Int J Adv Life Sci Res. Volume 9(1) 157-170
https://doi.org/10.31632/ijalsr.2026.v09i01.012

International Journal of Advancement in Life Sciences Research

Online ISSN: 2581-4877

Journal homepage http://ijalsr.org

Original Article

Screening and Profiling of Bioactive Compounds from Moringa Oleifera Fruit Interaction with Advanced Glycation End Product Protein: A Molecular Docking Approach for Anti-Atherosclerosis Candidate Identification

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Abstract

Diabetes mellitus represents a rapidly expanding worldwide health challenge, with estimates suggesting it will impact 643 million adults by 2030, with atherosclerosis representing a primary complication that substantially elevates disease burden and mortality rates. Chronic hyperglycemia compromises vascular equilibrium, resulting in endothelial impairment and atheromatous plaque development, potentially causing fatal thrombotic complications. Advanced glycation end products (AGEs) and their corresponding receptor RAGE are pivotal in diabetic vascular pathology, accelerating atherosclerotic development through both direct and indirect pathways. Moringa oleifera show potential as a natural therapeutic intervention with anti-glycation capabilities, although investigation of active constituents in M. oleifera fruit for RAGE protein inhibition remains insufficient. This study aimed to evaluate the capacity of bioactive compounds from the oleifera fruit to bind with the RAGE protein using computational analysis. The RAGE protein configuration (PDB ID: 3O3U) was retrieved from the RCSB database, while test compounds containing M. oleifera fruit metabolites were obtained from the Phytochem database. Molecular docking analysis was performed using PyRx software with AutoDock Vina. Drug-like characteristics were evaluated through SwissADME and pkCSM platforms, applying Lipinski's criteria. Protein-ligand visualization was performed using Biovia Discovery Studio and PyMol. This study revealed that nine bioactive compounds showed favourable RAGE protein binding with negative Gibbs free energy (-3.3 to -7.7 kcal/mol). Riboflavin demonstrated optimal binding affinity (-7.7 kcal/mol), followed by thiamine (-7.4 kcal/mol) and indole acetonitrile (-7.0 kcal/mol). These compounds established hydrogen bonds with 5-8 essential amino acid residues, resembling native ligand binding patterns. This study shows that riboflavin and thiamine exhibit strong RAGE protein binding affinity, representing promising therapeutic candidates for anti-atherosclerosis treatment targeting AGE-RAGE pathways in diabetic complications. Further studies should further validated using in-vitro, in-vivo, and clinic-based phase trials.

Keywords: *Atherosclerosis; Diabetes Complications; Molecular Docking; Moringa Oleifera Fruit; RAGE Protein*

Introduction

Diabetes mellitus represents a rapidly expanding worldwide health challenge, with estimates suggesting it will impact 643 million adults by 2030 (Ye et al., 2022). Among diabetic patients, atherosclerosis

Received on :17th July 2025; Revised version received on :14th December 2025; Accepted: 27st December 2025

stands out as a common complication that substantially increases illness and death rates arising (Poznyak et al., 2020). Research extensively documents how diabetic complications typically stem from atherosclerotic processes, indicating that persistent high blood sugar levels interfere with blood vessel stability and impair endothelial function, thereby initiating a cascade of harmful processes that result in atherosclerosis. During atherosclerotic progression, plaque accumulates within the inner arterial wall layer, and when this plaque eventually breaks apart, it triggers blood clot formation. The combination of reduced blood vessel diameter and clot-related incidents frequently results in life-threatening outcomes (Sala et al., 2019; Poznyak et al., 2020; Ye et al., 2022).

High blood sugar is the main factor causing diabetes complications through induction of oxidative stress which forms the basis of cellular and tissue damage (Nathan et al., 2013). This relates to the concept of "metabolic memory" or legacy effect, where biological molecular damage during periods of high blood sugar and long-term exposure to oxidative stress and advanced glycation end products (AGEs) and its cell receptor RAGE can cause lasting genetic changes (Ceriello et al., 2012). The risk of complications remains despite various combination treatments being provided (Ceriello et al., 2012). AGEs are a group of compounds formed through non-enzymatic reactions between reducing sugars and proteins, lipids, or nucleic acids, which accumulate in nearly all mammalian tissues and are associated with diabetes, cardiovascular disease and atherosclerosis (Pinto et al., 2022). Advanced glycation end products promote vascular damage and acceleration of atherosclerotic plaque progression mainly through two mechanisms: directly, altering the functional properties of vessel wall extracellular matrix molecules, or indirectly, through activation of cell receptor-dependent signalling pathways (Pinto et al., 2022). Targeting AGEs presents a viable therapeutic strategy for mitigating cardiovascular complications in diabetic populations, encompassing both preventive approaches to limit AGE synthesis and interventional methods to block AGE-mediated biological pathways.

Moringa oleifera has emerged as a promising natural therapeutic agent for inhibiting advanced glycation end-product (AGE) formation through multiple biochemical mechanisms (du Toit, 2021). The aqueous leaf extract of M. oleifera exhibits significant anti-glycation properties against both glucose and fructose-induced protein glycation, effectively preventing the formation of fluorescent AGEs (Aekthammarat et al., 2019). This AGE inhibitory capacity positions M. oleifera as a valuable therapeutic intervention for AGE-atherosclerosis pathology (Chumark et al., 2008). Previous research has shown that oleifera extract can inhibit AGE protein activity (Adeniran & Mogale, 2021). However, there has been no research on the active compounds in oleifera fruit to inhibit RAGE protein activity. Hence, the purpose of this study is to determine the ability of an active chemical found in oleifera fruit to interact with RAGE protein through in silico analysis.

Materials and Methods

Ligand and Protein Preparation

The structure of the RAGE protein was obtained from the RSCB protein data bank (https://www.rcsb.org/) (PDB Format ID 3O3U) (Berman, 2000; Park & Boyington, 2010). Meanwhile, the list of test ligands was the secondary metabolites of fruit oleifera obtained from Phytochem data (https://phytochem.nal.usda.gov/). A total of 23 phytochemicals were initially obtained from the phytochemical database for oleifera fruit. However, 14 entries were excluded because they represented inorganic elements/macronutrients (e.g., minerals and water) rather than bioactive phytochemical compounds. Therefore, 9 phytochemicals were selected and included for subsequent physicochemical property prediction (Lipinski's Rule of Five) and ADMET analysis. All the 3D structures and SMILES format of test ligands were downloaded from the PubChem website (http://pubchem.ncbi.nlm.nih.gov) (Kim et al., 2025). Open Babel online was used to convert the ligands from SMILES into PDBQT format in PyRx software (Dallakyan & Olson, 2014).

Drug Likeness Assessment and Toxicity Prediction

To find out the drug likeness assessment and toxicity of the test ligand as a candidate for drug ingredients using SwissADME (http://www.swissadme.ch/index.php) and pKcSM pharmacokinetic https://biosig.lab.uq.edu.au/pkcsm/ (Daina et al., 2017).

Molecular Docking and Visualization

All procedures were performed using the software Windows 11 Home Single Language 64-bit operating system, Intel ® Core (TM) i3-10110U CPU @ 2.10 GHz (8 CPUs), ~1.20 GHz. The binding/active site of the RAGE protein was defined based on the position of the co-crystallized native ligand in the PDB structure (3O3U). The docking grid box was centered on the native ligand to cover the binding pocket and surrounding residues. The grid box parameters in AutoDock Vina were set as center_x = 40, center_y = 46, and center_z = 40, with dimensions size_x = 21.041 Å, size_y = 20.25 Å, and size_z = 69.701 Å. Docking protocol validation was performed by redocking the co-crystallized native ligand into the binding site of the RAGE protein (PDB ID: 3O3U) using PyRx (AutoDock Vina) with the same grid box coordinates and docking parameters applied for the test ligands. The resulting docking pose of the redocked native ligand or best pose with the lowest binding energy was then superimposed with the original crystallographic native ligand, and the RMSD value was calculated to quantify the deviation between both ligand conformations. RMSD was calculated by comparing the heavy-atom coordinates of the redocked ligand against the crystallographic native ligand. The docking protocol was considered valid when the RMSD value was ≤ 2.0 Å. Molecular docking was performed in PyRx using AutoDock Vina via the Vina Wizard module. Ligand-protein interactions were visualized using Biovia Discovery Studio Visualizer v21.1.0.20298 software (Dassault Systèmes, San Diego, California, USA) and PyMOL software version 4.6.0 (Schrödinger LLC).

Result

Ligand Screening

A data set of bioactive compound oleifera fruit (ligands) was obtained from the phytochem database. From the phytochemical dataset, 23 compounds/entries were retrieved. After excluding 14 non-phytochemical entries (mainly inorganic elements/macronutrients), 9 phytochemicals were retained for further analysis. The structure and PubChem identity number are presented in Figure 1. Drug-likeness screening of the selected ligands was performed using the Lipinski rules of five requirements. Based on the screening results, all 9 compounds satisfied Lipinski's criteria. The result of this study shows that ligand molecular weights between 90.03 g/mol - 376.36 g/mol, with log P values spanning -1.86 to 4.22. Hydrogen bond donor counts varied from 1 to 4, while hydrogen bond acceptor values ranged from 1 to 8 (Syahputra et al., 2021). Therefore, these 9 compounds were chosen as potential candidate ligands for molecular docking analysis (Table 1).

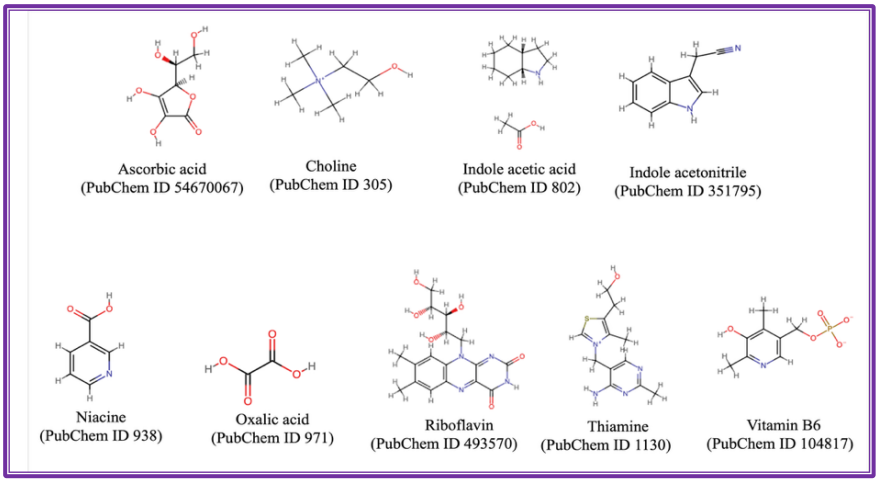

Figure 1: *Structure and Pubchem Identity Number of Bioactive Compound Oleifera Fruit*

Table 1: *Physicochemical and Drug Likeness Properties Prediction of Compounds Based on Lipinski Rule of 5 (Ro5)*

Compound	MW g/mol	LogP	H-bond donors	H-bond acceptor	Violation	Bioavailability
Ascorbic acid	176.12	4.22	4	6	0	0.56
Choline	104.17	-1.86	1	1	0	0.55
Indole acetic acid	185.26	1.30	2	3	0	0.55
Indole acetonitrile	156.18	1.83	1	1	0	0.55
Niacin	123.11	0.32	1	3	0	0.85
Oxalic acid	90.03	-0.79	2	4	0	0.85
Riboflavin	376.36	-0.19	5	8	0	0.55
Thiamine	265.35	0.53	2	3	0	0.55
Vitamin B6	231.14	0.17	1	6	0	0.56

ADMET Prediction

Pharmacokinetics investigates how a medicinal drug interacts inside biological systems, with a particular emphasis on four essential processes: absorption (A), distribution (D), metabolism (M), and excretion (E). Table 2 shows the ADME and toxicity (T) data for the most promising leads (MPLs). Drug candidates fail at a high rate during clinical development due to inadequate ADMET characteristics. Thus, assessing drug candidates' ADMET profiles before starting clinical trials is a sensible and cost-effective strategy for drug discovery. The investigation found that all of the bioactive compounds from oleifera fruit, which are tested ligands, could be absorbed through the gastrointestinal tract. In addition, the blood-brain barrier (BBB) is made up of specialized microvascular endothelial cells in the brain that form a selective barrier between cerebral tissue and systemic circulation, protecting the central nervous system (CNS) from dangerous substances (König et al., 2013; Wang et al., 2015).

The ADMET screening results for the selected ligands are presented in Table 2. All ligands showed predicted gastrointestinal absorption. BBB permeability prediction indicated that several ligands, including ascorbic acid, choline, oxalic acid, riboflavin, thiamine, and vitamin B6, were predicted as non-BBB permeant. All ligands were predicted as non-substrates of P-glycoprotein (P-gp), except thiamine, which was predicted as a P-gp substrate. Acute oral toxicity predictions showed low toxicity profiles, with LD50 values ranging from 1.063–2.672 mol/kg or 151.25–816.70 g/kg body weight. All compounds were classified as Class 6 (Relatively harmless ≥15 g/kg body weight) according to BPOM RI criteria and Category IV (LD50 > 5000 mg/kg) in standard toxicity frameworks (Gadaleta et al., 2019; Setiani et al., 2023).

Table 2: *ADMET Profiles of Bioactive Compound Oleifera Fruit*

Compound	Pharmacokinetic	Oral Rat Acute Toxicity (LD50) (mol/kg)	Oral Rat Acute Toxicity (LD_{50}) (g/kg)	Class	Classification
Ascorbic acid	P-gp substrate: No GI Absorption: High BBB permeation: No	1.063	187.22	6	Relatively Harmless
Choline	P-gp substrate: No GI Absorption: Low BBB permeation: No	1.939	201.99	6	Relatively Harmless
Indole acetic acid	P-gp substrate: No GI Absorption: High BBB permeation: Yes	2.104	389.79	6	Relatively Harmless
Indole acetonitrile	P-gp substrate: No GI Absorption: High BBB permeation: Yes	2.339	365.31	6	Relatively Harmless
Niacin	P-gp substrate: No GI Absorption: High BBB permeation: Yes	2.24	275.77	6	Relatively Harmless

Oxalic acid	P-gp substrate: No GI Absorption: High BBB permeation: No	1.68	151.25	6	Relatively Harmless
Riboflavin	P-gp substrate: No GI Absorption: Low BBB permeation: No	2.17	816.70	6	Relatively Harmless
Thiamine	P-gp substrate: Yes GI Absorption: High BBB permeation: No	2.672	709.02	6	Relatively Harmless
Vitamin B6	P-gp substrate: No GI Absorption: High BBB permeation: No	2.227	514.75	6	Relatively Harmless

Protein Preparation

The RAGE Protein was obtained from protein data bank on three-dimensional structure models using PDB ID 3O3U. The protein visualization depicted in the figure 2 illustrates the three-dimensional structure of the RAGE protein. Figure 2a shows the structural 3D RAGE protein before preparation that used to analyse molecular docking. This protein possesses a multi-domain architecture predominantly composed of β-sheet secondary structural elements, several α-helix segments, and loop regions connecting the structural elements. For molecular docking analysis purposes, the protein structure requires specific preparation to optimize computational processes and result accuracy. The preparation steps performed include removal of water molecules and elimination of bound ions, as well as selection of chain A (auth N) as the primary target domain relevant for ligand interactions. The RAGE protein structure that has undergone this preparation process is displayed in Figure 2B, which shows the single domain (chain A auth N) that has been cleaned of non-essential components and is ready for use in molecular docking analysis with a focus on specific binding sites.

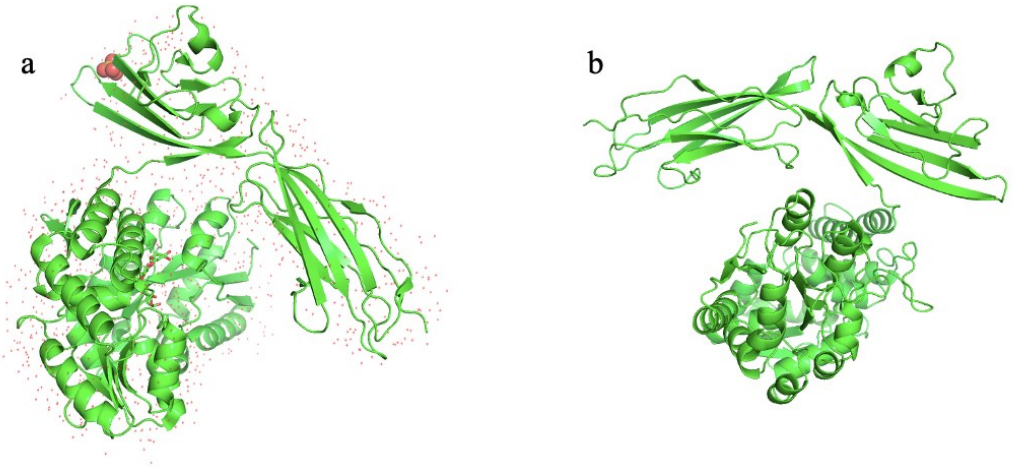

***Figure 2**: a. Three-Dimension Structure Protein AGE Before Preparation; B. Three-Dimension Structure Protein AGE After Preparation*

Before performing molecular docking on AGE proteins, a grid box is required in Autodock Vina. The binding site was determined based on the position of the native (co-crystallized) ligand in the protein structure. The grid box parameters used for docking were center X: 40, Y: 46, Z: 40, with box size x: 21.041, y: 20.25, and z: 69.70. This is important to explore the binding area between ligands/natural compounds from the oleifera fruit and AGE proteins. This binding area includes the protein's active site, binding pocket, and surrounding areas that allow for interaction. The three-dimensional structure is shown in Figure 2A. Protein preparation included removal of water molecules and bound ions, and selection of chain A (auth N) as the target docking chain. The prepared protein is shown in Figure 2B. The docking binding site was defined based on the position of the native (co-crystallized) ligand in the protein structure.

Molecular Docking

The molecular recognition between protein targets and small-molecule ligands constitutes the fundamental mechanistic basis underlying therapeutic intervention in drug development. Quantitative assessment of protein-ligand binding affinity, defined as the thermodynamic measure of association strength between a pharmacologically active compound and its cognate protein target, represents a critical computational parameter in structure-based virtual screening methodologies employed in rational drug design (Gu et al., 2023). The magnitude of binding affinity is governed by the cumulative contribution of non-covalent intermolecular forces, encompassing hydrogen bonding networks, van der Waals dispersion forces, electrostatic charge interactions, and hydrophobic clustering effects established between the ligand molecule and specific amino acid residues within the protein's active site architecture (Bulusu & Desiraju, 2020; Madushanka et al., 2023; Nivatya et al., 2025). Computational molecular docking simulations demonstrated that all nine bioactive phytochemical constituents isolated from the oleifera fruit exhibited thermodynamically favourable binding interactions with the receptor for advanced glycation end-products (RAGE) protein (PDB ID:3O3U), as quantified by negative Gibbs free energy values ranging from -3.3 to -7.4 kcal/mol, indicating spontaneous complex formation and stable protein-ligand associations.

All nine ligands demonstrated favourable docking interactions with the RAGE protein (PDB ID: 3O3U), with predicted binding free energies (ΔG) ranging from −3.3 to −7.4 kcal/mol. The native ligand showed the strongest binding affinity (−9.3 kcal/mol), used as a positive control. Among tested ligands, riboflavin exhibited the highest binding affinity (−7.7 kcal/mol), followed by thiamine (−7.4 kcal/mol) and indole acetonitrile (−7.0 kcal/mol). Moderate binding affinities were observed for vitamin B6 (−6.8 kcal/mol), ascorbic acid (−5.5 kcal/mol), and niacin (−5.3 kcal/mol). The weakest binding compounds were oxalic acid (−3.7 kcal/mol), choline (−3.4 kcal/mol), and indole acetic acid (−3.3 kcal/mol).

Docking protocol validation was conducted by redocking the native ligand, producing an RMSD value of ≤2.0 Å, indicating acceptable docking reliability (Nivatya et al., 2025; Pulido et al., 2014). The detailed interaction profiles, including hydrogen bond lengths, are summarized in Table 3, while key interaction diagrams are shown in Figures 3–4.

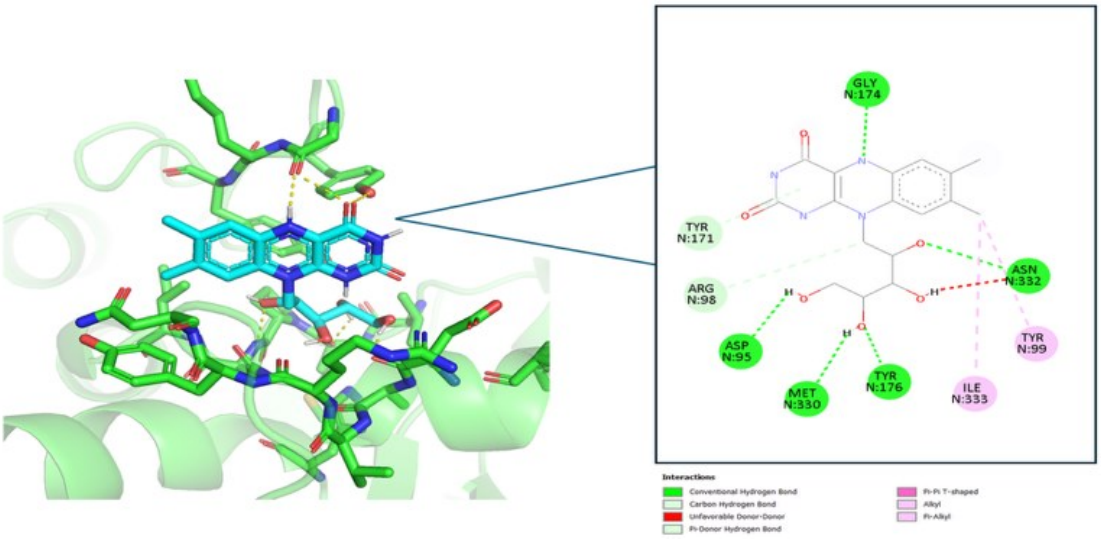

Figure 3: *2D Diagram of Interaction of Riboflavin as A Promising Bioactive Compound from The Oleifera Fruit*

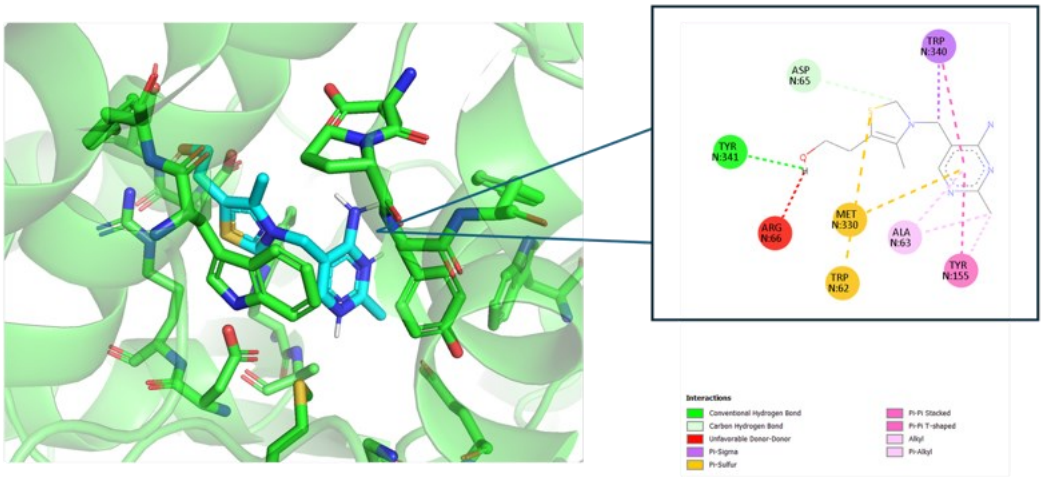

Figure 4: *2D Diagram of Interaction of the Thiamine as Promising Bioactive Compound from the Oleifera Fruit*

Table 3: *Binding Affinity (ΔG), RMSD, And Interaction Profiles of Oleifera Fruit Ligands with RAGE Protein (PDB ID: 3O3U)*

Ligand	ΔG (kcal/mol)	RMSD (Å)	Hydrogen bonds (distance Å)	π / Hydrophobic interactions	Electrostatic / other interactions	van der Waals contacts
Native ligand	-9.3	0.0	ASP14 (2.70); **TYR155** (3.15); GLU111 (2.71); LYS15 (3.03); **GLU153** (2.70); **GLU44** (2.83); **ARG66** (2.69); **TRP62** (2.74); **ASP65** (2.88)	**TRP340** (π–sigma, 3.85)	**PRO154** (C–H bond, 3.19)	**ALA63**; **MET330**; PHE156; ASN12; **ARG344**; **TYR341**; GLU45; TRP230
Ascorbic acid	-5.5	0.0	ARG98 (2.63); ASN332 (2.85)	–	–	ASP95; ILE329; ALA96; **MET330**; HIS64; TYR176; PRO331; ILE333; TYR99
Choline	-3.4	0.0	–	–	GLU328 (attractive charge, 4.10); PHE169 (C–H bond, 3.56)	LYS256; TYR171; LYS170; TYR167; TYR176; ALA168; PHE258; PRO331

Indole acetic acid	-3.3	0.0	–	–	PHE169 (donor–donor, 2.10); ALA168 (acceptor–acceptor, 2.74)	PRO331; ILE329; TYR176; PHE258; GLU328; TYR167; TRP158
Indole acetonitrile	-7.0	0.0	**GLU153** (2.53)	**TRP340** (π–π stacked, 4.14); **PRO154** (π–alkyl, 5.45)	SER337 (C–H bond)	**ASP65**; **TYR155**; **ARG344**; **GLU44**; **ARG66**; **TYR341; TRP62**
Niacin	-5.3	0.0	**ASP65** (2.34)	**TRP340** (π–π stacked, 4.01); **PRO154** (π–alkyl, 5.40)	–	–
Oxalic acid	-3.7	0.0	ALA168 (2.20); PHE169 (2.94)	–	–	TRP158; TYR167; GLU328; PHE258; TYR176; TYR171; PRO331; ILE329
Riboflavin	-7.7	0.0	ASP95 (2.03); **MET330** (2.21); TYR176 (2.84); ASN332 (3.04); GLY174 (2.93)	ILE333 (π–alkyl, 4.58); TYR99 (alkyl, 5.27)	TYR171 (acceptor–acceptor, 2.81); ARG98 (donor–donor, 2.32)	–
Thiamine	-7.4	0.0	**TYR341** (2.73)	**TRP340** (π–sigma, 3.72); **TYR155** (π–π stacked, 4.04); **ALA63** (alkyl, 3.98)	**ASP65** (C–H bond, 3.56); **ARG66** (donor–donor, 2.25)	–
Vitamin B6	-6.8	0.0	**TRP62** (3.10)	**TRP340** (π–sigma, 3.68); TYR341 (π–alkyl, 4.40)	**TYR155** (π–anion, 4.00); **ASP65** (C–H bond, 3.55)	–

***Bold**: Similar interaction amino acid in the active site among native ligands and the active compound of Oleifera fruit; Hydrogen bond lengths are reported in*

Discussion

Ligand screening and drug-likeness relevance

Drug-likeness is a qualitative description of a medicinal compound's oral bioavailability. This examination is critical because many medications are taken orally. This essential metric was analysed for the most promising leads utilizing the well-known Lipinski's rule of five. A framework containing five criteria that can forecast ligand solubility and permeability properties. The criteria specify: maximum 5-10 hydrogen bond acceptors, molecular weight below 500 Da, log P value under 5, polar surface area below 140 Å, and fewer than 10 rotational bonds for the ligand (Brito, 2011). In this study, all nine retained oleifera fruit phytochemicals complied with Lipinski's criteria, supporting their suitability as ligands for structure-based screening. The physicochemical ranges observed, including molecular weight (90.03–376.36 g/mol) and logP (−1.86 to 4.22) suggest that the selected ligands possess favourable properties for docking-based evaluation.

ADMET significance and blood-brain barrier considerations

Preclinical failure often results from inadequate pharmacokinetic and toxicity properties, highlighting the importance of early ADMET filtering before clinical development. In particular, blood-brain barrier (BBB) permeability and P-glycoprotein (P-gp) efflux are key determinants of drug distribution and safety, especially for compounds targeting peripheral pathways such as AGE-RAGE (Ameji et al., 2023; König et al., 2013; Wang et al., 2015). Based on Table 2, several ligands from oleifera fruit including ascorbic acid, choline, oxalic acid, riboflavin, thiamine, and vitamin B6, cannot pass the BBB. This could be advantageous since they may not have negative consequences when used as medications. Compounds aimed at the CNS may require BBB penetration. Furthermore, acute oral toxicity prediction indicated low toxicity, with all compounds classified as relatively harmless and demonstrating high safety margin based on LD50 estimates (Gadaleta et al., 2019; Setiani et al., 2023).

Docking interpretation and ranking of candidate ligands

Based on this study, the native ligands show the strongest binding affinity with a value of -9.3 kcal/mol. Native ligands serve as a positive control for protein-ligand interaction with the RAGE protein. Among the oleifera fruit compounds tested, riboflavin emerged as the most promising candidate, exhibiting the highest binding affinity at -7.7 kcal/mol, followed by thiamine (-7.4 kcal/mol) and indole acetonitrile (-7.0 kcal/mol). These three compounds demonstrated relatively strong interactions with the RAGE protein, suggesting their potential as lead compounds for anti-atherosclerosis therapy development (Guvatova et al., 2025; Vianello et al., 2025). The moderate binding affinity group included vitamin B6 (-6.8 kcal/mol), ascorbic acid (-5.5 kcal/mol), and niacin (-5.3 kcal/mol), which showed acceptable interaction strengths that warrant further investigation. However, three compounds displayed notably weaker binding affinities: oxalic acid (-3.7 kcal/mol), choline (-3.4 kcal/mol), and indole acetic acid (-3.3 kcal/mol). These lower affinity values indicate less favourable interactions with the RAGE protein binding site. Docking protocol validation showed an RMSD of ≤2.0 Å for the redocked native ligand, indicating that the docking method was valid, confirming successful docking conformations (Nivatya et al., 2025; Pulido et al., 2014). To determine binding affinity, researchers calculate the total intermolecular energy by combining multiple energy components (van der Waals forces, hydrogen bonds, desolvation energy, electrostatic energy, internal energy, and torsional free energy), then subtract the energy of the unbound system to obtain the final value (Auli et al., 2024).

The bioactive compound of the oleifera fruit was subjected to molecular docking analysis against the RAGE protein; three compounds demonstrated substantially reduced binding affinities compared to both native ligands and other test compounds. These weak-binding ligands comprised oxalic acid (ΔG = -3.7 kcal/mol), choline (ΔG = -3.4 kcal/mol), and indole acetic acid (ΔG = -3.3 kcal/mol), exhibiting binding energies that were 2.5-2.8-fold lower than the highest-affinity natural compound (riboflavin, ΔG = -7.7 kcal/mol). The data in Table 3 reveal that these compounds did not contain any amino acid residues that are similar to those reported in native ligands that interact with the RAGE protein active site. Molecular recognition studies have shown that efficient protein-ligand interactions necessitate exact geometric optimization of contact locations, with van der Waals attractions and hydrogen bonding networks working together to preserve binding stability (Wu & Huang, 2023). Molecular docking-based ligand evaluation examines a variety of computational factors, most notably the binding free energy and the degree of amino acid residue conservation in comparison to endogenous ligands (de Oliveira et al., 2022). The low binding affinities of these chemicals indicate minimal complementarity with the RAGE binding active site (Lang et al., 2025; Pantsar & Poso, 2018). To further understand binding stability and specificity, residue-level interactions were examined using 2D interaction profiling (Table 3 and Figures 3 and 4).

Residue-level interaction patterns and binding stability

This study shows that distinct binding interaction patterns between the native ligands and riboflavin with the RAGE protein, refer to Figure 3. The native ligands established a comprehensive hydrogen bonding network involving nine critical amino acid residues (ASP:14, TYR:155, GLU:111, LYS:15, GLU:153, GLU:44, ARG:66, TRP:62, and ASP:65), resulting in a high binding affinity of -9.3 kcal/mol. This

extensive hydrogen bonding pattern is consistent with previous findings demonstrating that specific hydrogen bonding interactions with conserved RAGE residues are fundamental for ligand recognition and binding stability (Chavakis et al., 2004). The computational identification of energetically favourable binding sites through hydrogen bonding analysis provides critical insights into ligand-protein interaction mechanisms and binding specificity (Nivatya et al., 2025). The differences in binding interactions at MET:330 have important consequences for ligand selectivity and therapeutic efficacy. The creation of hydrogen bonds between riboflavin and MET:330 may provide greater binding stability and specificity than the weaker non-hydrogen bond interactions found with native ligands at this location. This structural difference indicates that riboflavin may have a more competitive binding capability, potentially displacing native ligands and influencing RAGE-mediated inflammatory pathways more effectively. Furthermore, the hydrogen bonding contact at MET:330 may operate as a crucial anchor point, stabilizing the overall riboflavin–RAGE complex while compensating for the lower total number of hydrogen bonds compared to native ligands. This focused interaction at a conserved residue position suggests that MET:330 is a strategically important binding site that could be exploited in the rational design and optimization of RAGE inhibitors (Ferreira et al., 2015; Guvatova et al., 2025; Lin et al., 2025; Majewski et al., 2019; Vianello et al., 2025). A comparison of prior investigations suggests that riboflavin has flexible binding capabilities across a wide range of protein targets. According to (Ciftci et al., 2024), riboflavin forms hydrogen bonds with ACE inhibitor proteins at GLN:281, GLU:376, THR:282, and TYR:520, with a binding affinity of -8.1 kcal/mol. The therapeutic value of ACE protein interactions is well known, as ACE reduces atherosclerosis development in experimental models and recurrent myocardial infarction incidence in clinical populations (Ciftci et al., 2024). The amino acid residue numbering method utilized enables the precise identification and description of individual residues within protein sequences, which is particularly important for binding site analysis and enzymatic function assessment (Auli et al., 2024).

To further interpret the docking patterns, the residue-level interaction conservation between native ligands and test ligands was examined. Based on Table 3 and Figure 4, the convergent binding pattern of thiamine and native ligands at conserved residues ASP:65, ARG:66, and TYR:155 has various critical implications for therapeutic intervention. The shared use of these important binding sites suggests that thiamine may potentially act as a competitive inhibitor, perhaps displacing native ligands from the RAGE active site via molecular mimicry. Although thiamine contacts these residues via non-hydrogen bond interactions rather than the hydrogen bonding demonstrated by native ligands, this alternative binding mechanism may provide significant pharmacological benefits, such as reduced binding reversibility and altered dissociation rates. The constant engagement of hydrophobic anchoring residues TRP:340 and ALA:63 by both thiamine and native ligands emphasizes the structural significance of these sites in ensuring ligand orientation and stability inside the binding pocket. This similar hydrophobic interaction pattern indicates that these residues are critical structural factors for RAGE ligand recognition, regardless of the binding mode used. Thiamine's dual-mode interaction profile, which combines hydrophobic anchoring with electrostatic engagement at conserved polar residues, may give greater binding specificity and less off-target effects than drugs that rely simply on hydrogen bonding interactions. Furthermore, thiamine's ability to occupy the same binding sites as native ligands while using different interaction mechanisms implies the possibility of creating structure-based drug design strategies that take advantage of these conserved binding motifs to improve therapeutic effects (De Freitas & Schapira, 2017; Nivatya et al., 2025). In a previous study, thiamine reacted with an ACE inhibitor to form three conventional hydrogen bonds: ASP:415, HIS:513, and LYS:454 (Ciftci et al., 2024), demonstrating its versatility in binding to various protein targets and reinforcing its potential as a multi-target therapeutic agent.

On the other hand, the striking overlap of indole acetonitrile and native ligands at eight conserved amino acid positions has far-reaching implications for therapeutic targeting and drug discovery approaches. The common hydrogen bonding interaction at GLU:153 suggests that this residue may serve as a key recognition motif contributing to RAGE binding specificity and ligand orientation within the active site (Ferreira et al., 2015; Nivatya et al., 2025). This conserved association indicates that compounds

capable of forming hydrogen bonds with GLU:153 may exhibit improved binding affinity and selectivity toward RAGE (Guvatova et al., 2025).

The overlap of indole acetonitrile and native ligands at five key residues (ASP65, TYR155, GLU44, ARG66, and TRP62) suggests that this compound may engage conserved RAGE binding hot spots through a distinct interaction profile. This observation is consistent with previous structural evidence showing that the RAGE V-domain exhibits conformational plasticity and may accommodate ligands via multiple binding modes, rather than following a single rigid lock and key interface (Indurthi et al., 2020). Such binding mode variability may influence ligand orientation, binding stability, and potentially binding kinetics; however, this mechanistic interpretation remains hypothesis-generating and should be further validated using molecular dynamics simulation and kinetic studies (Nivatya et al., 2025).

The structural homology seen in amino acid residue interactions between experimental ligands and native substrates at the target protein interface raises the possibility of establishing optimum binding configurations. This happens because endogenous ligand recognition relies on precise intermolecular interactions with certain amino acid residues within the protein's active site architecture (Wu & Huang, 2023). The extensive overlap in binding site utilization between indole acetonitrile and native ligands suggests that the compound has high translational potential for therapeutic applications, as it can effectively compete for the same binding sites while potentially providing superior pharmacological properties. Although indole acetonitrile had the third-highest binding affinity of the substances tested, it is not suggested for further therapeutic research due to its propensity to cross the blood-brain barrier (BBB). Refer to Table 2, the BBB permeability of indole acetonitrile raises serious safety concerns about potential CNS effects and neurotoxicity risks. Compounds with reduced BBB penetration are often recommended for peripheral therapeutic targets such as RAGE in order to reduce unwanted neurological side effects and ensure targeted therapeutic activity. This pharmacokinetic limitation emphasizes the significance of complete ADMET profiling in drug candidate selection, as strong binding affinity alone is insufficient to ensure therapeutic appropriateness without considering safety and distribution profiles.

Implications and translational relevance

Molecular docking studies show that the two best ligands tested for the target protein bind with the identical amino acid residues as the native ligands, indicating their potential as promising candidates for modulation/inhibition of the AGE–RAGE pathway. Molecular docking demonstrated interactions between natural chemicals found in the oleifera fruit. The activity of a chemical against a target protein can be assessed using two parameters: bond energy and interaction pattern within the binding pocket (De Freitas & Schapira, 2017; Ferreira et al., 2015; Nivatya et al., 2025). With diabetic complications and atherosclerosis representing major global health challenges, scientists and clinicians are actively pursuing natural bioactive compounds to target the AGE-RAGE pathway. However, the existing therapeutic approaches have shown limited success in completely mitigating diabetic vascular complications (Guvatova et al., 2025; Vianello et al., 2025). Several researchers are screening drug-like compounds using in-silico, in-vitro, and in-vivo approaches to identify effective RAGE inhibitors, but breakthrough therapies remain elusive (Guvatova et al., 2025). A comparative analysis of binding affinity at the interface of riboflavin and thiamine with RAGE protein has revealed that these multi-functional compounds demonstrate superior binding characteristics compared to single-targeted approaches, particularly when optimized hydrophobic interactions and hydrogen bonding networks increase their binding affinity (Ferreira et al., 2015). Riboflavin (vitamin B2) plays an important role in vascular health through its involvement in redox homeostasis and oxidative stress regulation, which are closely associated with endothelial dysfunction and atherosclerosis progression. Thiamine significantly reduces vascular inflammation and negatively correlates with LDL cholesterol and triglycerides. Chronic vitamin B1 supplementation attenuates atherosclerosis progression (Al-Attas et al., 2014).

The binding affinity profiles of oleifera fruit compounds suggest that optimization of key non-covalent interactions, including hydrophobic contacts, hydrogen bonding, and residue-specific interactions at the RAGE binding pocket, may contribute to ligand–protein complex stabilization and potential therapeutic

relevance. These findings highlight the importance of mapping interaction patterns beyond binding energy alone and may support future structure-based optimization of natural product–derived candidates targeting the AGE–RAGE axis. However, it should be emphasized that these results are derived from an in silico approach and are therefore subject to the inherent limitations of docking prediction tools. Accordingly, the predicted therapeutic potential of riboflavin and thiamine cannot be regarded as definitive until validated through further experimental investigations, including in vitro, in vivo, and clinical studies. Nevertheless, this integrated computational strategy may provide a rational basis for the early-stage identification and prioritization of potential RAGE inhibitors for diabetic vascular complications and atherosclerosis.

Limitation and Future Scope

This study is limited by its reliance on molecular docking, which provides a static approximation of ligand–protein interactions and may not fully reflect dynamic physiological conditions. In addition, the predicted binding modes and affinities were not confirmed by in vitro assays, and complex stability was not evaluated using molecular dynamics simulations.

Future work should validate the top-ranked ligands (e.g., riboflavin and thiamine) using in vitro experiments, including RAGE inhibition assays and inflammation-related biomarker analysis. Molecular dynamics simulations are also recommended to assess the stability of ligand–RAGE complexes over time. Furthermore, biological target prediction tools (e.g., SwissTargetPrediction and PASS Online) may be used to support mechanistic interpretation and clarify whether the ligands are more likely to act as inhibitors or activators.

Conclusion

This study establishes a fundamental framework for elucidating the molecular mechanisms that govern the bioactive characteristics of compounds derived from the oleifera fruit. The findings reveal significant potential for therapeutic applications specifically targeting RAGE (Receptor for Advanced Glycation End products)-mediated signalling pathways in pathogenesis. There are two compound from the oleifera fruit that potential, riboflavin and thiamine. These compounds potentially function by interfering with natural ligand-protein interactions that are critical contributors to atherosclerosis pathogenesis, thereby offering a molecular basis for therapeutic intervention.

Conflict of Interest

The authors declare there are no conflicts of interest regarding the publication of this article.

Acknowledgement

The authors express sincere gratitude to Universitas Pembangunan Nasional Veteran Jakarta and Research Centre for Moringa Oleifera for providing the necessary computational resources and research facilities and also this research was supported by Hibah Internal Research Universitas Pembangunan Nasional Veteran Jakarta 2024 number 977/UN61/HK03.01/2024.

References

Adeniran, O. I., & Mogale, M. A. (2019, November). In vitro inhibitory effect of selected parts of Moringa oleifera against the formation of major types of advanced glycation end-products. In *II International Symposium on Moringa,* 135-142. *1306.* https://doi.org/10.17660/ActaHortic.2021.1306.16

Aekthammarat, D., Pannangpetch, P., & Tangsucharit, P. (2019). Moringa oleifera leaf extract lowers high blood pressure by alleviating vascular dysfunction and decreasing oxidative stress in L-NAME hypertensive rats. *Phytomedicine : International Journal of Phytotherapy and Phytopharmacology*, *54*, 9–16. https://doi.org/10.1016/j.phymed.2018.10.023

Al-Attas, O., Al-Daghri, N., Alokail, M., Abd-Alrahman, S., Vinodson, B., & Sabico, S. (2014). Metabolic benefits of six-month thiamine supplementation in patients with and without diabetes mellitus type 2. *Clinical Medicine Insights: Endocrinology and Diabetes*, *7*, 1–6. https://doi.org/10.4137/CMED.S13573

Ameji, P. J., Uzairu, A., Shallangwa, G. A., & Uba, S. (2023). Molecular docking-based virtual screening, drug-likeness, and pharmacokinetic profiling of some anti-Salmonella typhimurium cephalosporin derivatives. *Journal of Taibah University Medical Sciences*, *18*(6), 1417-1431. https://doi.org/10.1016/j.jtumed.2023.05.021

Auli, W. N., Husniati, H., Suprahman, N. Y., Fauziyya, R., Sarmoko, S., Ashari, A., ... & Fasya, G. A. (2024). Study of Gnetum gnemon metabolites as potential anti-breast and prostate cancer by metabolomic and molecular docking. *Journal of Applied Pharmaceutical Science*, *14*(12), 120-130. https://dx.doi.org/10.7324/JAPS.2024.170723

Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., ... & Bourne, P. E. (2000). The protein data bank. *Nucleic Acids Research, 28*(1), 235-242. https://doi.org/10.1093/nar/28.1.235

Brito, M. A. D. (2011). Pharmacokinetic study with computational tools in the medicinal chemistry course. *Brazilian Journal of Pharmaceutical Sciences, 47*, 797-805. https://doi.org/10.1590/S1984-82502011000400017

Bulusu, G., & Desiraju, G. R. (2020). Strong and weak hydrogen bonds in protein–ligand recognition. *Journal of the Indian Institute of Science, 100*(1), 31-41. https://doi.org/10.1007/s41745-019-00141-9

Ceriello, A., Gallo, M., Armentano, V., Perriello, G., Gentile, S., & De Micheli, on behalf of the Associazione Medici Diabetologi, A. (2012). Personalizing treatment in type 2 diabetes: A self-monitoring of blood glucose inclusive innovative approach. *Diabetes Technology & Therapeutics, 14*(4), 373-378. https://doi.org/10.1089/dia.2011.0233

Chavakis, T., Bierhaus, A., & Nawroth, P. P. (2004). RAGE (receptor for advanced glycation end products): A central player in the inflammatory response. *Microbes and Infection, 6*(13), 1219-1225. https://doi.org/10.1016/j.micinf.2004.08.004

Chumark, P., Khunawat, P., Sanvarinda, Y., Phornchirasilp, S., Morales, N. P., Phivthong-Ngam, L., ... & Pongrapeeporn, K. U. S. (2008). The in vitro and ex vivo antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of Moringa oleifera Lam. leaves. *Journal of Ethnopharmacology, 116*(3), 439-446. https://doi.org/10.1016/j.jep.2007.12.010

Ciftci, M. H., Turkoglu, V., Bas, Z., & Celikezen, F. C. (2024). In vitro inhibitor effect and molecular docking of thiamine (vitamin B1), riboflavin (vitamin B2), and reference inhibitor captopril on angiotensin-converting enzyme purified from sheep plasma. *Archives of Physiology and Biochemistry, 130*(6), 974-983. https://doi.org/10.1080/13813455.2024.2376814

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports, 7*(1), 42717. https://doi.org/10.1038/srep42717

Dallakyan, S., & Olson, A. J. (2014). Small-molecule library screening by docking with PyRx. In Chemical biology: methods and protocols (pp. 243-250). New York, NY: Springer New York. https://doi.org/10.1007/978-1-4939-2269-7_19

De Freitas, R. F., & Schapira, M. (2017). A systematic analysis of atomic protein–ligand interactions in the PDB. *Med. Chem. Commun., 8*, 1970-1981. https://doi.org/10.1039/C7MD00381A

de Oliveira, T. A., Medaglia, L. R., Maia, E. H. B., Assis, L. C., de Carvalho, P. B., da Silva, A. M., & Taranto, A. G. (2022). Evaluation of docking machine learning and molecular dynamics methodologies for DNA-ligand systems. *Pharmaceuticals, 15*(2), 132. https://doi.org/10.3390/ph15020132

du Toit, E. S. (2019, November). Horticultural research review on Moringa oleifera trees growing in South Africa under sub-optimal conditions. In *II International Symposium on Moringa 1306* (pp. 1-12). https://doi.org/10.17660/ActaHortic.2021.1306.1

Ferreira, L. G., Dos Santos, R. N., Oliva, G., & Andricopulo, A. D. (2015). Molecular docking and structure-based drug design strategies. *Molecules, 20*(7), 13384-13421. https://doi.org/10.3390/molecules200713384

Gadaleta, D., Vuković, K., Toma, C., Lavado, G. J., Karmaus, A. L., Mansouri, K., ... & Roncaglioni, A. (2019). SAR and QSAR modeling of a large collection of LD50 rat acute oral toxicity data. *Journal of Cheminformatics, 11*(1), 58. https://doi.org/10.1186/s13321-019-0383-2

Gu, Y., Zhang, X., Xu, A., Chen, W., Liu, K., Wu, L., ... & Luo, Q. (2023). Protein–ligand binding affinity prediction with edge awareness and supervised attention. *Iscience, 26*(1). https://doi.org/10.1016/j.isci.2022.105892

Guvatova, Z. G., Vakhrusheva, A., & Moskalev, A. (2025). A receptor for glycation end products (RAGE) is a key transmitter between garb-aging and inflammaging. *Ageing Research Reviews*, *113*, 102919. https://doi.org/10.1016/j.arr.2025.102919

Indurthi, V. S., Jensen, J. L., Leclerc, E., Sinha, S., Colbert, C. L., & Vetter, S. W. (2020). The Trp triad within the V-domain of the receptor for advanced glycation end products modulates folding, stability and ligand binding. *Bioscience Reports, 40*(1), BSR20193360. https://doi.org/10.1042/BSR20193360

Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., ... & Bolton, E. E. (2025). PubChem 2025 update. *Nucleic Acids Research, 53*(D1), D1516-D1525. https://doi.org/10.1093/nar/gkae1059

König, J., Müller, F., & Fromm, M. F. (2013). Transporters and drug-drug interactions: important determinants of drug disposition and effects. *Pharmacological Reviews, 65*(3), 944-966. https://doi.org/10.1124/pr.113.007518

Lang, S., Braz, N. F., Slater, M. J., & Kidley, N. J. (2025). In Silico Methods for Ranking Ligand–Protein Interactions and Predicting Binding Affinities: Which Method is Right for You? *Journal of Medicinal Chemistry, 68*(19), 19795-19799. https://doi.org/10.1021/acs.jmedchem.5c02582

Lin, H., Yang, Y., Wang, X., Chung, M., Zhang, L., Cai, S., ... & Pan, Y. (2025). Targeting the AGEs-RAGE axis: pathogenic mechanisms and therapeutic interventions in diabetic wound healing. *Frontiers in Medicine, 12,* 1667620. https://doi.org/10.3389/fmed.2025.1667620

Madushanka, A., Moura Jr, R. T., Verma, N., & Kraka, E. (2023). Quantum mechanical assessment of protein–ligand hydrogen bond strength patterns: insights from semiempirical tight-binding and local vibrational mode theory. *International Journal of Molecular Sciences, 24*(7), 6311. https://doi.org/10.3390/ijms24076311

Majewski, M., Ruiz-Carmona, S., & Barril, X. (2019). An investigation of structural stability in protein-ligand complexes reveals the balance between order and disorder. *Communications Chemistry, 2*(1), 110. https://doi.org/10.1038/s42004-019-0205-5

Nathan, D. M., Bayless, M., Cleary, P., Genuth, S., Gubitosi-Klug, R., Lachin, J. M., ... & DCCT/EDIC Research Group. (2013). Diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: Advances and contributions. *Diabetes, 62*(12), 3976- 3986. https://doi.org/10.2337/db13-1093

Nivatya, H. K., Singh, A., Kumar, N., Sonam, Sharma, L., Singh, V., ... & Mishra, A. K. (2025). Assessing molecular docking tools: understanding drug discovery and design. *Future Journal of Pharmaceutical Sciences, 11*(1), 111. https://doi.org/10.1186/s43094-025-00862-y

Pantsar, T., & Poso, A. (2018). Binding affinity via docking: fact and fiction. *Molecules, 23*(8), 1899. https://doi.org/10.3390/molecules23081899

Park, H., & Boyington, J. C. (2010). The 1.5 Å crystal structure of human receptor for advanced glycation endproducts (RAGE) ectodomains reveals unique features determining ligand binding. *Journal of Biological Chemistry, 285*(52), 40762-40770. https://doi.org/10.2210/pdb3o3u/pdb

Pinto, R. S., Minanni, C. A., de Araújo Lira, A. L., & Passarelli, M. (2022). Advanced glycation end products: A sweet flavor that embitters cardiovascular disease. *International Journal of Molecular Sciences, 23*(5), 2404. https://doi.org/10.3390/ijms23052404

Poznyak, A., Grechko, A. V., Poggio, P., Myasoedova, V. A., Alfieri, V., & Orekhov, A. N. (2020). The diabetes mellitus–atherosclerosis connection: The role of lipid and glucose metabolism and chronic inflammation. *International Journal of Molecular Sciences, 21*(5), 1835. https://doi.org/10.3390/ijms21051835

Pulido, R., Baker, S. J., Barata, J. T., Carracedo, A., Cid, V. J., Chin-Sang, I. D., ... & Leslie, N. R. (2014). A unified nomenclature and amino acid numbering for human PTEN. Science Signaling, 7(332), pe15. https://doi.org/10.1126/scisignal.2005560

Sala, L. L., Prattichizzo, F., & Ceriello, A. (2019). The link between diabetes and atherosclerosis. European *Journal of Preventive Cardiology, 26(*2_suppl), 15-24. https://doi.org/10.1177/2047487319878373

Setiani, L. A., Sari, B. L., & Muntaza, W. (2023). Prediction of Carcinogenic, Mutagenic, Hepatotoxic, and LD50 Toxicity of Herbs Euphorbia hirta and Camellia sinensis Leaf Compounds as In Silico Antihypertensive Agents. *Jurnal Penelitian Pendidikan IPA, 9(*SpecialIssue), 103-112. https://doi.org/10.29303/jppipa.v9iSpecialIssue.5900

Syahputra, G., Gustini, N., Bustanussalam, B., Hapsari, Y., Sari, M., Ardiansyah, A., ... & Putra, M. Y. (2021). Molecular docking of secondary metabolites from Indonesian marine and terrestrial organisms targeting SARS-CoV-2 ACE-2, M pro, and PL pro receptors. *Pharmacia, 68*, 533-560. https://doi.org/10.3897/pharmacia.68.e68432

Vianello, E., Beltrami, A. P., Aleksova, A., Janjusevic, M., Fluca, A. L., Corsi Romanelli, M. M., ... & Dozio, E. (2025). The advanced glycation end-products (AGE)–receptor for AGE system (RAGE): An inflammatory pathway linking obesity and cardiovascular diseases. *International Journal of Molecular Sciences, 26*(8), 3707. https://doi.org/10.3390/ijms26083707

Wang, Y., Xing, J., Xu, Y., Zhou, N., Peng, J., Xiong, Z., ... & Jiang, H. (2015). In silico ADME/T modelling for rational drug design. *Quarterly Reviews of Biophysics, 48*(4), 488-515. https://doi.org/10.1017/S0033583515000190

Wu, Q., & Huang, S. Y. (2023). HCovDock: an efficient docking method for modeling covalent protein–ligand interactions. *Briefings in Bioinformatics, 24*(1), bbac559. https://doi.org/10.1093/bib/bbac559

Ye, J., Li, L., Wang, M., Ma, Q., Tian, Y., Zhang, Q., ... & Sun, G. (2022). Diabetes mellitus promotes the development of atherosclerosis: the role of NLRP3. *Frontiers in Immunology, 13*, 900254. https://doi.org/10.3389/fimmu.2022.900254