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Structure-Based Virtual Screening of Bioactive Natural Polyphenols Towards Lead Discovery of the RAGE Inhibitor for the Eradication of Diabetes-Associated Pathology

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22014065).which included 6000 of natural polyphenolic compounds, optimized molecular structures, ADMET prediction data, and molecular docking results.

Structure-Based Virtual Screening of Bioactive Natural Polyphenols Towards Lead Discovery of the RAGE Inhibitor for the Eradication of Diabetes-Associated Pathology

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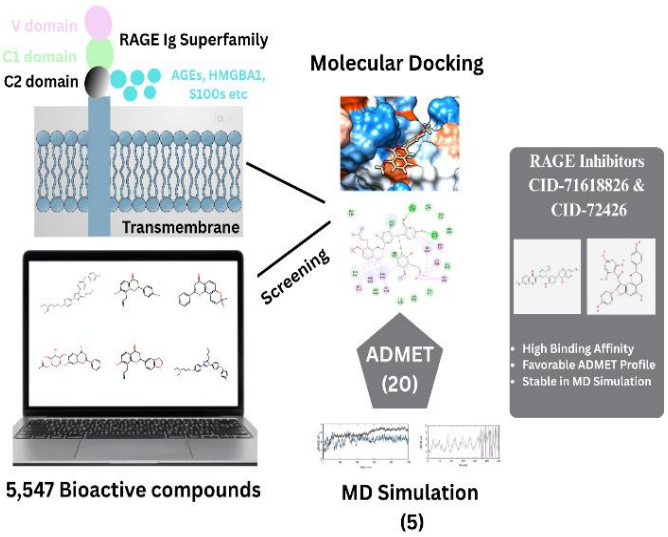
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Abstract:

Receptor for Advanced Glycation End Products refers to RAGE belongs to a superfamily of immunoglobulin (Ig) cell surface receptors that is currently considered to be central to the pathogenesis of diabetes and its complications. Various ligands to the pattern recognition receptor RAGE, which identifies endogenous stress signals, include High Mobility Group Box 1, Advanced Glycation End products, S100 proteins, amyloid-beta, some nucleic acids, phospholipids, and glycosaminoglycans. In this study, we used an *in silico* virtual screening approach, performing molecular docking with 6,000 bioactive molecules, and the top 5 were selected for ADMET and Molecular Dynamics Simulation (MDS) profiling based on the best docking scores. CID-72426 and CID-71618826 were found to exhibit strong binding affinities that were -7.1 and -6.9 kcal/mol, respectively, which is far better than the control drug Azeliragon (CID-11180124), which showed a binding affinity of −3.1 kcal/mol. Both substances exhibited favorable ADME properties and minimal toxicity and further evaluation was done by performing MDS simulations over 100 ns. These novel findings indicate CID-72426 and CID-71618826 as ideal candidates for inhibiting RAGE protein expression. Furthermore, *in vivo* and *in vitro* experiment need be conducted in the following series to confirm our computational identification as a potential therapeutic.

Keywords: RAGE, Virtual Screening, Molecular Docking, Natural Polyphenol, MD Simulation, ADMET.



Graphical Abstract

1. Introduction

Diabetes mellitus (DM) is one of the leading noncommunicable diseases characterized by hyperglycemia due to insufficient insulin release from pancreases and inadequate action due to resistance [1]. Most of the diabetes patients diagnosed are type 2 (T2DM), which has a strong relationship with genetics, being overweight, and not doing any physical activity [2]. The formation of advanced glycation end products (AGEs) is the critical molecular process underlying problems related to diabetes [3]. AGEs bind to a multi-ligand cell surface receptor, RAGE [4]. This interaction results in RAGE protein activation, leading to tissue damage, inflammation, and increased oxidative stress via the NF-kB signaling pathway [5]. This RAGE activation promotes several disorders, such as aggravating the effects of diabetes, and stimulates the growth of pathological conditions, for instance, renal fibrosis, cancer metastases, and atherosclerosis.

The protein structure of RAGE comprises three Ig domains, each with distinct functions that vary with changes in amino acid residues. The RAGE protein consists of three major sections: an extracellular part (residues 23- 342), a single transmembrane helix (residues 343- 363), and a cytoplasmic signaling domain. It consists of the extracellular part, that has two constant domains (C1 and C2) and one variable domain. V domain lies between residues 23 and 116; the C1 and C2 domains lie between residues 124-221 and 227-317, respectively, as the main ligand-binding domains. It regulates the primary activity of the RAGE protein, as it is the essential ligand-binding area [6]. RAGE, present in macrophages, smooth muscle cells, and endothelial cells, worsens atherosclerosis, particularly in diabetic individuals. The three-dimensional structure of the RAGE receptor was derived from a co-crystallized X-ray structure in the Protein Data Bank, indicating that there are several possible ligand-binding sites on its extracellular domain [7]. The VC1 and V domains have been used in several studies to look at RAGE inhibitors. However, the V domain is more commonly used in fragment-based binding approaches in these studies. Nevertheless, because of its dimeric nature, the VC1 domain is thought to be more constant than the V domain [8].

RAGE plays a critical role in facilitating AGE-induced cellular damage, making it a hopeful therapeutic target in diabetes and its associated diseases [9]. A macromolecule, Azeliragon (TTP488), has shown aptitude in preclinical and clinical trials by preventing tissue destruction and reducing inflammation [10]. Manufactured inhibitors of natural chemicals are considered alternative therapies due to their safety, bioavailability, and long-term tolerance.

Till date natural products remain the main source of drug discovery and development to prevent and irradiate diseases, and thousands of studies are undertaken to acknowledge their valuable role. Recently our group demonstrated that ursolic acid, a pentacyclic triterpene can inhibit AGEs formation [11]. Polyphenols are a heterogeneous family of secondary metabolites

produced by plants and are known to possess therapeutic effects, including antioxidant, anti-inflammatory, and antidiabetic properties. Many studies have shown that these compounds can inhibit the production of advanced glycation end products (AGEs), improve endothelial function, and alter signal transduction pathways linked to RAGE [12][13] [14] [15] [16]. Their noble safety profiles and multi-target perspective make them a lead candidate for drug development determinations intended for RAGE-mediated disorders. *In silico* methods are gaining popularity for discovering new drug leads, as traditional methods of synthesizing and characterizing chemical compounds derived from natural resources are much slower and often produced inconsistent outcomes.

Structure-based virtual screening (SBVS) is a powerful *in silico* screening method that can be used to screen a compound library for affinity with a protein target. The method can be used to evaluate large chemical libraries in a relatively short time using molecular docking. Moreover, the SBVS, in conjunction with molecular dynamics simulations and binding free energy calculations, can provide more information on the interaction of ligands and receptors and conformational stability in near-physiological conditions. This study used SBVS to identify potential inhibitors of the RAGE receptor, in particular, naturally derived polyphenolic compounds. A large-scale polyphenol library was screened to identify high-affinity ligands for the target protein that may disrupt AGE-RAGE interactions and have therapeutic potential to alleviate diabetes-related complications.

2. Result

2.1. Virtual Screening

We conducted a virtual screening of 6,000 selected phytocompounds against the RAGE receptor (PDB ID: 4LP5) using molecular docking techniques using Maestro (Schrodinger Release 2021-2). Azeliragon (CID: 11180124) was used as a control group. Gnetin C (CID: 21633857) demonstrated the highest binding affinity (–7.3 kcal/mol) and MM-GBSA (–57.41 kcal/mol) binding free energy. The analogous docking scores of Compound B (CID: 11410680) and Compound E (CID: 71618826) were -6.9 kcal/mol, and their MM-GBSA scores are -64.54 and -51.27 kcal/mol, respectively, in MM-GBSA energies. The control compound Azeliragon had a binding affinity of -3.1 kcal/mol and MM-GBSA energy of -44.99 kcal/mol. Daphnodorin A (CID: 72426) on the other hand had a valid binding affinity and free binding energy of -7.1 kcal/mol and -50.51 kcal/mol respectively (**shown in Table 1**).

	CID	Compound Name	Binding Affinity	MM-GBSA	MOLECULAR Weight
Control	11180124	Azeliragon	-3.1	-44.99	532.12
A	72426	Daphnodorin A	-7.1	-50.51	526.49

B	11410680	[(2S,3R,4R,5S)-5-[2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxochroman-3-yl]oxy-3,4-dihydroxyoxolan-2-yl]methyl 3,4,5-trihydroxybenzoate	-6.9	-64.54	586.45
C	21633857	Gnetin C	-7.3	-57.41	454.47
D	57333038	Morusyunnansins D	-7.1	-58.38	680.83
E	71618826	7-Hydroxy-3-[4-hydroxy-3-[2-hydroxy-5-(7-hydroxy-4-oxochromen-3-yl)phenyl]phenyl]chromen-4-one	-6.9	-51.27	506.46

Table 1: CID and name of Top Five hits

2.2. Interaction Profiling of Ligand-Protein Complexes

Daphnodorin A (CID: 72426) interacted with the amino acids SER74, VAL78, and GLN47 through hydrogen bonding, π-sigma interactions, and alkyl interactions. So that the complex was stabilized. Morusyunnansins D (CID: 57333038) interacted through H-bonds with SER74 and ARG77 and through van der Waals interactions with residues such as ARG42 and PHE85. The control drug, Azeliragon, had limited interactions in general, only one hydrogen bond (ILE91), and minimal hydrophobic interactions, which could contribute to lower binding affinity. Additionally, compound B (CID: 11410680) engaged in extensive interactions via π-π stacking and hydrogen bonding, notably with GLU69 and SER65, whereas compound E (CID: 71618826) interacted with residues including VAL78 and ARG57. These interactions indicate that the selected phyto-compounds are well-suited to the binding pocket and form a wider range of stabilizing contacts than the reference compound **(see supplementary Table S1 and S3**). Detailed interaction analysis revealed that the selected phyto-compounds have multiple stabilizing interfaces with significant residues within the RAGE binding pocket. Gnetin C (CID: 21633857) formed hydrogen bonds with ASP73, SER74, ARG72, and GLN47 and displayed significant van der Waals and alkyl interactions involving residues such as LEU49 and PRO87.

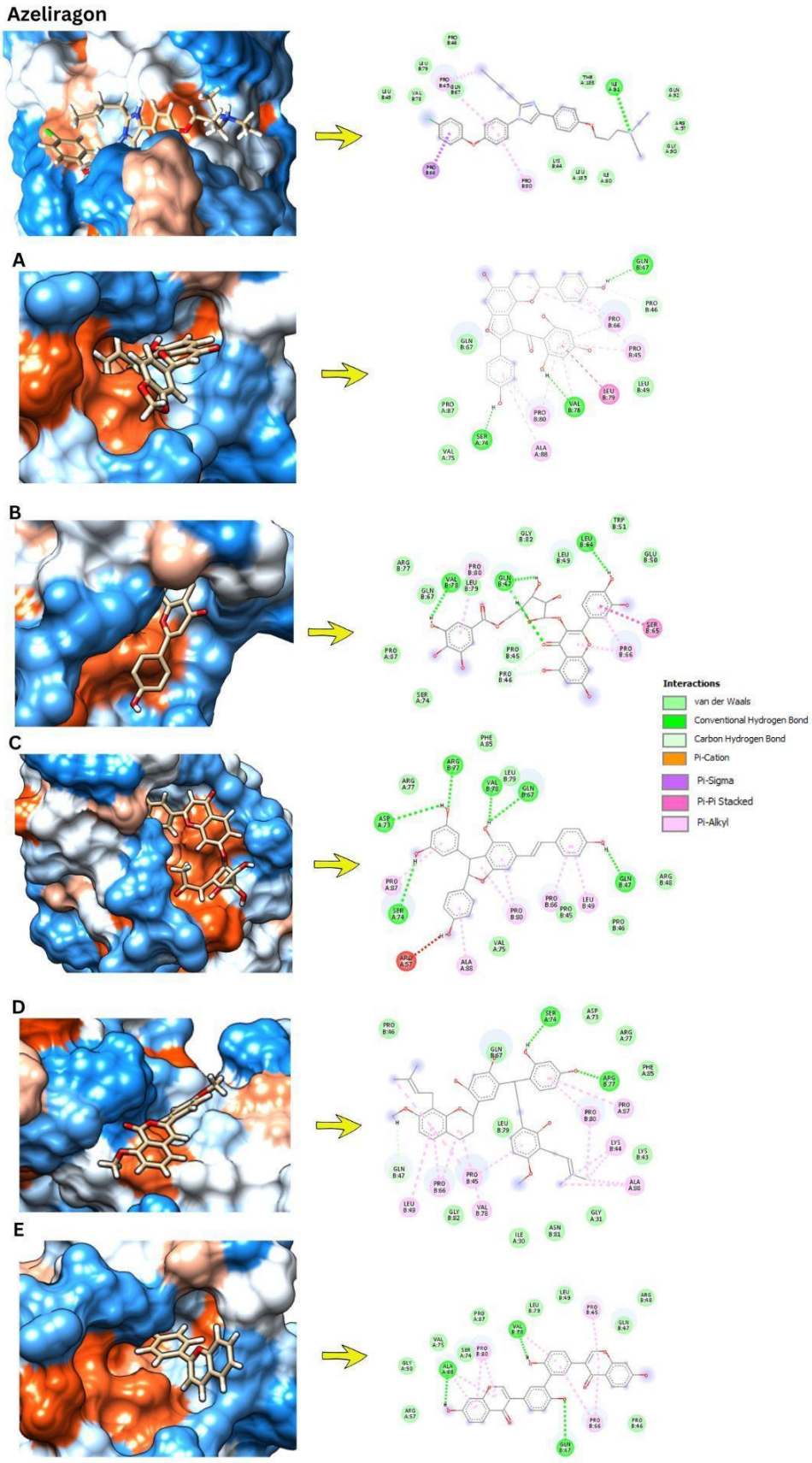

Azeliragon
A
B
C
D
E
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Cation
Pi-Sigma
Pi-Pi Stacked
Pi-Alkyl

Figure 1: Interactions of the RAGE protein (PDB ID: 4LP5) with the selected ligands. The left-most panels showcase the 3D structures of the protein-ligand complexes, and the right-most panels depict the 2D interaction patterns of the protein-ligand complexes. First, an interaction profile of the RAGE protein with the control molecule Azeliragon (CID-11180124) is presented, followed by interaction profiles of the selected ligands.

(A) Daphnodorin A (CID-72426); (B) (2S,3R,4R,5S)-5-[2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxochromen-3-yl]oxy-3,4-dihydroxyoxolan-2-yl methyl 3,4,5-trihydroxybenzoate (CID-11410680);(C) Gnetin C (CID-21633857);(D) Morusynnasins D (CID-57333038); and (E) 7-Hydroxy-3-[4-hydroxy-3-[2-hydroxy-5-(7-hydroxy-4-oxochromen-3-yl)phenyl]phenyl]chromen-4-one (CID-71618826).

2.3. Analysis of Pharmacokinetics

There are numerousnumbers of public and commercial available software tools to evaluate physicochemical and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of compounds. The SwissADME website was used to predict the drug-likeness properties of the top five molecules including the control molecules in this study **(see supplementary Table 2**). This popular web server provides reliable, flexible predictions to the research community. CID-11180124 (control), CID-21633857, CID-72426, and CID-71618826 were found to be the most promising compounds, indicating their potential use as chemical weapons. Their molecular weights were calculated as 532.12, 454.47, 526.49, and 506.46 g/mol, respectively. These molecules demonstrated similar bioavailability (0.55) and were anticipated to be non-toxic, as indicated by the Ames test. They also showed a few violations of Lipinski's rule and good drug-likeness. They also had lower topological polar surface area scores than the standard drug, which may enhance absorption. Moreover, they did not show any evidence of liver toxicity. **Table 2** also shows other physicochemical properties including number of hydrogen bond donors and acceptors, and rotatable bonds.

2.4. Molecular Dynamics Simulations

We performed 100 ns MD simulation to better understand the structural stability and dynamics of the protein-ligand complexes. Molecular dynamic modeling provides a time-resolved view of atomic motions, enabling assessment of conformational flexibility, intermolecular interactions, and overall stability within a simulated biological environment. This method is also useful for highlighting critical structural changes and forms of interaction that contribute to stability in complexity. We used terminal snapshots of the simulation trajectories at 100 ns to analyze the structural integrity of the complexes and the degree of intermolecular communication at that time.

2.4.1. Root Mean Square Deviation Analysis

RMSD is a frequently used method for evaluating the changes in atomic positions between two structures, in particular, between a structure and a reference structure. An RMSD value range of between 1 and 3 Å is generally considered acceptable for determining a stable protein-ligand complex. When the readings exceed this range, it usually indicates that the protein backbone is changing or is flexible. 100 ns of simulation were used to assess the dynamic immobility of protein–ligand complexes with five ligands (CID-21633857, CID-57333038, CID-72426, CID-71618826, and CID-11410680) and a control (CID-11180124). The RMSD curves showed that the average deviation for most complexes fell within the accepted range. This implies that the conformational stability was high during the simulation. The control complex (CID-11180124) and the complexes with the ligand (CID-72426, CID-71618826, and CID-11410680) had relatively stable RMSD values, suggesting a well-maintained structural integrity. In contrast, the complexes containing CID-21633857 and CID-57333038 exhibited greater RMSD fluctuations, particularly after 50 ns, with values exceeding 15 Å. This suggests that conformational changes or weaker binding may have caused these complexes to undergo structural reorganization or increased flexibility. The above outcomes suggest that the ligands CID-72426, CID-71618826, and CID-11410680 can confer greater stability to the target protein than others (**Figure 2**).

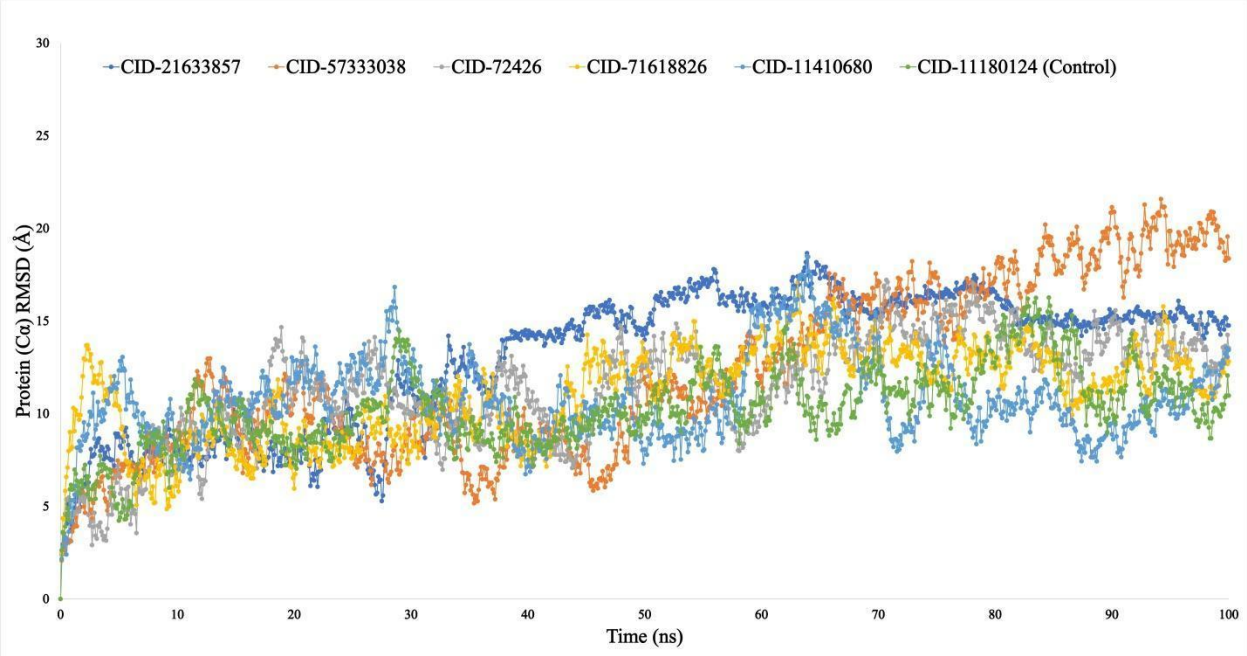


Figure 2: RMSD curves of 4LP5 protein backbone (C) atoms in complex with five ligands, CID-21633857 (blue), CID-57333038 (orange), CID-72426 (gray), CID-71618826 (yellow), CID-11410680 (sky-blue), and control CID-11180124 (green) during a 100 ns MD simulation. The differences show how stable each complex is relative to the others; the smaller the RMSD, the better established the protein-ligand interaction.

2.4.2. Root Mean Square Fluctuation Analysis

The RMSF method was used to calculate the average distance between each residue (usually the Cα atoms) and the average residue positions in a molecular dynamics (MD) simulation. It helps determine both flexible and rigid regions of a protein structure when bound to various ligands. In the RMSF graph provided, the 4LP5 protein was simulated in the presence of six ligand complexes, including a control (CID-11180124). The majority of variations are relatively stable across residues; however, there are sharp peaks, particularly around residues 200-320, where elevated flexibility is observed, presumably due to loop or unstructured regions. The N and C-terminal regions are also more mobile, as they are more open and less constrained. Conversely, the interior regions (primarily residues 6-230) that could probably comprise α-helices and 2-sheets were more stable, indicating low RMSF. These findings indicate that ligand binding does not significantly destabilize the core structure, although some ligands (such as CID-57333038 and CID-72426) increase the flexibility of the loop regions (**Figure 3**).

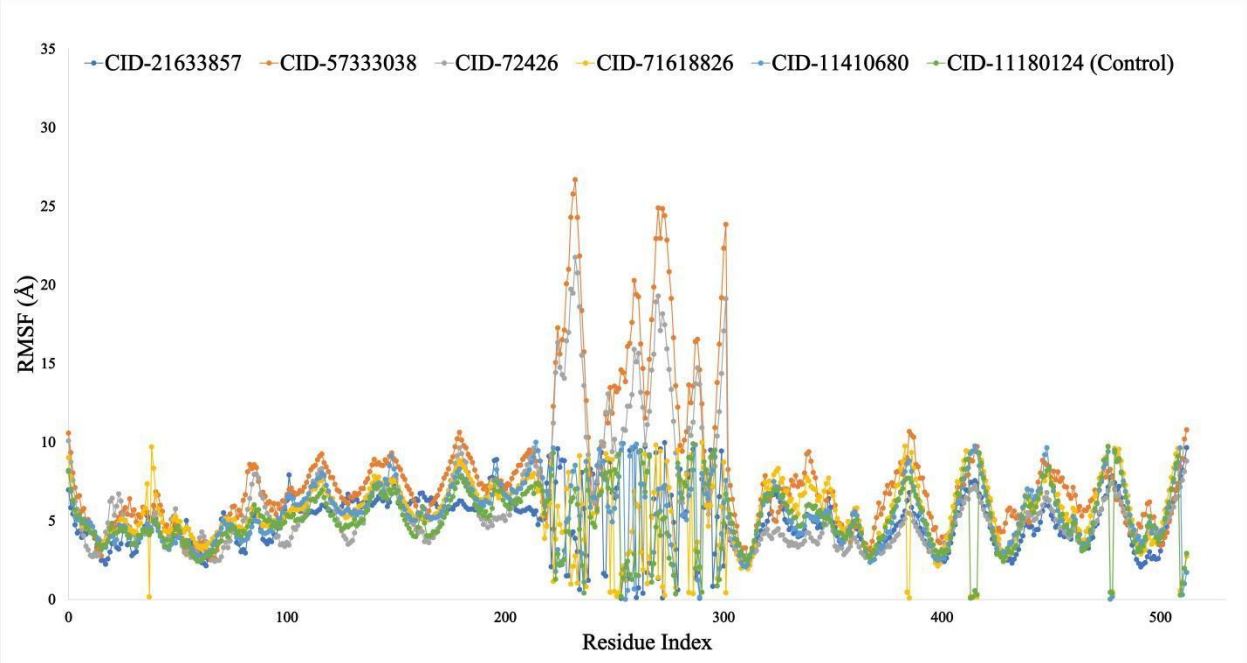


Figure 3: RMSF analysis of 4LP5 protein residues in complex with six ligands: CID-21633857 (blue), CID-57333038 (orange), CID-72426 (gray), CID-71618826 (yellow), CID-11410680 (sky-blue), and control CID-11180124 (green), illustrating flexibility variations across the amino acid chain. Maximum fluctuations were observed at the N- and C-termini, while core structural regions remained relatively stable, including α-helices and β-sheets (residues ~6–230).

2.4.3. Rg (Radius of Gyration) Analysis

The Rg represents how an MD simulation compresses a protein structure over time. It is used to determine the packing of a protein-binding complex. A reduction in Rg and a more constant value indicates a small and stable protein structure, whereas an increase in Rg or unstable values indicates structural laxity or instability. On the graph, we compare the Rg of six ligand-bound complexes of the 4LP5 protein (with the control CID-11180124) over a 100 ns simulation interval. The majority of ligands (CID-72426, CID-71618826) exhibit low Rg values (~3-5.5 Å), indicating a compact protein structure. Control (CID-11180124), on the other hand, has a more prominent and unstable Rg (approximately 6.575 Å), indicating it is less compact and can adopt a different shape (**Figure 4**). This comparison indicates that two ligands can better protect or improve the protein's structural integrity than the control.

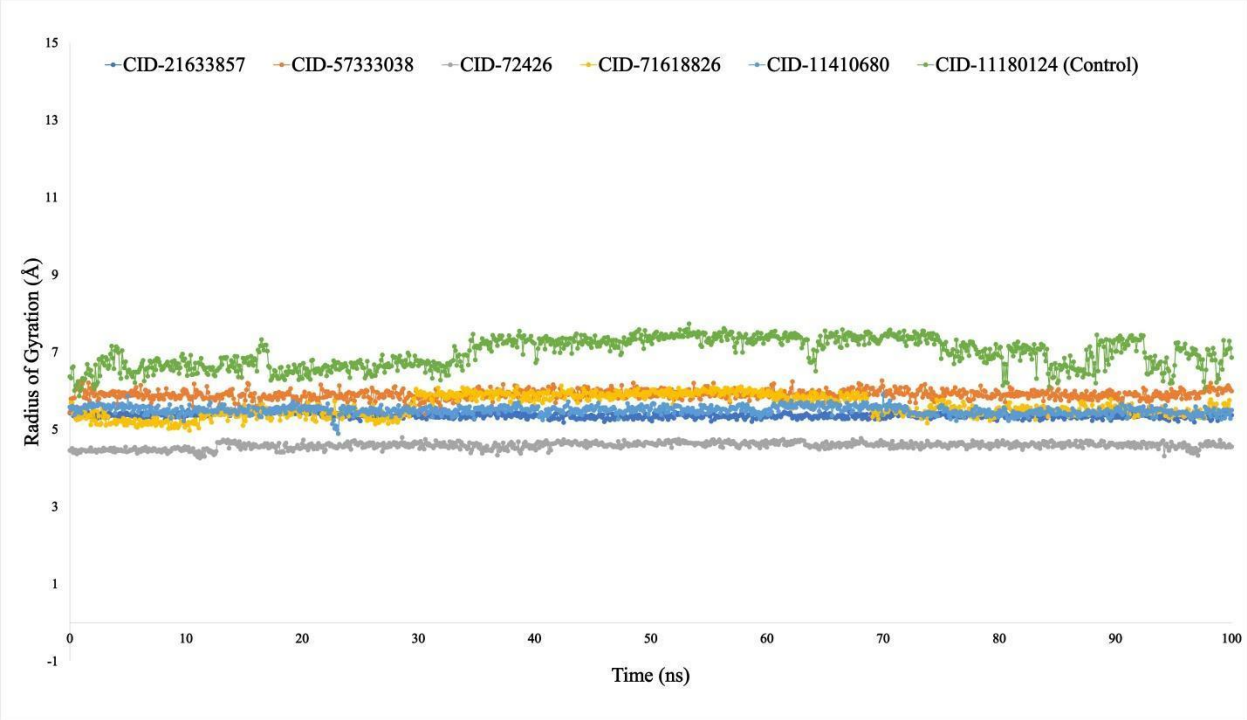


Figure 4: The radius of gyration (Rg) was determined in the 100 ns simulation to test the compactness of the complexes. The compounds CID-21633857, CID-57333038, CID-72426, CID-71618826, CID-11410680, and the control CID-11180124 are demonstrated with their respective colors: blue, orange, gray, yellow, sky-blue, and green.

2.4.4: Analysis of Solvent-Accessible Surface Area

To calculate SASA, we considered the flexibility of the protein, which helps to understand the shape of a protein when it is bound to different ligands and how many solvents it can accommodate. The behavior of the protein-ligand complexes was tracked for the entire time span of the simulation. **Figure 5** shows that the SASA values for the six complexes ranged from about 200 to 500 Å^2. The control compound (CID-11180124) had higher SASA values, particularly in

the later stages of the simulation (around 90-100 ns), indicating increased solvent exposure and a higher probability that the protein-ligand complex would be unstable. Of the test ligands, CID-21633857 and CID-11410680 exhibited reduced and more stable SASA profiles during the simulation, indicating tighter, more stationary interactions with the target protein. CID-72426 and CID-71618826 exhibited moderate changes, whereas CID-57333038 showed higher SASA values, indicating that it experienced relatively greater conformational changes. These results indicate that some of the ligands, especially CID-72426, CID-71618826, and CID-11410680, can retain the interactions and possibly desirable binding characteristics (Figure 5).

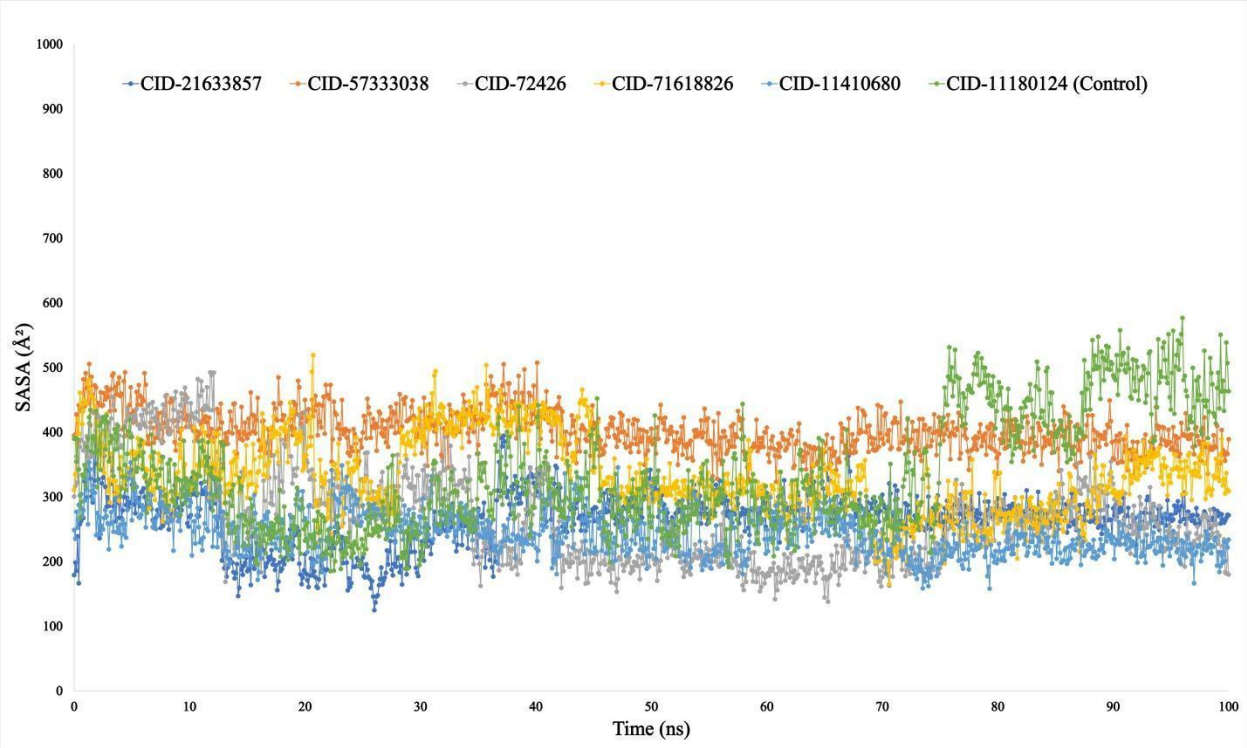


Figure 5. Solvent Accessible Surface Area (SASA) of the 100 ns simulation was also calculated to determine the solvent accessibility of the protein-ligand complexes. The compounds CID-21633857, CID-57333038, CID-72426, CID-71618826, CID-11410680, and control CID-11180124 are represented in blue, orange, gray, yellow, sky blue, and green, respectively.

2.4.5. MoLSA Analysis

The MolSA analysis was performed to evaluate the surface area stability of the complexes of ligand-RAGE during a 100 ns molecular dynamics simulation. The MolSA values for top-hit complexes were found to be very low and also did not change significantly throughout the simulation period in Figure 6, suggesting that the surfaces do not change much during the simulation time. The MolSA values of the compounds were highest for CID-57333038, averaging

approximately 630 Å², indicating a relatively larger molecular surface area. The control compound Azeliragon (CID-11180124) had slightly lower MolSA, averaging around 530 Å², followed by CID-72426 at around 510 Å², suggesting compactness and stability. CID-21633857, CID-11410680, and CID-71618826 had lower MolSA values (~420450 A²) due to their relatively small surface areas. The most active natural polyphenolic compounds, CID-72426 and CID-71618826, as evident in docking and ADMET studies, exhibited consistent MolSA paths with no sharp deviations during the simulation (**Figure 6**). This stability makes them an appropriate choice as potential RAGE inhibitors, since changes in the structure of protein-ligand complexes tend to correlate with binding instability.

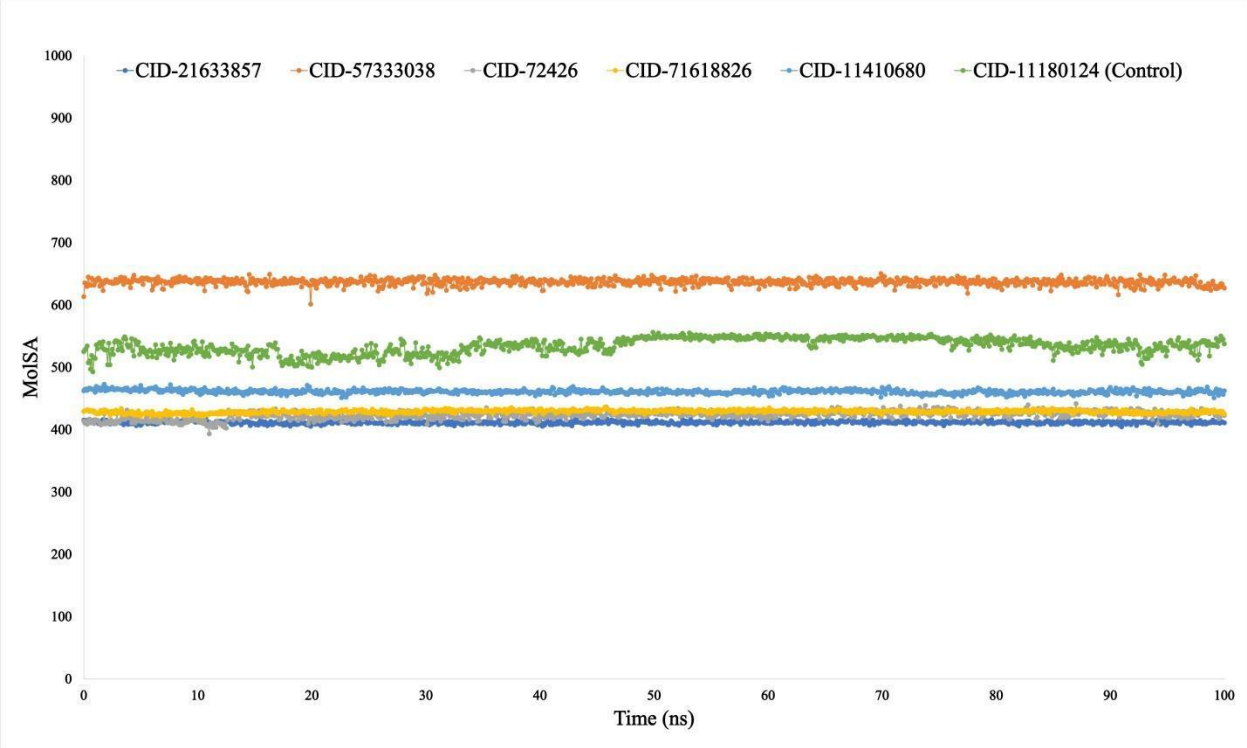


Figure 6: To analyse the stability of the surface area, the molecular surface area (MolSA) of the protein-ligand complexes was calculated during the same 100 ns MD simulation. The compounds CID-21633857, CID-57333038, CID-72426, CID-71618826, CID-11410680, and the control CID-11180124 are depicted using the same color scheme: blue, orange, gray, yellow, sky-blue, and green, respectively.

2.5.6. PSA (Polar Surface Area) Analysis

The PSA analysis indicates polar features of ligand-protein complexes, which may affect the solubility and membrane permeability. The PSA of all six ligand-RAGE interactions for the 100 ns simulation is shown in Figure 9, confirming their stability. CID-11410680 had the highest PSA,

with values continuously in the range of 480-500 Å²; this substance is a highly polar material. The best polyphenolic candidates identified in the study were CID-72426 and CID-71618826. They had a stable profile at 295 Å² and 270 Å² in PSA. This demonstrates that they were medium-polar molecules and remained at the surface throughout the simulation. The PSA of CID-57333038 (approximately 180 Å²) was significantly lower, and the PSA of CID-21633857 (approximately 260 Å²) was somewhat higher, but there were hardly any changes with time. The control drug, Azeliragon (CID-11180124), had the lowest PSA values (about 40–60 Å²), suggesting it is more lipophilic than the polyphenolic compounds (Figure 7). The PSA stability of Daphnodorin A (CID-72426) and CID-71618826 in the RAGE binding pocket suggests that the structures remained polar and unchanged over the simulation period, supporting their potential as bioavailable and efficacious inhibitors.

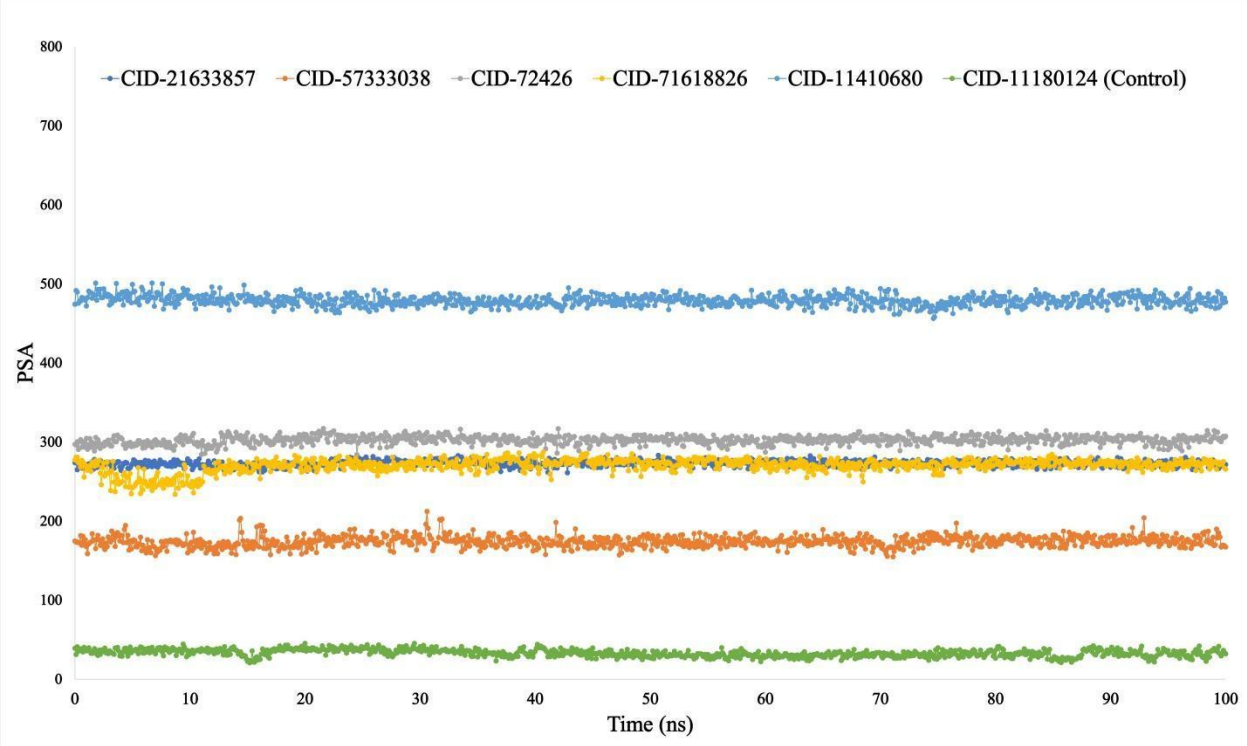


Figure 7: To assess the polarity of the protein-ligand complexes, a 100 ns molecular dynamics (MD) simulation was performed that employed the protein polar surface area (PSA) in the simulation. The compounds CID-21633857, CID-57333038, CID-72426, CID-71618826, CID-11410680, along with the control CID-11180124, are shown in blue, orange, gray, yellow, sky blue, and green, respectively, in complex with the target protein.

3. Discussion

RAGE belongs to a large family of molecules (Ig superfamily) that is known to be quite important in oxidative stress, chronic inflammation, and the pathogenesis of neurological disorder like atherosclerosis, Alzheimer's disease and diabetic complication [16]. In this study, an integrated *in silico* approach such as molecular docking and 100-ns molecular dynamics simulations (MDS) were used to find polyphenolic compounds that can modulate the receptor for advanced glycation end products (RAGE), a key mediator of oxidative stress, chronic inflammation and various neurological and metabolic diseases. Our results show that compounds CID-72426 and CID-7161826 have better binding stability, pharmacokinetics and molecular interactions with RAGE, which makes them better candidate molecules to act as effective modulators of RAGE-mediated pathogenic activationFive hit compounds (shown in Figure 8; CID-72426, CID-11410680, CID-21633857, CID-57333038, and CID-71618826) were identified along with the control (CID-11180124), showing diverse structural features and molecular weights. Many of the hits contained hydroxyl- and polyphenolic-rich functional groups, which are known to increase interactions via hydrogen bonding and electrostatic interactions with target proteins. Specifically, CID-57333038 is the most complicated molecule with the highest molecular weight, which may lead to high interaction but relatively low bioavailability. In contrast, CID-21633857 is more drug-like, with improved ADMET properties.

CID-72426 and CID-71618826 were the most effective phytochemicals for all metrics. These included protein-ligand interactions, pharmacokinetic analysis, RMSD, RMSF, Rg, SASA, and intramolecular hydrogen bond formation. The RMSD mean values for the following complexes were kept significantly below 3.5 Å throughout the simulation, showing that they had not changed much and were, on the whole, stable. The RMSD curves showed that the protein-ligand complexes were stable during the 100ns simulation period. The RMSD (CID-11180124) of the control complex showed mild oscillations after 60 ns with minor structural changes. Among the chemicals studied, CID-72426 had the least variation in RMSD profile (less than 0.3 nm) followed by CID-71618826. This procedure resulted in minimal structural perturbations and a constant binding mode in the RAGE-binding pocket. However, CID-57333038 and CID-71618826 showed greater variation, suggesting a lack of conformational restriction during binding.

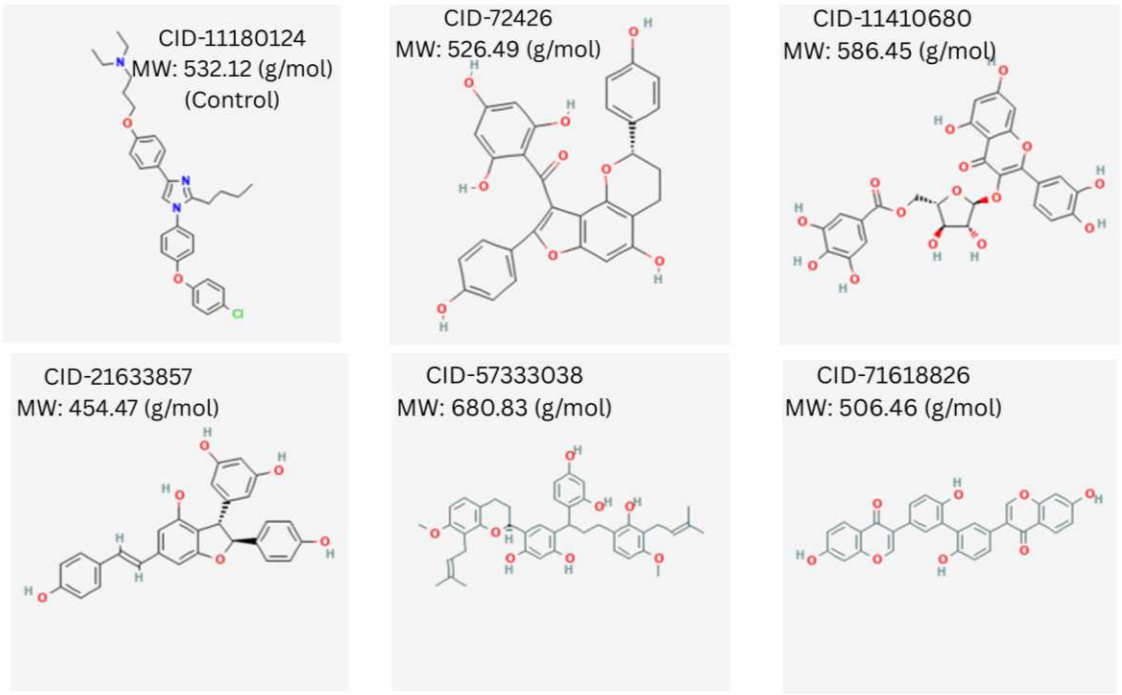


Figure 8: Top Six Compounds Obtained Based on MMGBSA and Binding Affinity

RMSF data also revealed that the RAGE protein was flexible at the residue level. The increase in variation was more noticeable in the loop regions across all complexes, as well as in those regions that are commonly less structured and solvent-accessible, such as 45–65 and 110–130. Complexes with CID-72426 and CID-71618826 showed reduced alterations in these loop domains compared to the control, suggesting that ligands stabilize flexible segments, a key feature of inhibitor development that reduces receptor plasticity.

The Rg values from the top two ligands showed that the protein packing was not destabilized by the ligand, and the two ligands indicated that there was a close packing with the protein. Previous studies have suggested a correlation between Rg and the protein–ligand complex density and folding stability, and an enhanced Rg would have suggested unfolding or disorder. CID-72426 and CID-71618826 maintained a constant Rg (approximately 1.75 nm) and showed a small, stable protein structure throughout the simulation. The mixture of CID-57333038, in contrast, also exhibited additional changes in Rg, which is an indicator of a more unstable protein structure after binding. A decrease in size of a structure means an increase in the affinity of a ligand or decrease in entropy.

Moreover, SASA studies provide insights into protein surface exposure. In the case of control chemical (CID-11180124), SASA increased with time, which implies unfolding and/or partial exposure. The other way around, however, is true of CID-72426 and CID-71618826 as always had a relatively low SASA in the simulation. This parameter is useful for predicting its more

hydrophobic interaction in drug design, the burial of hydrophobic residues and the stable formation of protein-ligand complexes. The hydro-gen-bonding profile of small molecules is important for bioactivity. Long-range hydrogen bonds can be made between ligands and key RAGE residues, especially in the V-domain ligand binding region, to effectively inhibit downstream pro-inflammatory signaling. The complexes CID-72426 and CID-71618826 made stable hydrogen bonds, most likely due to low RMSD and SASA. The simulated trajectory showed that the two top ranked ligands possessed better intramolecular hydrogen-bond network than the control compound CID-11180124, suggesting that they are more specific and possibly have greater binding ability. The present *in silico* study is able to shed light on the therapeutic effects of polyphenolic compounds against RAGE.

The importance of this study is that it advances the rational design of novel RAGE-targeting therapeutics, which is generally performed using computational techniques that minimize reliance on ethically and time-consuming in vivo models. In this study, information regarding the molecular mechanism of polyphenol binding to RAGE and the protein conformational changes are provided, which will help to better understand the ability of polyphenols to stabilize the flexible parts of RAGE such as loop domains and thus inhibit receptor plasticity and pro-inflammatory signaling. This mechanistic understanding adds to and builds upon previous reports concerning the antioxidative, anti-inflammatory, and neuroprotective effects of polyphenols [17] [18] by offering a structural and dynamic explanation of these effects at the molecular level.

Our results are consistent with the previous studies, which emphasize the significance of hydrogen bonding and hydrophobic interactions on the affinity of a ligand–receptor interaction, and extend the analysis by incorporating the predictions of the Swiss ADME and pkCSM computer programs to evaluate drug-likeness and bioavailability. Interestingly, among these, CID-72426 and CID-71618826 have not only stable RMSD and Rg values, reflecting minimal structural perturbation, but are also good examples of molecules with favorable ADMET properties as compared to more complex molecules such as CID-57333038, which, although higher in molecular weight and interaction potential, may have poorer bioavailability. It is a new and complete screening paradigm, based on both molecular dynamics and pharmacokinetics, which adds to the translatability of computational hits.

The observed stabilization of the flexible loop regions for RAGE and the stabilization of compact protein folding (same Rg and lower SASA values) with the best ligands might help to reduce receptor flexibility, which is important for activation and receptor signal transduction. This observation is in line with the idea that conformational restriction of the ligand could be a way to regulate the activity of the receptor, a concept that has been suggested in other receptor–ligand systems. The intramolecular hydrogen-bond networks in both CID-72426 and CID-71618826 also exist in a strong form, thus supporting their high specificity and binding affinity, which are essential for the development of selective RAGE inhibitors.

The antioxidative properties of organic compounds, as well as their anti-inflammatory and neuroprotective effects, have been well established [19] [18] [17]. This study offers a scientific and reliable approach to the identification and characterization of natural polyphenolic compounds as potential candidates to develop therapy targeting RAGE. The integration of docking, molecular dynamics, and pharmacokinetic modeling offers a robust platform for accelerating drug discovery while minimizing animal testing. The findings not only corroborate the therapeutic potential of polyphenols documented in previous experimental studies but also introduce novel insights into their molecular interactions with RAGE, laying the groundwork for subsequent in vitro and in vivo validation.

4. Conclusion

RAGE protein is a significant contributor to complications associated with diabetes, and our experiment aimed to investigate whether natural polyphenolic compounds inhibit the protein. We first filtered 6,000 compounds by their physicochemical and ADMET properties. Among these, good candidates were selected that possessed a high binding affinity of -7.1 kcal/mol and -6.9 kcal/mol when compared to Daphnodorin A (CID-72426) and 7-hydroxy-3-[4-Hydroxy-3-[2-hydroxy-5-(7-Hydroxy-4-Oxochromen-3-ylphenyl) Phenyl]chromen-4-One (CID-71618826). We found that our proposed compound has a more negative binding value to the diabetic protein RAGE protein than the control, Azeliragon (−3.1 kcal/mol). Both lead compounds were also satisfactory in terms of pharmacokinetics, including satisfactory lipophilicity (logP 3.79 and 4.06) and no anticipated toxicity.

Furthermore, molecular dynamics simulations supported the idea that our lead compounds were structurally stable in the RAGE binding site, demonstrating greater conformational integrity and consistency in interaction with the protein compared with the control drug. Our results indicate that natural polyphenolic compounds have enormous potential as lead therapeutics for the control of diabetes, particularly targeting the RAGE protein. The hits have more diverse functional groups than the control, which may help contribute to the strength of these interactions. Additional experimental studies, including chemical isolation, mechanistic investigations, and in vivo confirmations, are indispensable for determining their therapeutic potential.

5. Methodology:

5.1. Platforms for this Study

Databases such as PubChem, the Natural Product Activity and Species Source Database (NPASS), and Coconut; online servers, including Swiss-ADME and ProTox-II; and commercial software, including Schrodinger software (Maestro 11.4, Schrodinger 2020-3), were used in the study.

5.2. Obtaining and Target Protein Isolation

RCSB (Research Collaboratory for Structural Bioinformatics) PDB provided with high-resolution 3D X-ray diffraction crystal structures of the receptor-binding domain of the vertebrate RAGE protein (PDB ID: 4LP5, less than 2 Å resolution, an R-Value Work of less than 0.25, and an R-Value Free of less than 0.30) [7][20] [21]. The protein preparation wizard of the Maestro panel was used to prepare the protein. The preparation involved the addition of hydrogen bonds; protein bond orders were provided as required. The groups were separated by 3 Å of water molecules [22]. Lastly, the OPLS-2005 force field (Schrodinger LLC, NY, USA, 2020-3) was used to minimize the structural protein [23]. We also prepared receptor grid boxes to simulate the active site using the Receptor Grid Generation feature of Glide.

5.3. Ligand Library Selection and Preparation

To construct a database of 6000 natural bioactive polyphenol compounds, we searched across numerous online databases like NPASS (Natural Product Activity and Species Source Database) [24], Coconut [25], and PubChem [26]. Natural polyphenols were also collected from several articles that discussed various kinds of polyphenols in plants, animals, and the sea [1-187 (**Supplementary file**)]. The PubChem database provided the 3D chemical structures of the desired chemicals in SDF format. They carried out 3D and geometry optimizations with Schrödinger Maestro 11.4, minimizing ligand energy [27]. The Ligand preparation was performed using the LigPrep 2.5 module (Schrodinger, LLC, NY, USA), where structural optimization was conducted using the OPLS 2005 force field within the Schrodinger Suite. In the process, ligand ionization was adjusted to physiological conditions (pH 7.0 ± 2.0) using the Epik 2.2 program to obtain accurate ionization states and favorable energetic conformations.

5.4. High Throughput Virtual Screening

By implementing several virtual screening strategies, including Standard Precision (SP) and Extra Precision (XP), Schrödinger 2020-3 software was used to obtain valuable hits. SP refers to a rapid filter that adopts a balanced approach, aiming to deliver accurate results while maintaining an acceptable speed [28]. Whereas XP is a detailed examination, it provides higher-accuracy virtual screening performance than other methods, using sophisticated algorithms and scoring functions [29]. This method also includes High Throughput Virtual Screening (HTVS) (Figure 9), creating a receptor grid box on the active side of the protein where all ligands undergo side-specific binding. The top-hitting inhibitors identified by the XP analysis were selected based on their MMGBSA-estimated binding free energies.

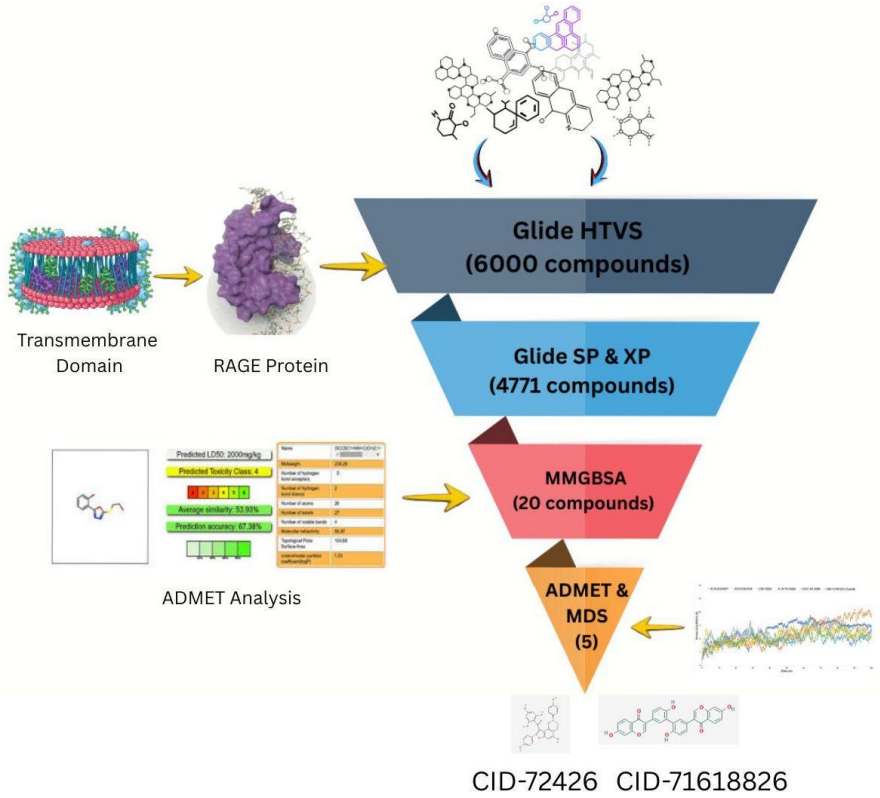


Figure 9: Workflow for high-throughput virtual screening of natural polyphenols for promising RAGE inhibitor(s)

5.5. Evaluation of drug-likeness

The substances chosen for ADMET analysis had binding affinities ranging from 0 to -6.85 kcal/mol. The ADME parameters of potential drug candidates were calculated using the SwissADME webserver [30] (http://www.swissadme.ch/) and Pro-Tox II [31] (https://tox-new.charite.de/protox-II) to determine toxicity. The web server used oral absorption, Lipinski's rule of five, and QlogP [30] to estimate the characteristics and pharmacokinetic properties of the control drugs and ligands. The maximum oral absorption rates, set at 50 cutoffs, were selected as the best candidates for future research.

5.6. MD Simulation.

The stability and dynamics of the RAGE protein, along with the highest-ranking ligand conformations identified during docking and Prime MM-GBSA analyses, were further investigated using MD simulations to understand the complex's sustainability The extra-precision docking results were further optimized to protein-ligand complexes and simulated using Desmond from the Schrodinger Suite (2018). The systems were built in the Desmond system builder, optimizing the

hydrogen-bonding framework, and the complexes were docked in a cubic simulation box. Furthermore, YASARA Dynamics was used to facilitate system configuration and protein-ligand environment optimization [32]. All simulations were conducted under conditions simulating the physiological environment, with temperature maintained at 298 K and pH at 7.4, using the TIP3P water model and the AMBER14 force field, so that the parameters were correctly parameterized [33]. The ionic equilibrium of the system was restored by adding sodium (Na^+) and chloride (Cl^-) to the solvated system. The steepest descent algorithm was then used to reduce energy minimizing steric clashes and stabilizing the initial structure. For computing long-range electrostatic interactions, the Particle Mesh Ewald (PME) method was used, using a short-range cut-off of 8 Å [33].

Binding free energy of each system was estimated using the Molecular Mechanics Generalized Born Surface Area (MM-GBSA) approach. Furthermore, the last 100ns of the equilibrated trajectories were used to calculate MM-PBSA in order to get more information about the binding affinity. The simulation paths were analyzed using different methods such as RMSD (root mean square deviation), RMSF (Root Mean Square Fluctuation), Rg (Radius of Gyration), and SASA (solvent-accessible surface area) [34] [35]

5.7. Analysis of Simulation Trajectories

Maestro (version 9.4) was used in the Schrodinger platform to perform trajectory analysis. To evaluate the stability of the system and the stability of interactions in the simulation, a Simulation Interaction Diagram tool provided as a part of Desmond module was used to assess this. A range of structural and dynamic parameters were measured, such as protein-ligand contacts, Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), solvent-accessible surface area (SASA), intramolecular hydrogen bonding, radius of gyration (Rg), molecular surface area (MolSA), and polar surface area (PSA).

RMSD

Structural deviations were measured using RMSD analysis by comparing atomic coordinates at each time frame to a reference structure. This computation factored in various atomic groups of the protein, such as C-alpha atoms, backbone atoms, side chains, and heavy atoms, to provide a holistic evaluation of conformational stability [36].

$$RMSD_x = \sqrt{\frac{1}{N}} = \sum_{i=0}^{N} (r'i(t_x) - r_i\left(t_{ref}\right))^2$$

Following the completion of the reference system setup, the complete system has been superimposed over system X, and the symbol r′ indicates the location of the chosen atom, N is the total number of atoms, and the time interval, t_{ref}.

RMSF

The local structural variability of the protein complex was studied using RMSF, which revealed the dynamic behavior of the regions. By computing the variation of atomic locations from the average structure over time, RMSF measures each residue's flexibility [37]. The following formula was used to find the RMSF for the amino acid residue I:

$$RMSF_t = \sqrt{\frac{1}{T}} = \sum_{t=0}^{T} < (r'i(t) - r_t\left(t_{ref}\right))^2 >$$

Where the total simulation duration is denoted by T, the time frame reference is t_{ref}, the residue's position is denoted by "ri." The position of the residue i atoms after alignment at the reference time is denoted by "r'." The areas of greater flexibility and structural variety within the protein are highlighted by the conic brackets, which show the average of the squared displacement across all atoms in the residue.

Radius of Gyration (Rg)

The Rg analytical tool was used to track structural compactness and folding stability throughout the simulation [38]. Rg values were also measured throughout the 100 ns pathway to assess the effects of ligand binding on the protein's overall structural integrity. Rg changes with variations in the spatial distribution of atoms around the center of mass, where constant and reproducible Rg values can be used to indicate that a compact and well-folded protein conformation is maintained throughout the simulation.

Solvent Accessible Surface Area (SASA)

The SASA calculations were performed to determine changes in solvent exposure upon ligand binding and the impact on protein stability [39]. The SASA values were calculated from simulation trajectories to measure the extent of protein-solvent interactions. Changes in SASA were measured to estimate conformational changes, where increases or decreases in the exposed surface area to the negative or positive indicated structural rearrangements caused by phytochemical binding, thereby providing insights into protein stability and folding pathways.

Principal Component Analysis (PCA):

The simulation trajectories of 100 ns were also analyzed using PCA to isolate the major motions in the state space, thereby allowing evaluation of conformational space sampling and the dynamic changes induced by the ligand [40]. First, the covariance matrix of atomic positional fluctuations

was constructed from backbone (main-chain) atoms using both trajectory (XTC) and structure (PDB) data. The initial few key components (PCs) with the largest eigenvalues were selected to describe the system's significant conformational variations. This study enabled characterization of large-scale structural motions, flexibility, and ligand-induced conformational changes in protein–ligand complexes.

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Author contributions

Toma Shadhu: investigation, data collection, formal analysis, visualization, and writing-original draft.

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Competing interests

All the authors in the manuscript declare no competing interests.

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Data availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request. The datasets are also available in Zenodo under DOI

10.5281/zenodo.22014065 (https://doi.org/10.5281/zenodo. 22014065)which included 6000 of natural polyphenolic compounds, optimized molecular structures, ADMET prediction data, and molecular docking results.

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