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Computational Identification and Evaluation of Potential Bace1 Inhibitors for Alzheimer's Disease Via Molecular Docking and Dynamics Simulations

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ABSTRACT:

Alzheimer`s disease (AD) is a progressive neurodegenerative brain disorder characterized by cognitive decline, memory impairment, and neuropathological features such as amyloid-beta (Aβ) plaque accumulation and neurofibrillary tangles. The chances of Alzheimer's disease increase with age, and it is thought to affect about 4-8% of the population in elderly people. The β-secretase enzyme BACE1 plays a very crucial role in Aβ peptide formation or the amyloidogenic pathway, making it a crucial therapeutic target. In this study, in silico study methods, including virtual screening, molecular docking, and molecular dynamics (MD) simulations, were used to identify the potential BACE1 inhibitors. A compound library of about 77,000 CNS-permeable molecules was utilized. Following ADME filtering and applying Lipinski`s rule of five, 86 candidate ligands were selected. Molecular Docking using Auto Dock Vina identified five potent compounds with high binding affinities to BACE1 (PDB ID: 6EQM), with compound five showing the most favourable interactions with key active site residues. The compound was further subjected to a 10 ns MD simulation using GROMACS, demonstrating stable binding interactions. The study highlights the potential of computational approaches in the discovery of novel BACE1 inhibitors for the treatment of Alzheimer`s Disease

KEYWORDS: Alzheimer`s disease, Molecular docking, Virtual screening, Molecular dynamics

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative brain disorder characterized clinically by a progressive decline of cognitive function, resulting ultimately in death. AD is currently the leading cause of dementia in the elderly and represents a major unmet medical need. The incidence of AD increases exponentially with age and is thought to affect 4–8% of the population over 60 years old. In 2015, there were 46.8 million sufferers worldwide. Alzheimer's disease is predicted to affect 131 million people globally by 2050.[1] AD is a prevalent neurodegenerative disorder characterized by a progressive deterioration in memory and

cognitive abilities, ultimately leading to dementia. This condition is marked by cognitive impairment, neuropsychiatric disturbances, the accumulation of amyloid plaques, neurofibrillary tangles, and reduced cholinergic transmission. [2] The primary hallmark of Alzheimer's disease is the accumulation of β-amyloid proteins in the synapses of the cortical neurons and the formation of tau tangles inside the neurons due to the twisting of tau protein fibers. These accumulations lead to the neurons' death by hampering the transfer of signals among the neurons. [3] There are two widely accepted hypotheses of AD, namely the cholinergic hypothesis and the amyloid cascade.[4] The cholinergic hypothesis is supported by the observation that acetylcholine levels, a neurotransmitter that plays an important role in neuromodulation of learning, memory, and cognitive functions, are decreased in the cerebral cortex of AD patients compared with those in healthy brains. [5] The amyloid hypothesis states that the increasing aggregation of β-amyloid (Aβ) is another element in the pathogenesis of AD. [6] In the amyloid cascade, the β-Secretase-1 (BACE1) enzyme is responsible for the cleavage of amyloid precursor protein (APP), resulting in the formation of amyloid-β 1 - 40 (Aβ1-40) and Aβ1-42, followed by secretase cleavage. [7] Given the critical role of BACE1 in the production of Aβ peptides, inhibiting the activity of BACE1 has been a target of interest for potential therapeutic interventions for Alzheimer's disease. By inhibiting BACE1, it is hoped that the production of Aβ peptides will be reduced, which could potentially slow down or halt the progression of Alzheimer's disease. Therefore, the development of BACE1 inhibitors as a treatment for Alzheimer's disease remains an area of active research and investigation. [8] In this study, we used existing molecular modeling-based techniques to target the Alzheimer's disease BACE1 protein by docking, molecular dynamics simulations, and virtual screening. By the Lipinski rule of five, screened compounds were examined for ADME characteristics. To further assess top docking complexes, 10 ns Molecular Dynamics Simulations were used.

MATERIALS AND METHODS

MATERIALS

Biovia Discovery Studio, Pyrex, Data Warrior, UCSF Chimera, PyMol, Gromacs.

METHODS

Protein preparation

The BACE1 structure was downloaded from the PDB with the PDB ID: 6EQM (resolution: 1.35 Å) in PDB format.[9] The Biovia Discovery Studio is used for removing the heteroatom and other residues. The 6EQM structure file was stripped of water molecules, and hydrogen bonds were added.[10] Using Pyrex software, Kollman charges were added to the 6EQM structure during docking.[11]

Ligand preparation

In this study, we have used the library from the Mcule database.[12] The name of the library was "Two libraries containing compounds with favorable CNS MPO or BBB scores". In this, we have chosen the library with 77,000 compounds. These compounds are selected because of their ability to cross the blood-brain barrier. These compounds were from a wide range of parameters. To get the compounds that show the activity of BACE 1 inhibitors, we have filtered these 77,000 compounds. For the filtering of these compounds, we have used the software Data Warrior.[13] Filtering is done on the different parameters that belong to the approved BACE 1 drugs. Those parameters are log p, log s, druglikeness, PSA, molecular weight, and H bond acceptors – donors. On this, we have gained 86 compounds. These 86 compounds can act like BACE 1 inhibitors. These 86 compounds are further processed for virtual screening. The three-

dimensional structures of all 86 ligands were retrieved in SDF format from the PubChem database and then translated into PDB format using PyMol.[14] The AutoDock tools were used to convert each of the ligands to PDBQT format.[15]

Virtual screening and molecular docking

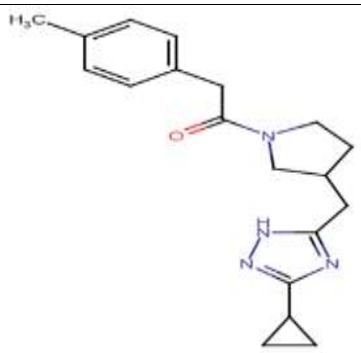
Virtual screening is one of the computational techniques applied in drug development, which finds candidates for drugs from large libraries of compounds. Computer approaches are employed to list compounds that may bind to a specific target protein, such as BACE1 (PDB ID: 6EQM), in this case, a critical enzyme responsible for the production of amyloid-beta peptides linked to Alzheimer's disease, through these libraries. All compounds were filtered out using the BACE1 (PDB ID: 6EQM) structure by AutoDock Vina. Identifying target structures and active site residues allows for the potential development of potent therapeutic drugs through ligand-protein docking. Then, the predicted active site was determined on the three-dimensional structure of the target protein. The active sites of the protein were obtained from the PDB records by Biovia Discovery Studio. And the dimensions of active sites of the protein were 26.853, 78.442, 20.684. For the virtual screening, we have used the software Pyrex. On Pyrex, all 86 compounds are screened against the 6EQM protein. After screening all 86 compounds, we have found the top 5 compounds that showed the greatest affinity towards the BACE 1 protein. Redocking of those 5 compounds was done again. The names of the top 5 compounds are compound 1,2,3,4,5, respectively. Compound 5 is selected for MD simulations.

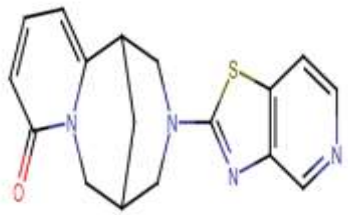
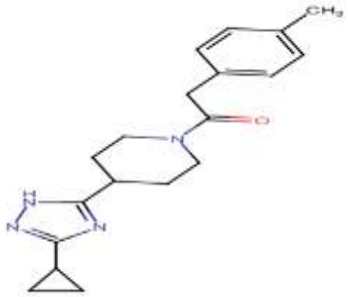
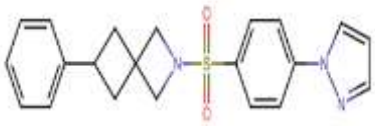
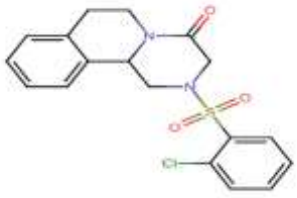
MD simulations

MD modeling of ligand-protein complexes remains a popular approach for providing physicochemically realistic structures of physiologically relevant species. MD stimulation is a simulation process to create a model of a biological system similar to the real-world situation. The solvent representation is seen throughout the protein. The protein structure is quite flexible, with native water molecules solvating the ligand-protein complex. To obtain insight into binding stability and interactions with key amino acids within the BACE 1 protein's drug-binding pocket in a dynamic state. MD simulations were performed for compound 5. The software used for MD simulations was GROMACS.[16] The force field used in the simulation was CHARMM27, and the water model was TIP3P. To see the MD movie, UCSF Chimera was used.[17] We put the MD run for 10 ns for the ligand-protein complex. To get the result of RMSD, RMSF, Radius of Gyration, Hydrogen bonds, and energies, we used the GRACE application.[18]

Results and Discussion

Compound Structure and PubChem ID

Sr. No	Compound Structure	PubChem CID
Compound 1		PubChem CID: 163340211

Compound 2		PubChem CID: 146077915
Compound 3		PubChem CID: 163340269
Compound 4		PubChem CID: 154831446
Compound 5		PubChem CID: 52985605

Dock score

The docking score of the top 5 compounds was found to be -8.7, -8.4, -8.6, -7.6, -8.6. The negative dock score showed a greater affinity towards the protein. More the docked score greater will be the interactions. After docking, we have seen the interacting amino acids of proteins that form hydrogen bonds with the ligand.

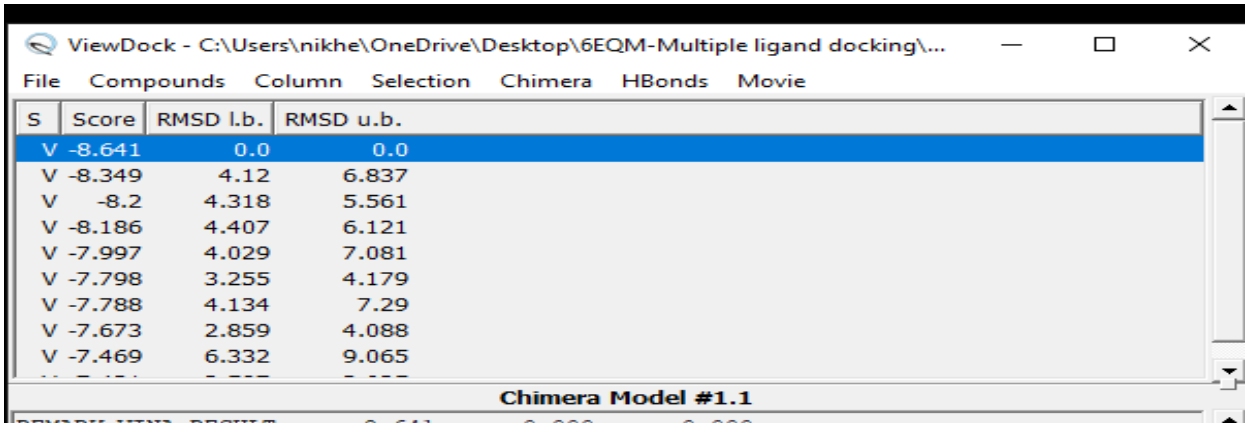


FIGURE 1. Dock score table of compound number 5

Amino acid interaction

For the protein BACE 1, the active sites for interaction of amino acid with ligand are Asp 32, Thr 32, Gln 73, Phe 108, Asp 228, Thr 231, Arg 235 are for 10 S loop and Gly 11, and Tyr 71, Thr 72, and Gln 73 are for the flap region. The drugs that act as BACE 1 inhibitors have shown this interaction with the protein. For compound 1 interaction was ALA 335; for compound 2, no interaction was found; for compound 3, ILE 110, TYR 71, GLY 13; for compound 4, ILE 110, THR 72, SER 229, and GLY 13, THR 232; for compound 5, TRP 115, GLN 73, THR 72, and TYR 71. Based on the interactions, compound 5 shows a good interaction with the BACE 1 protein.

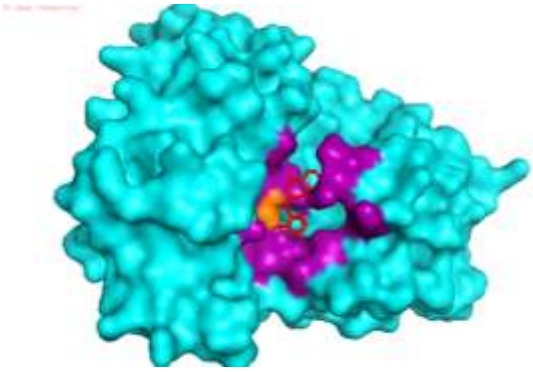
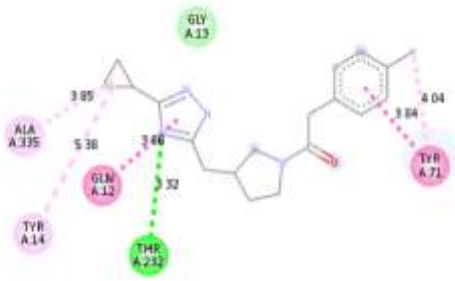
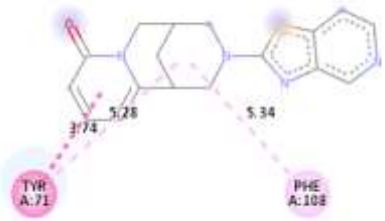


FIGURE 2. Ligand 5 fits into the protein cavity (orange colour showing polar region and purple region showing nonpolar region)

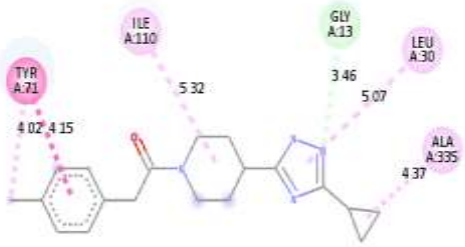
A) 2D Ligand residue interactions



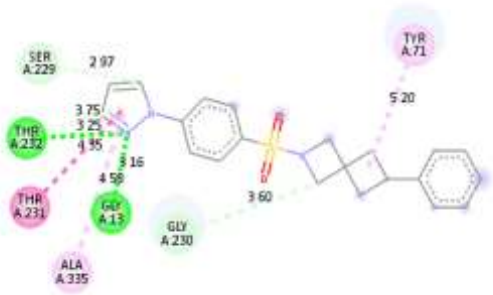
Compound 1



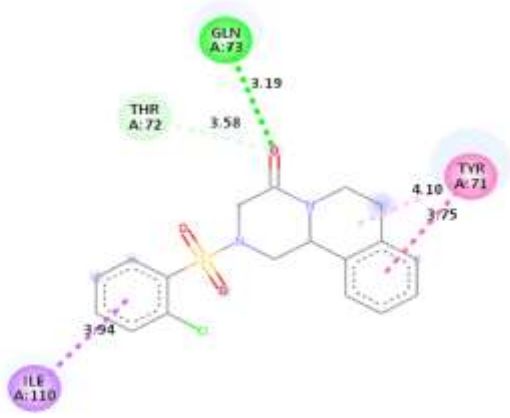
Compound 2



Compound 3

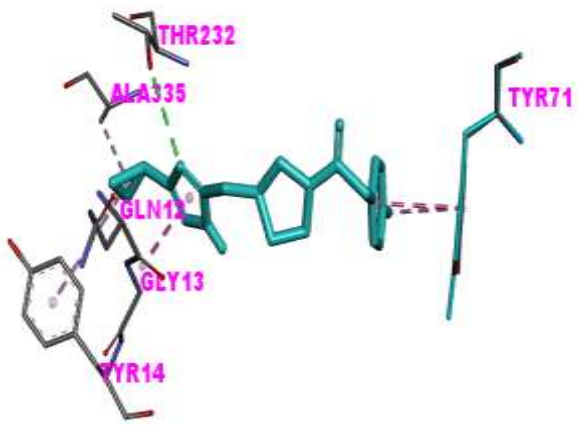


Compound 4

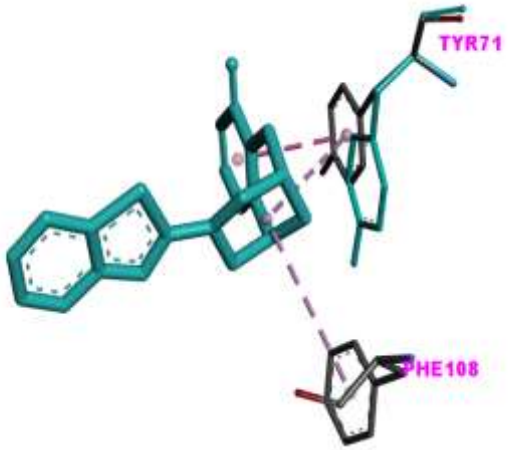


Compound 5

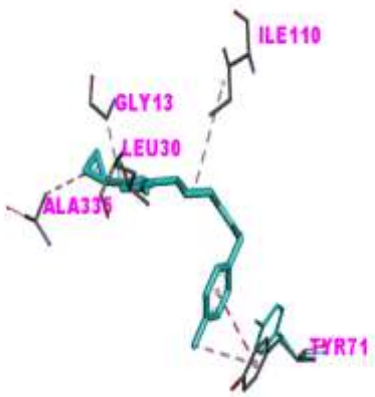
B) 3D ligand residue interaction



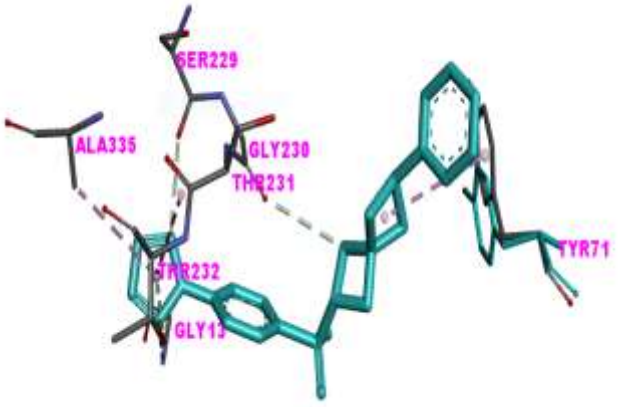
Compound 1



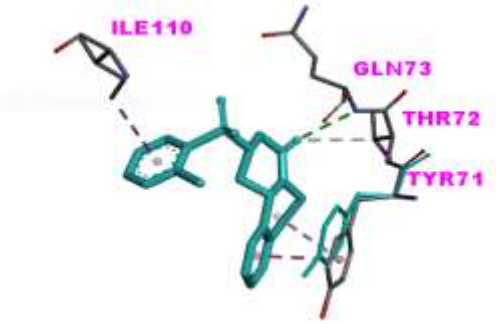
Compound 2



Compound 3



Compound 4



Compound 5

RMSD Analysis

It measures how much the structure deviates from the initial structure over time. RMSD values range from about 0.13 to 0.2 nm of the protein backbone complex. The RMSD grows during the first few nanoseconds (0–1 ns), then becomes constant. After approximately 1 ns, the numbers vary but stay fairly constant, suggesting that the structure is stable. No apparent drift or increasing deviation, implying that the protein backbone remains stable throughout the simulation. The overall conformation of this protein structure is also preserved during the 10ns simulation. The backbone fluctuation is minor (less than 0.1 nm variance), consistent with a stable protein system in MD simulations.

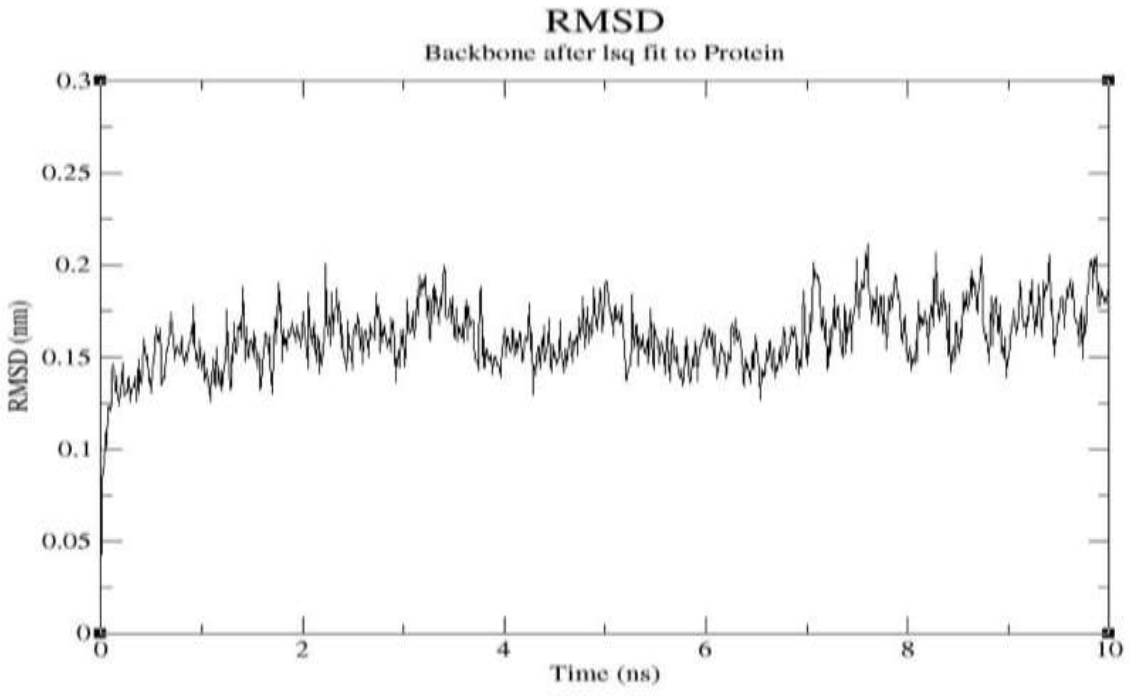


FIGURE 3. Protein Backbone RMSD Over Time (10 ns Simulation)

RMSF Analysis

The RMSF analysis indicates that the protein maintains structural stability throughout the simulation, with localized flexibility in specific regions. These flexible segments likely correspond to loops or terminal

residues, potentially playing roles in functional dynamics or interactions with ligands. Here, less RMSF value is 0.02-0.03 nm, indicating rigid regions, and the maximum RMSF value is 0.28-0.3nm, representing highly flexible regions. Notable peak regions around 1000-1500 and 3000-3500, showing a moderately flexible region and strong peaks near 4800-5000, indicate a highly dynamic region, most likely a terminal region or disordered loop, which could be crucial for binding dynamics, signaling, or flexible docking functions. Another significant increase after 5500, suggesting a gradual increase in flexibility in the terminal region, contributing overall stability of the protein.

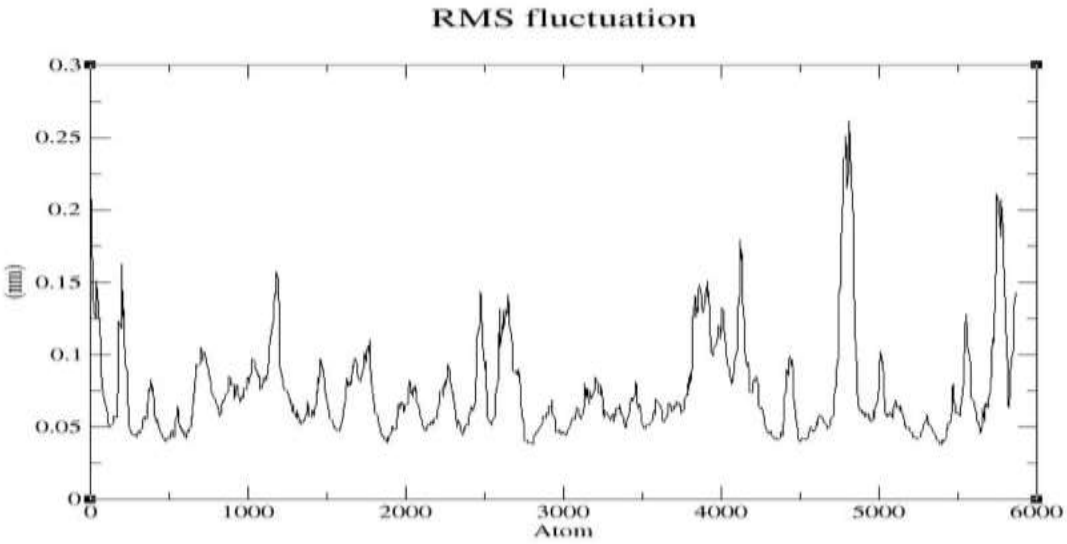


FIGURE 4. Root Mean Square Fluctuations of Protein Backbone Atoms

H-Bonds Analysis

The number of intermolecular hydrogen bonds is essential for the stability of protein complexes. The number of hydrogen bonds fluctuates over the 10 ns of simulation time, and is distributed mostly over 0–3. A distinct time window of enhanced hydrogen bonding, discernible from about 3.5 ns to 7.5 ns, where the number of hydrogen bonds hovers around 2 to 3. This may represent a well-interaction phase, meaning that in this period the protein and ligand are probably in an advantageous binding and conformation held by hydrogen bonds. 3 hydrogen bonds seen intermittently during the 4- 6ns window.

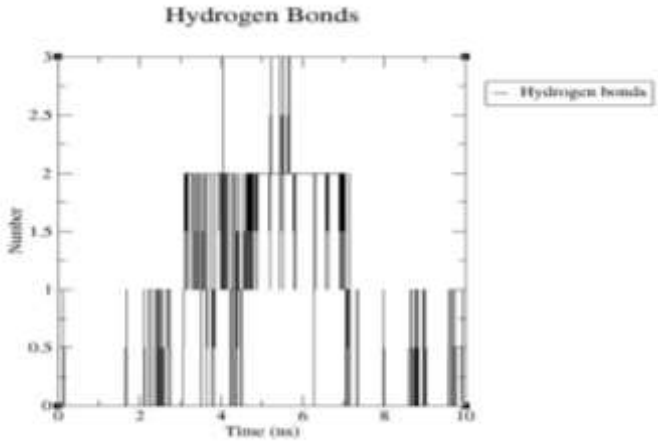


FIGURE 5. Hydrogen bonds formed between the protein and the ligand during the simulation steps

Radius of Gyration

The radius of gyration of the ligand as a function of the simulation time, showing how crowded and folded the ligand remains in its molecular state over the trajectory. The overall black line Rg value fluctuates within a small range at around 2.1–2.2 nm, suggesting a stable and compact conformation. The red line RgX factor was observed to be constant at a value of 0.4–0.5 nm, indicating a similar spatial characteristic along the X-axis, without significantly unfolding or collapsing large peak appears, and Rg shifts from 2.15 nm. This shows that the molecule has a compact and intact structure during the whole simulation. A stable Rg profile is a good indication of structural robustness. The flat trend indicates good structural stability over time, meaning the ligand did not undergo major unfolding or distortion during the simulation.

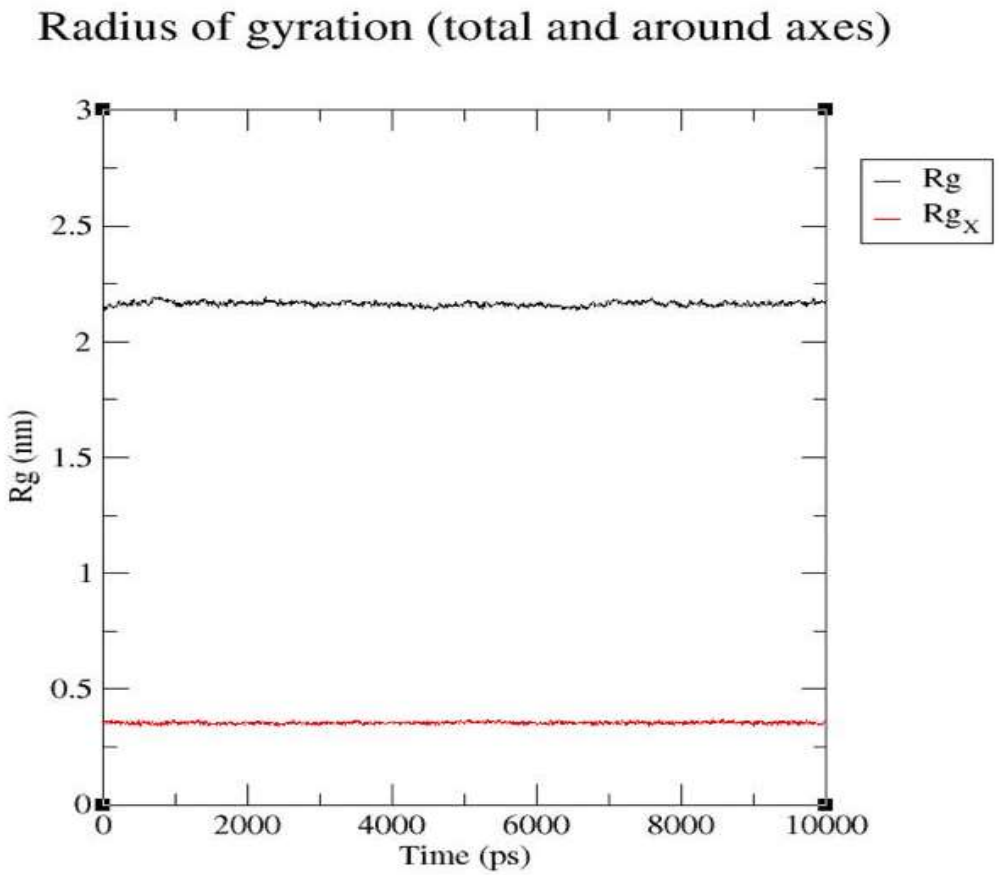


FIGURE 6. Radius of Gyration of the Protein-Ligand Complex Over Time

Bond energies

The variation range of the bond energy is small, approximately 4700~5100 kJ/mol. It's stable in that there are no sudden spikes or drops over the simulation. This means the bond lengths of the covalent bonds in the system were hardly changed during the dynamics. Stability in this graph indicates that no bond breaking or anomalous stretching took place, an important consideration for simulation reliability. The bond energies are kept reasonably constant during the 10 ns simulation. This suggests that the bond length was preserved, in agreement with the structural Integrity of the protein ligand complex during the MD run.

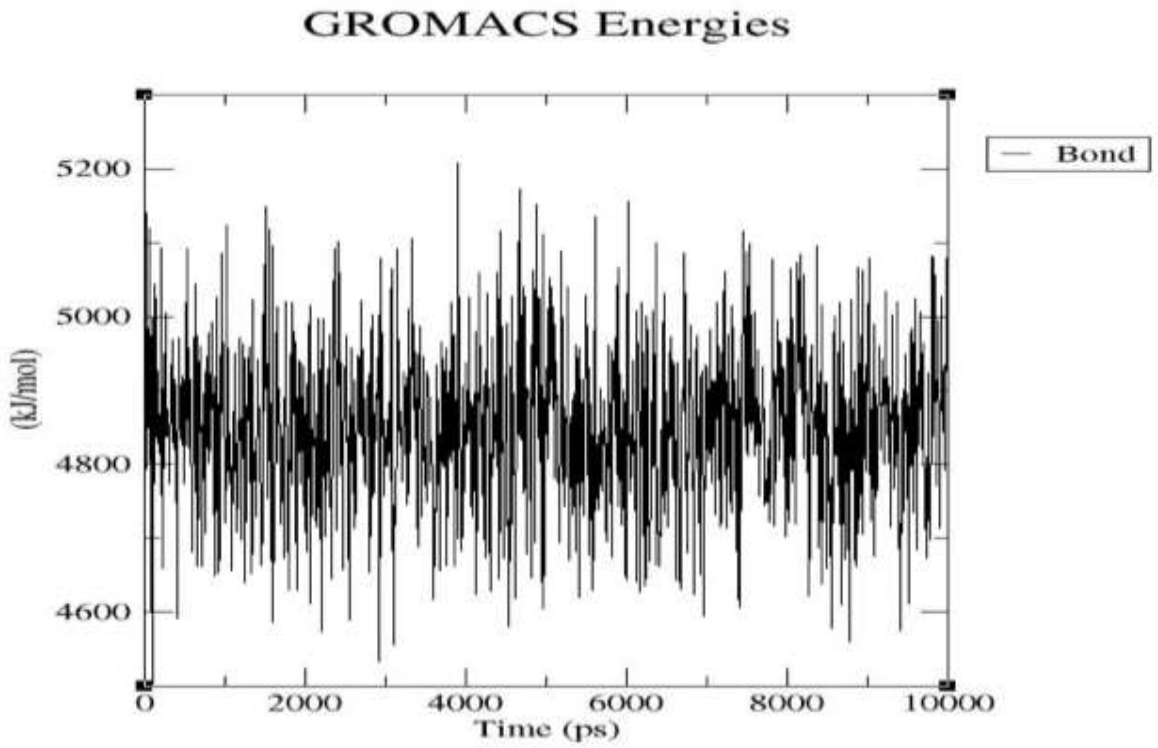


FIGURE 7. Bond Energy Fluctuations of Protein–Ligand Complex During 10 ns MD Simulation

CONCLUSION:

In summary, our present study successfully employed computational methodologies to identify potential BACE1(PDB ID: 6EQM) inhibitors as therapeutic candidates for Alzheimer`s disease. A CNS permeable compound library comprising approximately 77,000 molecules was screened based on drug likeness and ADME properties, selecting 86 lead compounds based on ADME properties and drug likeness criteria. Virtual screening and molecular docking revealed five top candidates with strong binding affinities to the BACE1 active site. Among these, compound 5 demonstrated the most favorable interaction profile and underwent molecular dynamics simulations, confirming its stable and sustained binding within the BACE1 catalytic pocket. These findings support the utility of structure-based drug design approaches in accelerating the identification of novel BACE1 inhibitors and provide a foundation for further in vitro and in vivo validation toward the development of effective Alzheimer`s Disease therapeutics.

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