 1077–1080. https://doi.org/10.2991/itms-15.2015.260

Yayla M, Binnetoğlu D (2022) Experimental approaches to diabetes mellitus. Eurasian J Med 54: 145–153. https://doi.org/10.5152/eurasianjmed.2022.22304

Yuliantini A, Ocktavyanie S, Febrina E, Asnawi A (2024) Virtual screening using a ligand-based pharmacophore model from

ashitaba (*Angelica keiskei* K.) isolates and molecular docking to obtained new candidates as -glucosidase inhibitors. Trop J Nat Prod Res 8: 5811–5819. http://www.doi.org/10.26538/tjnpr/v8i1.15

Zhang X, Xu L, Chen H, Zhang X, Lei Y, Liu W, Xu H, Ma B, Zhu C (2022) Novel hydroxychalcone-based dual inhibitors of aldose reductase and α-glucosidase as potential therapeutic agents against diabetes mellitus and its complications. J Med Chem 65: 9174–9192. https://doi.org/10.1021/acs.jmedchem.2c00380

Zheng X, Zhang L, Zhai J, Chen Y, Luo H, Hu X (2012) The molecular basis for inhibition of sulindac and its metabolites towards human aldose reductase. FEBS Lett 586: 55–59. https://doi.org/10.1016/j.febslet.2011.11.023

Zimmet P, Alberti KGMM, Shaw J (2001) Global and societal implications of the diabetes epidemic. Nature 414: 782–787. https://doi.org/10.1038/414782a

AUTHOR CONTRIBUTION:

Contribution	Susilawati Y	Indradi RB	Asnawi A	Febrina E
Concepts or ideas	x			x
Design	x	x	x	x
Definition of intellectual content	x	x	x	x
Literature search	x	x	x	x
Experimental studies	x	x	x	x
Data acquisition	x	x	x	x
Data analysis	x	x	x	x
Statistical analysis	x	x	x	x
Manuscript preparation	x	x	x	x
Manuscript editing	x	x	x	x
Manuscript review	x	x	x	x

Citation Format: Susilawati Y, Indradi RB, Asnawi A, Febrina E (2024) Molecular docking and dynamics studies of 8,9-dimethoxy ellagic acid contained in *Peperomia pellucida* (L.) Kunth against various diabetes mellitus receptors. J Pharm Pharmacogn Res 12(5): 929–942. https://doi.org/10.56499/jppres23.1936_12.5.929