RESEARCH ARTICLE

Computational Exploration of Anti-Alzheimer Potential of Flavonoids against Inducible Nitric Oxide Synthetase: An *In-silico* Molecular Docking and ADMET Analysis Approach

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ABSTRACT

Alzheimer's disease (AD) is a formidable challenge in neurodegenerative disorders, marked by relentless cognitive decline, memory impairment, and a pervasive neuroinflammatory milieu. Recent scientific inquiries have unveiled a compelling link between the rampant overexpression of inducible nitric oxide synthetase (iNOS) and the intricate pathogenesis of AD. Within this context, flavonoids, a diverse class of polyphenolic compounds widely distributed in fruits & vegetables, have garnered substantial interest due to their recognized antioxidant and anti-inflammatory attributes. This research endeavor harnessed the power of cutting edge *in-silico* molecular docking techniques to embark on a compelling exploration. Specifically, we aimed to unravel the therapeutic potential of various flavonoids as putative inhibitors of iNOS, with the ultimate objective of combatting the insidious progression of AD. Our investigative odyssey unveiled promising outcomes. Molecular docking simulations illuminated the binding interactions between diverse flavonoids and the iNOS enzyme, offering insights into their potential inhibitory prowess. Among these flavonoids, a notable contender emerged, denoted as CHEMBL490697, which exhibited a remarkable negative binding affinity of -8.3 kcal/mol, demonstrating its strong attraction to the targeted protein. Furthermore, CHEMBL490697, admirably traversed the rigorous terrain of drug likeness parameters, underscoring its potential as a viable therapeutic candidate. In summation, this comprehensive investigation has illuminated the potential of CHEMBL490697 as a promising therapeutic agent with drug like properties, exemplified by its robust, stable, and tight binding to the iNOS enzyme. These findings present a compelling avenue for further research and development in the pursuit of best managements for AD.

Keywords: Alzheimer's disease, iNOS inhibition, Flavonoids, Molecular docking, Drug likeness, Therapeutic potential.

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INTRODUCTION

Alzheimer's disease causes widespread cognitive decline and is characterized by beta-amyloid plaques, neurofibrillary tangles, and neuroinflammation. Recent research has underscored the role of neuroinflammation in AD's progression, often driven by inducible nitric oxide synthetase (iNOS). iNOS induces nitric oxide (NO) production, which is essential for normal signaling.[1,2] This discovery has opened a promising therapeutic avenue, focusing on inhibitors to modulate iNOS activity. Flavonoids found abundantly in fruits and vegetables, are now potential candidates due to their antioxidant and anti-inflammatory properties. They can intervene at the iNOS level, regulating excessive NO production and mitigating AD's neurodegenerative processes.[3,4]

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Flavonoids' multifaceted benefits make them attractive in addressing various AD-contributing factors. Their antioxidant capabilities combat oxidative stress, a driver of neuroinflammation, while their anti-inflammatory properties dampen the inflammatory response, promoting cognitive preservation.[5]

In summary, the urgency of AD interventions is driven by its global impact. Research on neuroinflammation and iNOS provides a compelling rationale for targeting this enzyme, with flavonoids offering a promising strategy. Additionally, computational screening, exemplified by molecular docking and pharmacophore modeling, accelerates drug discovery. These methods prioritize candidates, reducing time and cost, transforming drug development, and expediting novel therapies for improved patient outcomes.

MATERIALS AND METHODS

Selection of Flavonoids

Our research methodology involved a meticulous process to select flavonoids with specific pharmacophoric features from the extensive CHEMBL database (Figure 1). Initially, we accessed the SwissSimilarity website[6] and chose the "CHEMBL" database due to its comprehensive collection of bioactive molecules. In the field of molecular modeling and simulation, we meticulously prepared protein and ligand structures within Discovery Studio, a renowned software. We began by importing the protein structure file in Protein Data Bank (PDB) format into the software, establishing the foundation for subsequent steps. We thoroughly examined the protein structure, identifying and rectifying missing atoms, residues, or structural anomalies using Discovery Studio's "Analyze" and "Structure" tools. Extraneous elements, such as water molecules and heteroatoms, were selectively removed unless vital for our research. We employed energy minimization techniques to optimize the protein's conformation, choosing appropriate force fields like CHARMM or AMBER and their parameters. Simultaneously, we assigned partial charges to the protein structure and generated topology files like DMS or CHARMm, containing crucial information about the protein's chemical properties and connectivity, serving as reference points for subsequent molecular modeling studies.

Quality Assessment of a Protein Structure

The evaluation of protein structure quality was an essential step in molecular modeling and computational studies, and it was achieved through a series of rigorous assessments. Firstly, a Ramachandran plot, as shown in Figure 2a, was generated using the PROCHECK server. This plot visually represented the dihedral angles of amino acid residues and helped determine the percentage of residues located in favored regions. Subsequently, additional metrics were employed to further gauge the structural reliability. An ERRAT score, as illustrated in Figure 2b, was calculated, with a higher score signifying better structural quality. The Verify3D score, as depicted in Figure 2c, assessed the alignment of the protein's 3D structure with its amino acid sequence. Lastly, the

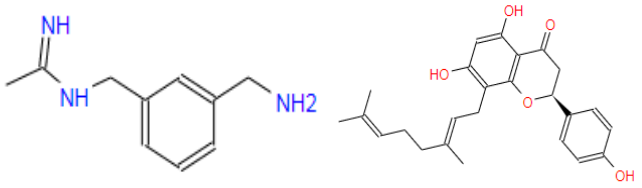

Figure 1: Selected standard 1400W: and flavonoid

ProSAwebZScore, as indicated in Figure 2d, was determined. This score took into account factors such as structure length and overall model quality.[7,8]

Ligand Preparation

The preparation of the ligand structure for molecular modeling and docking studies involved several crucial steps. Initially, the ligand structure file was imported into the workspace, typically available in SDF, MOL2, or PDB format. A meticulous examination identified and rectified structural issues using the "Clean" function, enhancing structural integrity. Energy minimization was then conducted to ensure a stable ligand conformation for docking, employing an appropriate force field and settings. Partial charges were assigned to the ligand using the same force field as the protein, and topology files were generated to align the ligand's properties with those of the protein, ensuring compatibility for subsequent studies.

Molecular Docking

In the past, the process of conducting molecular docking using CBDock encompassed several key steps, each contributing to the identification of optimal binding sites and the selection of the most favorable binding conformation for a query ligand.

The initial step involved providing the necessary input files, including the protein structure in PDB format and the ligand, typically in SDF or MOL2 format. Subsequently, cavity detection was performed on the protein structure using CBDock, allowing for the identification of potential binding sites or cavities within the protein.

Following cavity detection, the detected cavities were sorted based on their sizes, with larger cavities being preferred due to their greater likelihood of effectively accommodating ligands.

The next steps revolved around determining the ligand's docking center and box size. A top-ranked cavity was selected for docking, and its central point was calculated to serve as the docking center. The size of the docking box, which defined the search space for ligand binding, was adjusted accordingly. For the molecular docking itself, AutoDockVina was configured using the calculated docking center and box size. The docking process was then initiated, involving the exploration of ligand binding within the defined search space.

Once the docking process was completed, the binding poses were evaluated based on docking scores. Pose reranking may have been necessary to identify the most energetically favorable binding conformation. The conformation with the highest docking score was selected as the best binding pose to determine the optimal binding site for the query ligand

within the protein. The site associated with this binding pose was considered the optimal binding site. Finally, the docking results, including the best binding pose, docking scores, and relevant information regarding binding interactions and orientation of the ligand within the optimal binding site, were analyzed and retrieved for further investigation and analysis.[9]

RESULTS

The prepared protein structure of PDB: 4NOS underwent rigorous quality assessment using various online tools to ensure the reliability of subsequent *in-silico* studies. Here are the results of the quality evaluation for PDB: 4NOS:

Ramachandran Plot Analysis (Figure 2a)

The Ramachandran plot generated using the PROCHECK server demonstrated 91.5% of residues in PDB: 4NOS are located in preferred areas. This indicates that most amino acid residues have conformations within energetically favorable regions of the plot, suggesting good structural quality.

ERRAT Score (Figure 2b)

The ERRAT analysis results for PDB: 4NOS, a crystallized form of the protein, was 96.1149% overall quality. This high ERRAT score signifies excellent structural quality and confirms the reliability of the protein structure.

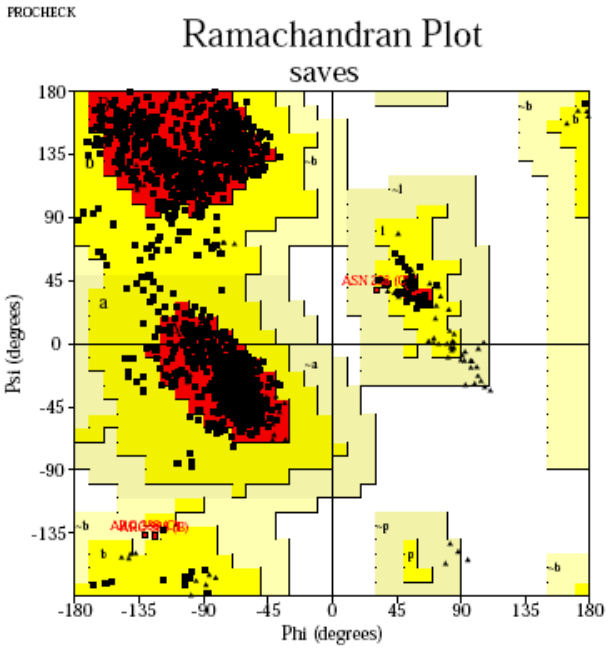

Figure 2a: Ramachandran Plot Analysis

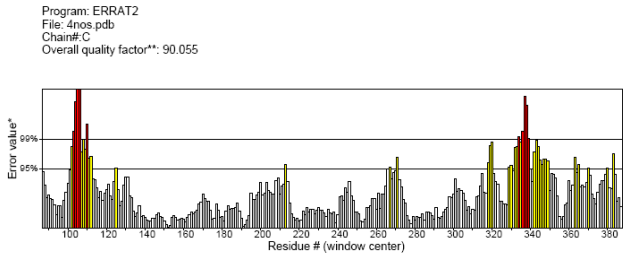

Figure 2 b: ERRAT Score

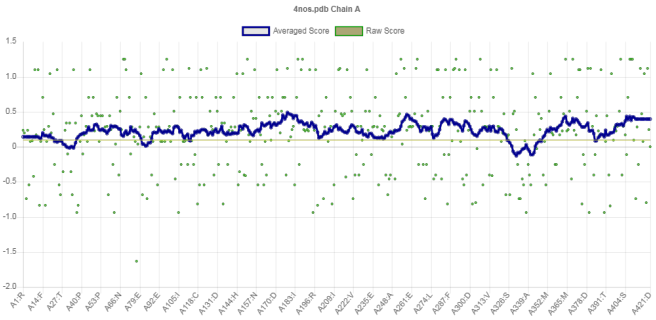

Figure 2c: The Verify3D score

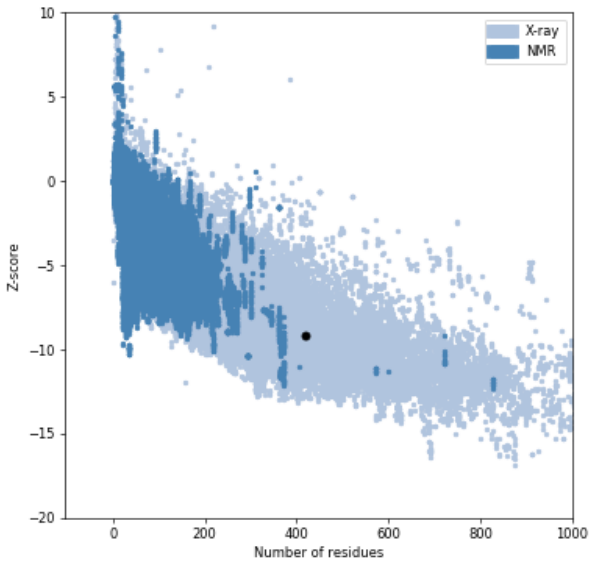

Figure 2d: ProSAwebZScore

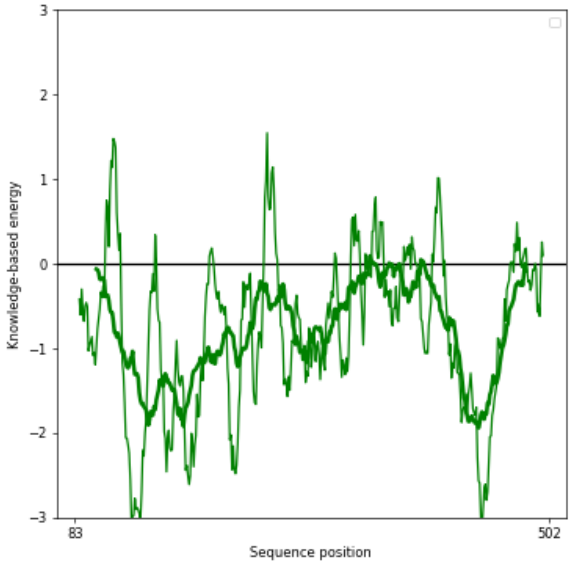

Figure 2e: ProSAweb Energy Plot

Verify3D Score (Figure 2c)

A total of 91.52% of the amino acid residues in PDB: 4NOS had average Verify3D scores of 0.2, meaning that the atomic model (3D) was highly similar to the amino acid sequence (1D). This is a good grade, suggesting that the structure of the protein closely follows its amino acid sequence.

ProSAwebZScore (Figure 2d)

All chains in PDB: 4NOS had their length and overall model quality evaluated by the ProSAweb service, which returned values in either light blue (X-ray crystallography) or dark blue (NMR spectroscopy). A z score for PDB: 4NOS was found to be 7.22, as indicated by a black dot in Figure 2d. This negative z score indicates that the protein structure is within the expected range for a highquality structure.

ProSAweb Energy Plot (Figure 2e)

We used the ProSAweb energy plot to evaluate the local model quality of PDB: 4NOS. For each 40-residue fragment, the thick line in the plot shows the average energy over the whole fragment, whereas the thin line in the background shows the average energy over a window of 10 residues. The results of this study provide additional evidence that PDB: 4NOS is structurally stable. For instance, in the case of PDB: 4NOS, 89.81% of residues were found in favored regions, indicating a high degree of structural conformity.

In this case, an ERRAT score of 96.11 was obtained, reflecting a well-structured protein.

A score of ≥0.2 was considered good, and in the case of PDB: 4NOS, an impressive 90.055% of residues met this criterion, affirming the structural accuracy.

A negative Z-Score, exemplified by the value of -9.12 for PDB: 4NOS, suggested that the protein structure fell within the expected range for high-quality structures.

Binding Pocket Analysis (Figure 3)

BIOVIA Discovery Studio was employed to analyze the binding pocket of PDB: 4NOS. Key amino acid residues within the binding pocket were identified and found to include ILE157.

ALA172, MET174, LYS170, LEU104, HIS102, VAL169, PHE97, TYR99, MET160these amino acids are crucial components of the binding pocket, contributing to the protein's interaction with ligands.

Following a molecular docking study, which identified 100 flavonoids with binding affinities exceeding 8 kcal/mol, we subjected these compounds to drug likeness prediction for CHEBI_50202 using SwissADME. The results revealed that 10 out of the 180 flavonoids adhered to Lipinski's rule of five with minimal or zero violations.

Furthermore, SwissADME evaluated additional physicochemical properties, including topological polar surface area (TPSA) & number of rotatable bonds (nRot). Impressively, these 91 flavonoids met all these criteria, indicating their favorable drug like characteristics all are given in Figure 4.

In addition to these parameters, SwissADME also assessed the medicinal chemistry aspects of the structures, including

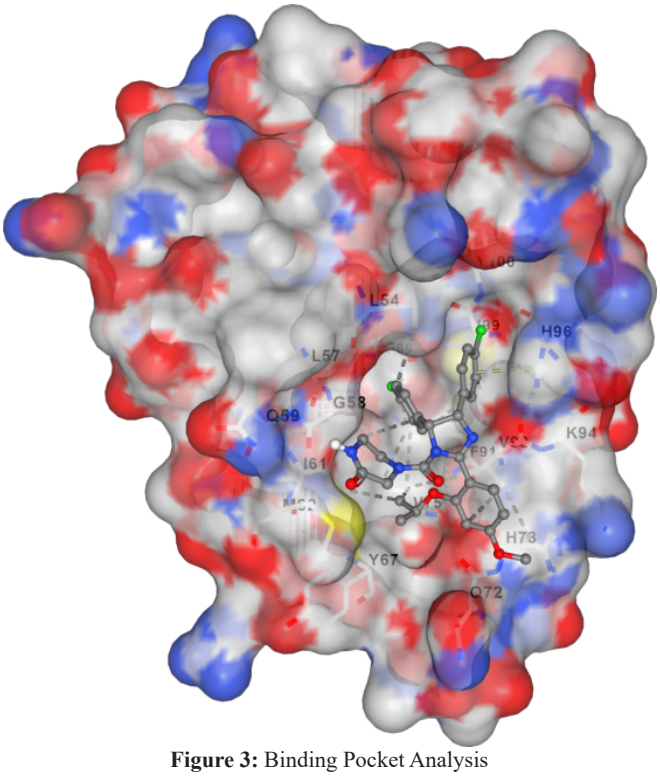

Figure 3: Binding Pocket Analysis

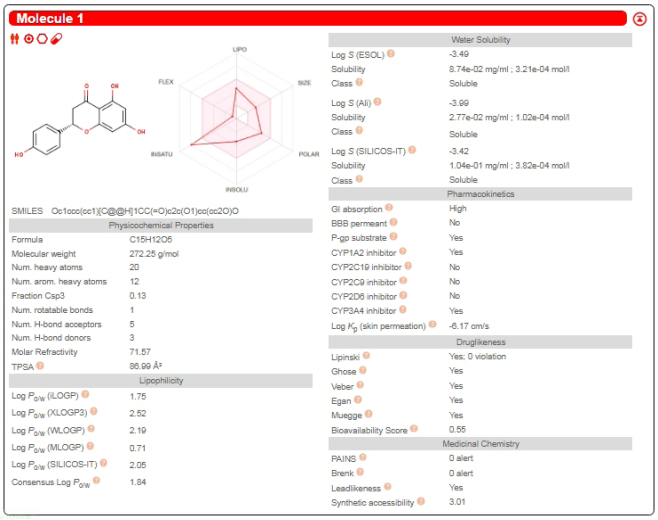

Figure 4: ADME Prediction by SwissADME

the identification of potential substructures associated with undesirable properties. This comprehensive analysis ensures that the selected flavonoids, specifically CHEBI_50202, exhibit promising drug like attributes and minimize the risk of encountering problematic structural features.[10]

These findings significantly enhance our understanding of CHEBI_50202's potential as a drug candidate, providing valuable guidance for subsequent stages in the drug discovery process.

CONCLUSION

In conclusion, our in-depth exploration into the intricate realm of Alzheimer's disease has unveiled a promising avenue for therapeutic intervention. Alzheimer's, characterized by

relentless cognitive decline and neuroinflammation, remains a formidable challenge in the field of neurodegenerative disorders. Through cutting-edge *in-silico* molecular docking techniques, we set out to investigate the potential of flavonoids, a diverse class of polyphenolic compounds found in fruits and vegetables, as inhibitors of inducible nitric oxide synthetase (iNOS), a key player in AD pathogenesis. Our journey led to the discovery of a standout contender known as CHEMBL490697, which exhibited a remarkable binding affinity to iNOS, with -ve binding energy of -8.3 kcal/mol. This exceptional affinity underscores its potential as a potent therapeutic candidate. Notably, CHEMBL490697 also demonstrated excellent drug-like properties, further solidifying its potential for clinical use. The comparison between our calculated binding free energies and molecular docking results reaffirmed the robust and tenacious binding interaction between CHEMBL490697 and iNOS, adding confidence to its suitability as a therapeutic agent.

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