VIRTUAL SCREENING, *IN SILICO* ADME PROFILING, MOLECULAR DOCKING, AND DYNAMICS SIMULATIONS OF ANTI-ALZHEIMER LEADS ON *β*-SECRETASE 1

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ABSTRACT

The amyloid-cleaving enzyme β –secretase 1 (BACE1), a membrane-associated, aspartic protease, has emerged as a key target for creating anti- Alzheimer's disease (AD) medications being developed to reduce the amyloid burden. Currently, there are limited marketed drugs available against BACE-1 based on limited ADME. In our present study, we used a virtual screening approach to scan the Pubchem database for BACE-1 inhibitors. Studies on pharmacodynamics and pharmacokinetics were conducted to further dock and simulate to select the top hit molecules to assess protein-ligand complex stability. The study reveals that potent, selective molecules that interact and form hydrogen bonds with amino acids Asp 93, Trp 137, and Tyr 132 are recognizable as established inhibitors for BACE-1. The ADME profiling was better with comparable interaction energy values and passed the Lipinski rule of 5 evaluations, Drug likeness, and Physico-chemical properties that include Molecular weight (MW) less than 400 Kilo Daltons; blood-brain barrier (BBB), and Gastrointestinal (GI) absorption. Molecular dynamics studies were performed to validate our findings; the results suggested that the stabilities of the dynamic equilibriums for the complexes were reliable, and the trajectories can be applied to collect snapshots for further analyses. In addition, the complex's stability also proved the credibility of the docking results.

Keywords: BACE-1, Virtual screening, ADME, Molecular dynamics, Alzheimer's disease

INTRODUCTION

Alzheimer's disease is a brain disease that worsens over time and has no apparent cause. It is the most common type of dementia over the age of 65. It is not a specific disease in and of itself; instead, it is a broad word that encompasses a wide range of signs and symptoms. It is a term used to describe a collection of symptoms that arise when brain cells stop functioning properly. This symptom occurs within certain parts of the brain, and it has the potential to alter

the ability to think, remember, and communicate. (McKhann *et al.,* 2011; Huang and Mucke, 2012). According to the Alzheimer's Association, It is estimated that 10 percent of all persons over the age of 65 have Alzheimer's disease and that as many as 50 percent of people over the age of 85 have it. Every five years beyond the age of 65, the number of persons who have the condition doubles. Every three seconds, someone somewhere around the globe develops dementia. In 2020, more than 50 million people lived with dementia worldwide. If this trend continues, the number of people will reach 78 million in 2030 and 152 million in 2050. (Lynch, C. 2020). The majority of the growth will occur in developing countries. Dementia affects 60 percent of the population in low and middle-income countries today; by 2050, this number will increase to 71 percent. The fastest growth in the older population occurs in China, India, and the southern Asian and western Pacific neighbors (Alzheimer's Disease International, www.alzint.org).

Alzheimer's disease (AD) is a terminal neurodegenerative disorder classified into four stages: pre, early, moderate, and advanced dementia. The pathophysiology of Alzheimer's disease includes the deposition of beta-amyloid peptide (Ab-42) in the form of plaques, the creation of neurofibrillary tangles, and the formation of paired helical filaments. The accumulation and aggregation of Ab-42 (Ab-40) oligomers result in the production of amyloid fibrils. (Herzig, M. C., *et al* 2004; Michaels TCT, *et al* 2020; Dear AJ *et al* 2020). Despite recent advancements in understanding Alzheimer's disease (AD) biology and neurology, there is currently no viable treatment or prevention for the condition. Many proteins have been linked to this disorder, but their involvement is still a mystery. According to neuropathologists, patients with Alzheimer's disease (AD) who have had brain autopsy have had "amyloid plaques," which are extracellular proteinaceous debris found in their brains (Huang and Mucke, 2012).

It is essential to provide a brief description of Aβ because many of the current therapies now being investigated focus on reducing Aβ production or preventing the formation of Aβ amyloid fibrils. Aβ is a small protein found in mammals produced by a gene identified on chromosome 21 in humans (Selkoe DJ 1994). In addition, because of its association with Alzheimer's disease, Aβ is a very popular target protein for investigating amyloid fibril production and inhibition. The amyloid precursor protein (APP) is a membrane-bound protein responsible for the formation of Aβ. There are two probable cleavage sites for APP; one site is

acted on by α -secretase, which produces the peptides APPs and C83, while APP acts on the other site. The other site is cleaved by the enzyme β -secretase, which results in the formation of APPs and C99 (Hamley IW, 2012).

It appears to be a strong therapeutic target in the upstream region of Aβ generation. In order to combat Alzheimer's disease, beta-secretase is considered the most promising target for pharmaceutical development, and beta-secretase inhibitors have the potential to reduce the amyloid deposition (Selkoe DJ.2001; Rochette MJ & Murphy MP 2002). Chemoinformatics and Bioinformatics have advanced to the point where various software tools can predict the preferred orientation, among other applications, by interactions implicated in diseases against the docking of one or more small molecule compounds. The demand for new pharmaceuticals has increased molecular docking methods for the computer prediction of possible drugs. These methods assist in investigating the intermolecular interactions between the ligand and the target protein. However, in this study, structural models of ligands and the β –secretase 1 (BACE-1) may be used to produce even more effective inhibitory medicines in the future as in other studies.

MATERIALS AND METHODS

Data Collection and Curation: Identifying new chemical entities by virtual screening of compounds similar to active compounds in AD progression reported in the literature is crucial for the industrial context. There are several ligands with available biological activity and 3D structure data from the PubChem database (https://pubchem.ncbi.nlm.nih.gov), (It is an accessible free repository of small molecules and their structure, physicochemical properties, and improved methods for retrieval of query data) were selected and investigated in this study.

Selection of Protein: The 3D structure of macromolecule BACE1, which was modeled using MODELLER (http://www.salilab.org/modeler) (Sali and Blundell, 1993), in our earlier published research work (Sowjanya K *et al.* 2022). The three-dimensional coordinates and the PDB format of the structure were used for docking analysis.

Molecular Docking: Molecular docking is one of the best filtering methods to identify how two molecules fit together in a 3D space. It is a widely used tool in computer-aided drug design and structural biology (Morris, GM. *et al.* 1998). Hence, docking was carried out to find each lead's most suitable orientation and interactions (hydrogen bonds and hydrophobic interactions) at the

protein's active site. Using PyMOL (www.pymol.org), an easy-to-use chemical interface, the limits of ligands were observed and converted into two-dimensional to three-dimensional structures with physicochemical properties to analyze and promote activity. The Auto Dock vina (Trott, O., & Olson, A. J. 2010) was used for the molecular docking analysis as it is an integrated platform for predicting the protein-ligand interactions. Also, it handles all aspects of the development, from drawing the molecules to shaping the potential prediction of the binding mode of the ligand and binding site of the target protein to focus on usability and productivity. After the docking process, the docking score and ligand binding conformation were studied using Discovery Studio software (Pawar, SS., & Rohane, SH. 2021).

Prediction of ADME Properties: Absorption, Distribution, Metabolism, and Excretion (ADME) parameters were calculated using the Swiss ADME open-access web tool (http://www.swissadme.ch), which offers a set of rapid predictive models for the assessment of physicochemical, pharmacokinetic, and pharmacological properties. Though their use has been criticized for being oversimplified, the evaluation of various Physico-chemical properties in relevance to drug-likeness (such as the "Lipinski rule of 5") has been employed (Cox P *et al.* 2012). In order to find therapeutics that are easily accessible, Lipinski rules assign specified cut-off values to physicochemical parameters (molecular weight, lipophilicity, the number of H-bond donors or acceptors). In silico models are frequently used to assess drug bioavailability (Hou T *et al.* 2009). Standard physical and chemical parameters such as solubility (Wang and colleagues, 2011) or the solubility coefficient (pKa) were assessed to describe the substance regardless of its biological environment.

Molecular dynamics: Based on the findings of the docking procedure and ADME properties screening, Molecular Dynamics (MD) simulation was performed for the complexes of modeled Beta secretase-1 structure obtained (with the ligands selected after ADME screening) using the GROMACS computational package (Berendsen 1995; Van der spoel *et al.* 2006). Topology files and other force field parameters for ligands were generated using the Swiss Param. An AMBER force field method was used to minimize the energy levels and thus relax the water molecules in each system. Protein and protein-ligand complex was simulated by incorporating space-filling cubic boxes of 8 nm x 8 nm x 8 nm and filled with optimal point charges (OPC) water molecules. Counter ions were added to neutralize and equilibrate the system. As a result, the

protein and its complexes were minimized; Calculations based on the NPT and NVT canonical ensembles were performed for thermostability. Analysis of Trajectories was observed using a Gromacs plug-in g_rmsd to analyze the RMSD (Root Mean Square Deviation) parameter of MD Simulation. Other MD Simulation parameters such as RMSF (Root Mean Square Fluctuation) and a number of intermolecular hydrogen bonds were also analyzed using plug-ins like g_rmsf, and g_hbond of Gromacs, respectively. The detailed results are provided in Figures respectively.

RESULTS AND DISCUSSION

Virtual screening: A virtual screening method based on rigid docking was used to screen out probable protein ligand complexes against BACE1 from the PUBCHEM database chemical compounds. In total, 908 compounds were identified through the screening process. Based on their docking score average values, the molecules with a Dock score greater than - 6 Kcal/mol were selected for consideration as probable hits. The resulted 320 compounds were further analyzed for Physicochemical Properties.

Prediction of ADME Properties: Further selection of compounds based on their ADME properties led to the identification of 3 molecules. Any lead chemical compound that has the potential to operate as a potent Alzheimer's disease inhibitor must first be screened for its ability to cross the blood-brain barrier (BBB). Lipinski's rule of five was also considered when determining which molecules would be used. The top three ligands from the virtual screening results were Pubchem ID's 11594966 (-9.3), 17749735 (-8.7), and 68107850 (-8.2), respectively (Table 1). These three compounds out of 320 docked compounds displayed extremely high penetration for blood-brain barrier permeability, indicating that they have the potential to be used as Central Nervous system (CNS) therapeutics. Furthermore, the ADME properties corroborated the findings and demonstrated acceptable intestinal absorption and improved aqueous solubility. The TPSA (The topological polar surface area) is used as a metric for the ability of the (drug) molecule to permeate the cell membrane as it summates the surface of all polar atoms, suggesting that the selected three compounds had a high permeability.

Table 1: The top 3 compounds selected against BACE-1 protein from Swiss ADME analysis with its physicochemical Properties

S.NO	Pub chem. ID	Formula	MW	#Rotatable bonds	#H-bond acceptors	#H-bond donors	TPSA	GI absorption	BBB permeant
1	11594966	C19H21N3O2	323.39	3	3	1	67.92	High	Yes
2	17749735	C19H21N3O2	323.39	3	3	1	67.92	High	Yes
3	68107850	C16H17ClF3N5O	387.79	5	6	2	77.46	High	Yes

Molecular Docking Analysis: The top three compounds from the ADME screening were submitted to Re-Docking tests against the BACE1 protein by AutoDock vina, which served as the receptor for the experiments. Most significantly, the participation of amino acids in the formation of hydrogen bonds was shown to be consistent among the established screened compounds. There is also a role for Asp 93, Trp 137, and Tyr 132, residues commonly involved in the compounds 1, 2, and 3, respectively. The docking scores of these compounds are comparable (Table 2).

Table 2: Molecular docking results of selected compounds against BACE 1 receptor.

S.No	Pubchem ID	Docking Score (Kcal/Mol)	Amino acid involved in hydrogen bond formation
1	11594966	-9.3	**Asp 93**, Gly 291, **Trp 137**, **Tyr 132,** Val 130.
2	17749735	-8.7	**Asp 93**, Asp 289, Gly 95, **Trp 137**, **Tyr 132**.
3	68107850	-8.2	**Asp 93**, Asn 98, Phe 169, **Trp 137**, **Tyr 132**.

The binding modes of selected ligands with BACE 1 protein interactions were shown in Figures 1 to 3, respectively. The bonds between ligands and residues are indicated as hydrogen acceptors (Green color) and donors (Pink color). The two-dimensional interactions are indicated as broken lines. Interaction color represented with green shows conventional hydrogen bond formation, and purple shows alkylation.

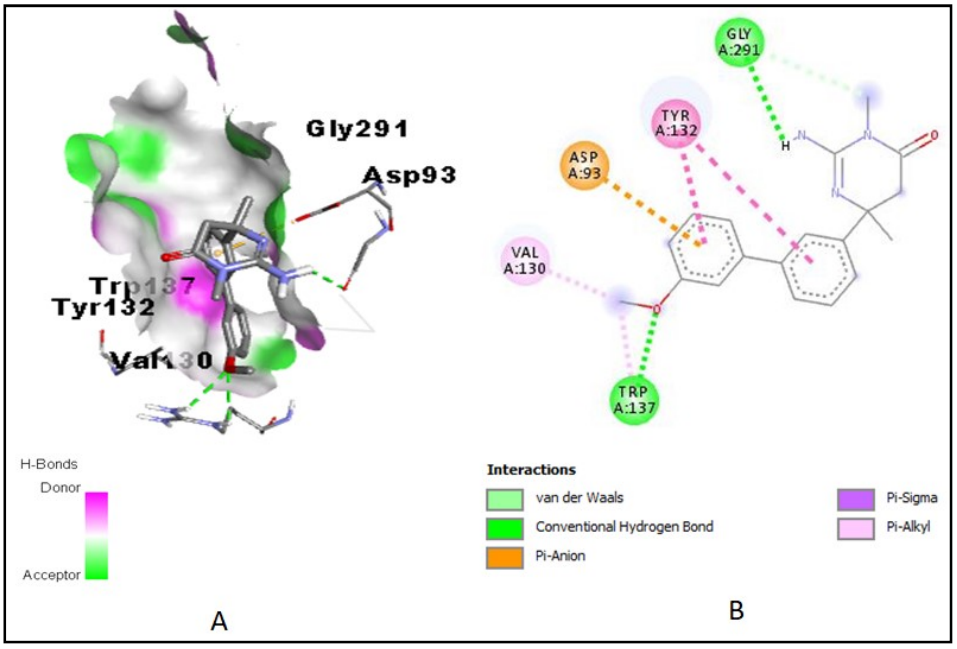

Figure 1: Visualization of interaction studies between ligand 11594966 against BACE 1 protein receptor. A) 3D binding modes B) 2D interactions

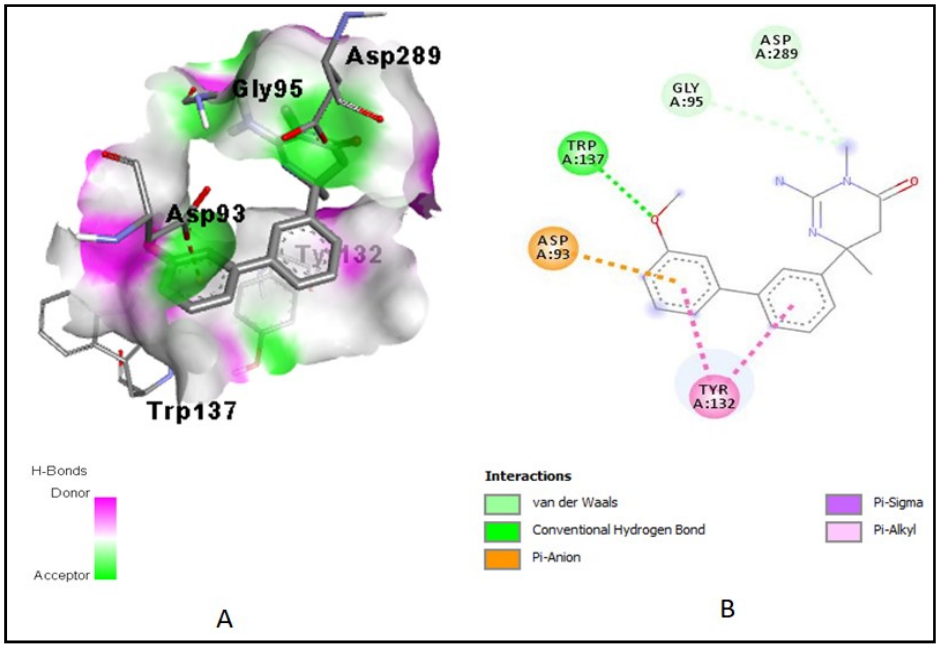

Figure 2: Visualization of interaction studies between ligand 17749735 against BACE 1 protein receptor. A) 3D binding modes B) 2D interactions

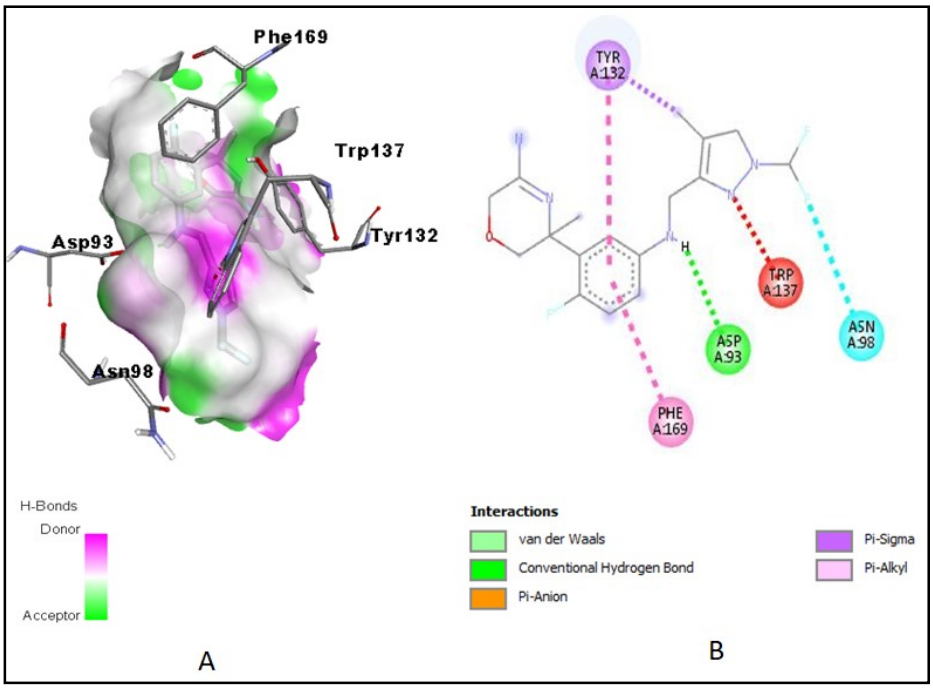

Figure 3: Visualization of interaction studies between ligand 68107850 against BACE 1 protein receptor. A) 3D binding modes B) 2D interactions

Molecular Dynamics simulations: Molecular dynamics investigations were carried out to gain a more in-depth understanding of the thermodynamic parameters related to the development of the ligand-binding complex. For BACE 1 with ligand, the total simulation time was 10,000 ps. It is commonly acknowledged that hydrogen bonds play a crucial role in molecular recognition and overall protein complex stability. Therefore, h-bond analysis was carried out to determine the intermolecular H-bond strength in the protein complex. Even though the most significant number of hydrogen bonds formed is five, it is also shown that at least two hydrogen bond formations offered stability over the entire run, which contributed to the development of complex molecules.

For the simulation, the overall backbone heavy atoms (C- Alpha, N, and C) RMSD value measured in the complex against time in Nanoseconds (ns) for all three simulations, the RMSD values were found to be within the permitted ranges of variation (< 2 Å) (Figure 4 A, C, and E).

Therefore, the trajectory analysis of the drug-target complex was performed using the RMSF analysis. From the starting structure to the end of the simulation, we discovered that the third complex with Pubchem ID 68107850 has highly fluctuating residues (Fig 4F), followed by the second (17749735) (Fig 4D) and first complexes (11594966) (Fig 4B), as shown in below Figure 4.

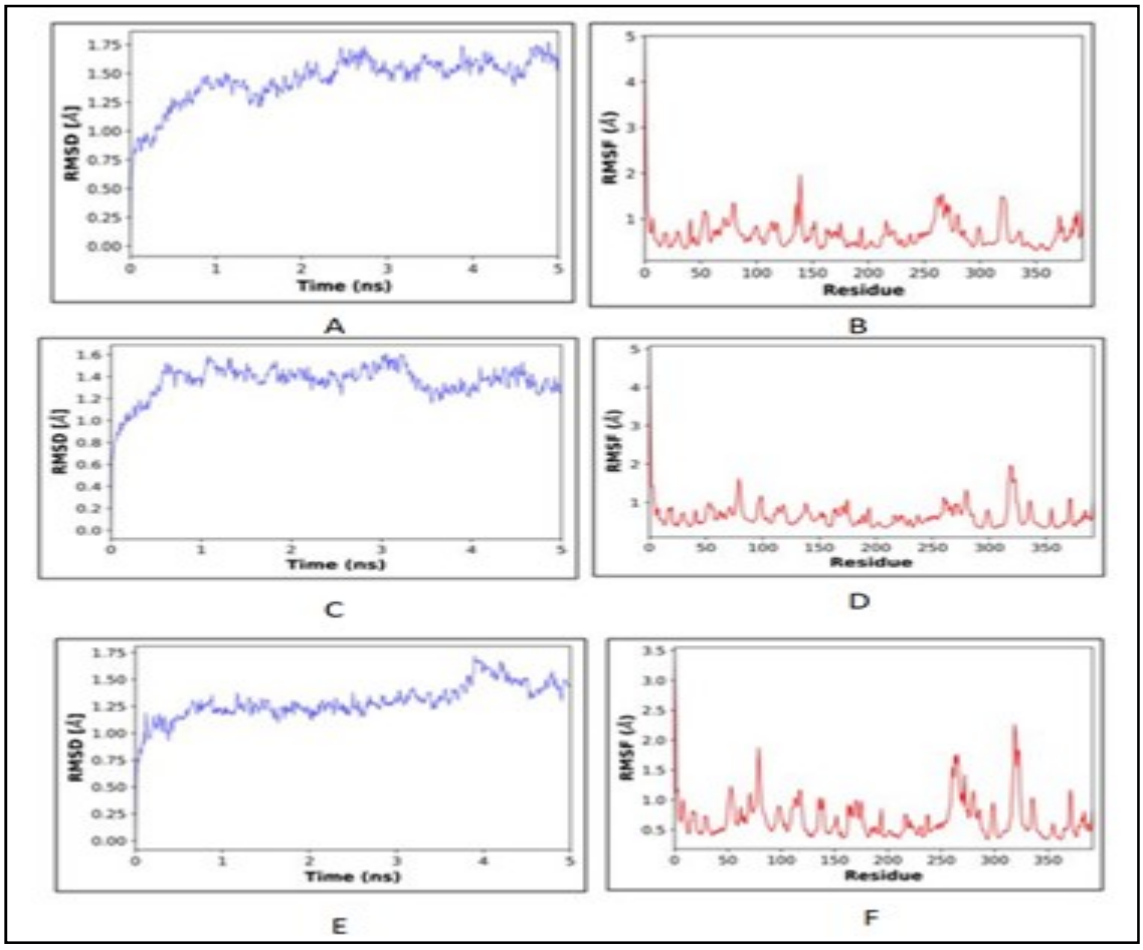

Figure 4: Trajectory Analysis of Complexes: (A) RMSD of 68107850 B) RMSF of 68107850 C) RMSD of 17749735 D) RMSF of 17749735 E) RMSD of 11594966 F) RMSF of 11594966.

In other words, it explains the role of a key amino acid in the binding of ligands to active sites. The binding strength of amino acids in this area (hydrogen bonds and hydrophobic contacts). Ligands were found to cause considerable fluctuations in amino acid levels (Fig. 4). As the run progresses, the fluctuation becomes less noticeable and more consistent. Furthermore, these findings support further investigation into the protein-ligand complex's stability by validating the docking experiment's role and position of critical amino acids in forming hydrogen bonds.

CONCLUSION

The current work has made an effort by virtual screening to find suitable ligand molecules as potent inhibitors against Beta secretase-1 targeted for Alzheimer's disease as BACE-1 has been the choice for AD. As a result, they showed better interaction results to match the ADME profiling highlighting the essential pharmacokinetics and pharmacodynamics, which possess blood-brain barrier and GI Absorption properties.

The selected three molecules bonded with the active sites and with the participation of important amino acids for BACE-1 from the rest of the molecule. Molecular dynamics analyses confirm docking experiments. Furthermore, the protein complex's conformational flexibility was observed, and the structural indicators, including RMSD and RMSF plots, confirmed the protein complex's stable structure. In addition, to the stable thermodynamic characteristics and acceptable RMSD values, this research may be used to develop even more effective inhibitory therapies in the future and in other studies.

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