Computational screening of promising beta-secretase 1 inhibitors through multi-step molecular docking and molecular dynamics simulations - pharmacoinformatics approach

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Highlights

- CNS-Asinex database was virtually screened to identify potential BACE1 inhibitors.
- Through docking and VSW five potential molecules were identified as BACE1 inhibitors.
- Different pharmacokinetics parameters were checked for final proposed molecules.
- MD simulation was performed of BACE1 complex with proposed molecules.
- The binding energy was calculated using MM-PBSA approach.

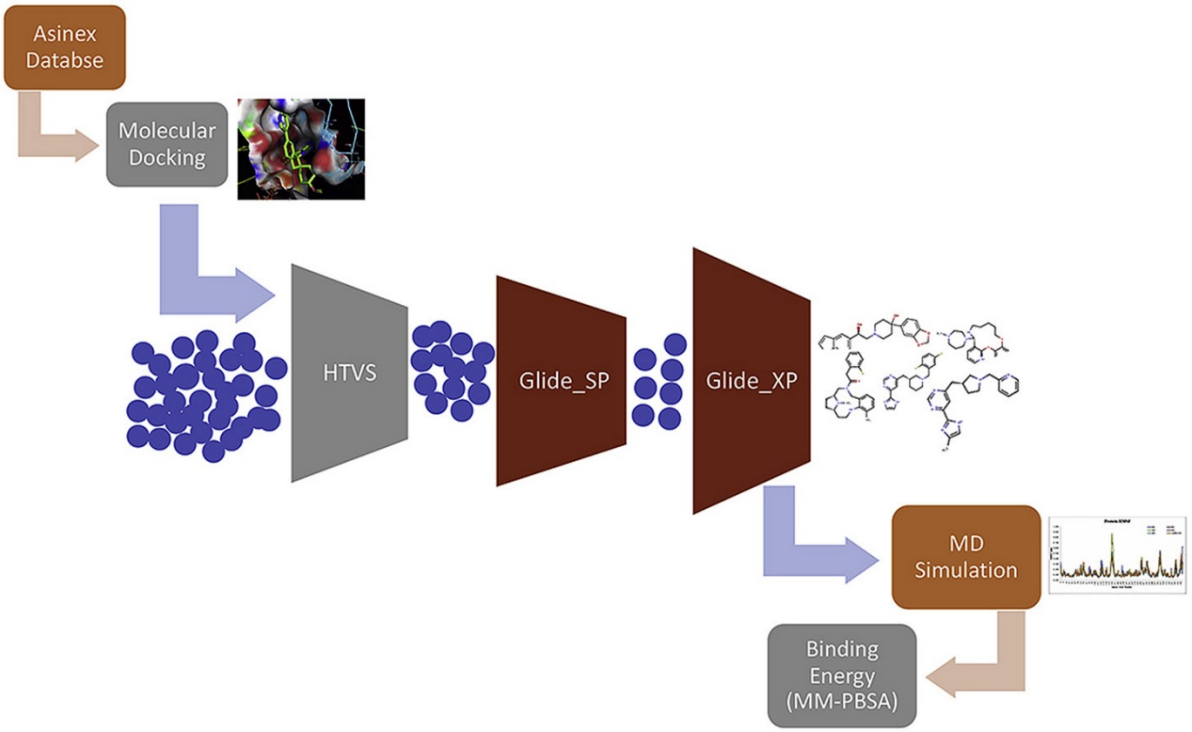
Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder generally develop with aging. AD slowly hammers the memory and cognitive abilities which eventually leads to abnormal behaviour, and ultimately left with disability and dependency. It is anticipated that by the year 2050, world population will experience the incidence of 100 million AD cases. It has

been more than hundred years passed since the AD recognized as a dreadfull disease, but there is no effective curative agent discovered against AD to date. One of the major hallmarks of the AD development is the accumulation of extracellular amyloid-beta (Aβ) plaques in the brain. In the amyloidogenic process, an extensively studied beta-secretase enzyme, known as BACE1, plays a key role in the accumulation and production of Aβ fragments. Therefore, successful inhibition of BACE1 by small molecular chemical entities can be an effective approach for anti-AD drug development. Hence, the current study has been perceived to find out potential BACE1 inhibitiors by virtual screening of entire Asinex chemical library database through multi-step molecular docking methodologies. Further, sequential screening of *in-silico* pharmacokinetics, molecular dynamic (MD) simulations analyses along with binding free energy estimation were performed. Comparative analyses and characteristics of molecular binding interactions assessment finally suggests that five molecules (B1-B5) to be most promising BACE1 inhibitors. Molecular interactions analyses revealed that either one or both the catalytic dyad residues (Asp32 and Asp228) of BACE1 has formed strong molecular interactions with all the proposed molecules. Not only the catalytic dyad residues are involved in the formation of molecular binding interactions but also other important non-Asp binders residues such as Gly34, Tyr71, Trp115, Arg128, Lys224, Gly230, Thr231, Thr232, Arg235, Thr329, and Val332 found to interact with the selected compounds. Moreover, the dynamic behavior of proposed molecules and BACE1 was explored through all-atoms MD simulation study for 100 ns time span. Analyses of MD simulation trajectories explained that all identified molecules are efficient enough to retain the structural and as well as molecular interactions integretiy inside the receptor cavity of BACE1 in dynamic environment. Finally, the binding free energy of each molecule was calculated from MD simulation trajectories through MM-PBSA method and found that all molecules possess a strong binding affinity towards the BACE1. The high negative binding free energies are found to be within the range of -994.978 to -561.562 kJ/mol for identified compounds. Henceforth, analyses of extensively studied multi-cheminformatics approaches explained that proposed molecules might be promising BACE1 inhibitors for therapeutic application in AD, subjected to experimental validation.

Keywords: Alzheimer’s disease; BACE1; Virtual screening; Molecular docking; Molecular dynamics simulations

Graphical abstract



1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative brain disorder which rapidly increasing in the world's population [1]. According to the report of "*2019 Alzheimer's disease facts and figures*", approximately 5.8 million Americans have Alzheimer's dementia. It is also estimated that by mid-century, in the United States itself the Alzheimer's dementia may grow up to 13.8 million [2]. According to the official death certificates, 121,404 deaths were recorded from AD in the year 2017, making AD as the 6th leading cause of death in the United States. It is also ascertained that AD is highly common at the age ≥65 years in the Western European population followed by the North American population [3]. With the increasing frequency of AD, it is now becoming a major public health burden. AD is the main cause of dementia which usually begins with memory loss, difficulties in speaking or writing, judgment making, changes in mood and behavior, problems with abstract thought, and disorientation with respect to time and place, etc. [3, 4]. Although, the AD was identified more than a hundred years ago by Dr. Alois Alzheimer – a German psychiatrist, pathologist and neurologist [5], but no definite curative measures except few treatment strategies have been developed so far for AD management [1]. At present, almost 132 chemical entities are in clinical trials for the treatment of AD, among them 28, 74 and 30 entities are in clinical trial phase 3, 2 and 1, respectively [1]. For example, entities like Elenbecestat, Lanabecestat, Verubecestat, JNJ-54861911, CNP520, and LY3202626 are entered in human clinical trials

and presently being tested on different types of populations with mild-to-moderate AD patients or individuals at risk for getting AD [6, 7]. Among them, Verubecestat progressed to enter in clinical trial phase 3 and confirmed its safety and efficacy of beta-secretase 1 (BACE1) inhibition. However, their success rate will be accounted in time.

Although, a tremendous effort is being given in terms of identifying new targets for the treatment of AD, however, since long, BACE1 - a membrane-bound aspartyl protease, has been demonstrated to be an appealing therapeutic target for controlling AD. The BACE1 is found to be highly expressed in the brain and responsible for 40 or 42 amino acid-long β-amyloid peptide (Aβ) production. It is widely believed that AD pathogenesis is majorly driven by the production, accumulation and deposition of Aβ that aggregates in the brain. The aggregated form of neurotoxic Aβ oligomers requires consecutive cleavage of β-amyloid precursor protein (APP) by two aspartyl proteases, beta-site APP cleaving enzyme BACE1 and finally by γ secretase [8, 9]. Since the proteolytic cleaving of APP is rate-limiting step in the production of Aβ, this BACE1 enzyme is considered as the major therapeutic target for the development of direct or disease-modifying drugs for AD treatment. Therefore, inhibition of BACE1 can prevent the deposition of Aβ, which is thought to be a prominent or alternative way to prevent or stop neuronal failure and death. BACE1 starts with 21 amino acids long NH_2-terminal signal peptide and followed by a pro-protein domain which is ranging from residues 22-45. The catalytic or lumenal domain of a mature BACE1 protein extends from residues 46-460 [10]. Moreover, the BACE1 active site contains few highly conserved or active site motifs (DTGS (residues 93-96) and DSGT (residues 289-292)) [10] and two aspartic acid residues (Asp32 and Asp228) as a catalytic dyad essential for exhibiting catalytic activity [11, 12]. Therefore, targeting the BACE1 catalytic dyad has gained special attention to academic and industrial researchers for designing novel inhibitors for BACE1. Earlier a number of studies have been identified small molecules inhibitors of BACE1 using various approaches including *in silico* analyses [3, 13-24]. For instance, few small molecule derivatives of amino/iminohydantoins were identified with high selectivity as BACE1 inhibitors using structure-activity relationship (SAR) and *in vitro* study analyses [25-28]. Similarly, a variety of aminooxazolines and aminooxazines derivatives also have been reported as potent BACE1 inhibitors using a similar approach [29, 30]. Stamford et al. identified a new class of iminopyrimidinone scaffold as BACE1 inhibitors which are orally bioavailable and blood-brain barrier (BBB) permeable [31]. Genistein - an isoflavone identified to exert a notable BACE1 inhibition using biological evaluation, kinetic analysis, and molecular docking simulation analysis [16]. Another study explores the binding pattern

and potentiality of AZD3293 and Solanezumab as anti-AD agents using molecular docking and dynamics simulation techniques targeting the BACE1 [32].

In this study, we have employed an extensive and rigorous virtual screening strategy for identifying potential drug-like small molecules capable of interacting with BACE1, and thus can confer BACE1 inhibition effectively. In particular, a multi-step molecular docking, classical molecular dynamic (MD) simulations and prediction of ADME (absorption, distribution, metabolism and excretion) profile were carried out for identifying five hit compounds. Moreover, binding free energy (ΔG) of each hit compound bound with BACE1 was estimated for evaluating the strength or characteristics of binding affinity in terms of different types of energy contributions. The adopted multiple molecular modeling approaches may provide an opportunity for extending and expediting the developmental process for finding out the treatment measures for AD considering the BACE1 as a potential target.

2. Materials and Methods

The multiple virtual screening paradigms such as *in-silico* pharmacokinetics analysis, molecular docking and dynamics simulations, and MM-PBSA based protein-ligand binding free energy calculations were implemented to identify the promising BACE1 inhibitors. In this regard, from the Asinex database, central nervous system (CNS) biased compounds were downloaded. This Asinex-CNS database consisting of 131014 compounds which are freely available (http://www.asinex.com/cns/). Compounds deposited in the Asinex database are ready to dock form and therefore, highly used for virtual screening purposes. Moreover, all selected compounds consisting of natural product-like scaffolds with the presence of polar functional groups. The above functionalities of the molecules make suitable for hit-to-lead identification and optimization, fragment-based drug design (FBDD), and structure-based drug design (SBDD), etc. In the current study, primarily molecules were screened out through molecular docking study in AutoDock Vina [33] followed by another round of multi-step docking protocol using the 'Virtual Screening Workflow' (VSW) [34] in Maestro.

2.1.Preparation of molecular structures obtained from Asinex-CNS library database

The entire CNS chemical library dataset of Asinex was available in two-dimensional (2D) representation and downloaded the structural data format (sdf) file format of the same. To remove duplicate molecules, repair the inappropriate valency and generate the 3D coordinates all molecules were prepared using the Discovery Studio [35]. Another open-source tool

widely accepted for file format conversion, the Open Babel [36], was used to convert each ligand compound into PDBQT format. The AutoDock Vina/AutoDock accepts the PDBQT format of the molecules [33], which is pretty similar to PDB representation but it includes partial charges and AutoDock 4 atom types. To assess the outcome from pharmacoinformatics approaches and for comparing purposes, an experimentally evaluated known standard or control molecule AZD3293 [37] was considered in this study. AZD3293 is an established BACE1 inhibitor that was drawn and prepared using the same parameters described above.

3. Virtual screening

3.1. Initial screening using AutoDock Vina

The crystal structure of BACE1 protein having the resolution of 2 Å and R-value of 0.223 was obtained from the RCSB-Protein Data Bank (PDB), the PDB ID: 6OD6 [38]. The size of the receptor cavity and date of deposition were considered to select the best BACE1 protein molecule. The selected BACE1 protein was deposited in March 2019 in the PDB, and to the best our knowledge this one was most recently deposited BACE1 crystal structure with overall accuracy when our study conceived. In order to prepare the protein structure for molecular docking, the polar hydrogen atoms and the suitable number of Gasteiger charges were added in the crystal structure of BACE1 protein using AutoDock tool (ADT). Water molecules were deleted from the protein crystal structure and the crystal structure saved in PDBQT format. The catalytic aspartate (Asp) dyad (Asp32 and Asp228) present in BACE1 protein at the interface of the two lobes was reported to be crucial for inhibition. Hence, the grid box was generated by confining both catalytic dyad residues with the specified coordinates of center as -24.693, -95.985 and 6.459 along the X-, Y- and Z-axis, respectively. By manual inspection, the size of X-, Y- and Z-axis dimension of the grid box was considered as 60x60x60 Å. In order to execution of molecular docking, the above mentioned information of the receptor, ligand and grid center and sizes were kept inside a configuration (.conf) file. Maximum 9 numbers of binding modes were allowed to generate for each ligand during docking execution. No others specific constraint were implied for molecular docking. The AutoDock Vina program [33] is installed on the Linux platform at the CHPC Lengau server (https://www.chpc.ac.za/index.php/resources/lengau-cluster) was used for docking execution of all 131014 compounds. Moreover, before analyzing the docking output of the 131014 compounds, the docking protocol was validated following the re-docking of co-crystalized ligand (ligand ID: M7D) attached with BACE1. Therefore, similar protocol for

ligand and protein preparation was followed and re-docking of co-crystallized ligand was carried out. The RMSD value was found to be 1.920Å after superimposing the original ligand structure over the re-docked co-crystal ligand structure. The superimposed structure is given in Figure S1 (Supplementary file). The molecular docking protocol can be considered suitable if the RMSD value between co-crytsal ligand and docking pose of the same found to be < 2Å [39]. Hence, the selected docking protocol was successfully validated and can be used to dock any molecule. Upon validation of the docking protocol, the binding affinity score of all successfully docked molecules was analyzed critically and ranked to reduce the chemical space of the CNS dataset. The binding affinity score of control ligand (AZD3293) was considered as threshold and molecules having binding affinity score less than or equal of AZD3293 considered for subsequent modeling study employing VSW in Maestro.

3.2.Screening using 'Virtual Screening Workflow'

The screened out molecules through AutoDock Vina were further submitted for 3 phase step-wise docking method using VSW, an extensive and rigorous protocol used for virtual screening. The VSW utility is available in the Maestro interface of the Schrodinger suite [40]. There are basically four steps involved in the VSW which include HTVS (high-throughput virtual screening), SP (standard precision), XP (extra precision) docking, and followed by MM-GBSA based binding free energy calculation for top-scored ligand-protein complexes. It is illustrated that the successful application of VSW is efficient and reliable to achieve the set of ligands with high accuracy and potency [41]. The systematic search is performed through the three stages of molecular docking in VSW through analysis of orientational, conformational, and positional space of the docked ligand. The VSW protocol was executed under some specific set of considerations in the CHPC server. The compounds considered in the AutoDock Vina screening step were selected by browsing of .sdf compounds for the source of ligands under 'Input' tab. To prepare ligands the 'Preparation' tab was selected. The grid confining the catalytic dyad was selected through the 'Receptor' tab. In each of HTVS, SP and XP docking filtering step, the best 60% molecules were retained and considered for used in subsequent steps. The outcome of XP-docking was written for further analysis. Maximum of six binding poses were allowed to generate in XP-docking protocol for each ligand. However, the 'all good scoring states' in each step was selected to retain the best-scored molecules only. The remaining parameters of the VSW panel were kept as default. The Prime MM-GBSA method was used in the final stage to estimate the binding free energy of the selected compounds retained in XP-docking protocol.

3.3. In-silico ADME and drug-likeness prediction

The ADME analysis is one of the important and as well as critical assessment to screen out a few promising chemical entities from a large chemical dataset. Compounds obtained from the VSW were therefore allowed for ADME profile predictions using an online accessible web tool: SwissADME, publicly available at http://www.swissadme.ch [42]. A number of physicochemical, lipophilicity, water-solubility, pharmacokinetics, and drug-likeness properties including Lipinski's rule of five (Ro5) [43] were documented. The SwissADME is widely popular to the scientific community for fast predictive power and spontaneous straightforward interpretation. Moreover, a number of characteristics such as *n*-octanol and water (log $P_{o/w}$) partition coefficient or lipophilicity, molar solubility in water, BBB permeability, skin permeation, human gastrointestinal absorption (HIA) capability and other important medicinal chemistry properties were also examined to select the best BACE1 inhibitors and further assessment.

3.4.Molecular dynamics simulation

The dynamic behavior of finalised molecules, each bound bound with BACE1 protein was extensively analyzed through conventional all-atom 100 ns of MD simulation study with a time step of 2 fs at the constant pressure of 1 atm and constant temperature of 300 K. The Gromacs 2018.2 software tool (http://www.gromacs.org/) available at the Lengau CHPC server was used for MD simulation. The ligand topology file was generated using the SwissParam tool [42]. The CHARMM36 all-atom force field was applied and the TIP3P water model considered to solvate the system. A 10 Å distance from the surface of the center of the protein was maintained for defining the system size for simulation. Appropriate amount of Na^+ and Cl^- ions were added to neutralize the system. The steepest descent algorithm of 10,000 steps was applied and executed to equilibrate and minimize each system. To consider the long-range interaction of van der Waals and electrostatic, the cut off was set to 0.9 and 1.4 nm, respectively. To obtain trajectory information upon MD simulation execution, the snapshots were saved in each 1 ps interval. A number of parameters included root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF) and radius of gyration (Rg) were calculated from each MD simulation trajectory to explore and analyze the conformational and stability of each molecular complex system in the dynamic environment. The MM-PBSA (Molecular Mechanics Poisson-Boltzmann Surface Area) method was employed using g_mmpbsa utility tool [44] to calculate the binding free energy of each molecule to explore the relative binding affinity towards the BACE1. The protocol of the

MM-PBSA procedure can be found in one of the previous publications by our research group [3].

4. Results and discussion

4.1.Virtual screening using multi-step docking and MM-GBSA analysis

With the advancement in computational techniques, the drug discovery research platform has received an exceptional strength for the identification of pharmacologically active lead-like chemical compounds against a specific biological target. The computational technique such as structure-based virtual screening (SBVS) primarily employs the molecular docking method to predict the potentiality of a ligand [45]. Typically, a large chemical library of potential compounds can be screened through SBVS to select a small subset of the potential chemical dataset that can further be considered for biological assessment. Keeping that view, nowadays the SBVS strategies are widely used in drug discovery and developmental projects.

In the present study, virtual screening was carried out for identifying potential drug-like molecules as BACE1 inhibitors from Asinex database - "CNS Library", containing 131014 compounds. To select a control compound for comparison of outcomes from the current study, a total of 9 reported standard BACE1 inhibitors [37, 46] were considered for molecular docking analysis. The binding energy and inhibitory activity of all standard molecules are given in Table S1 (Supplementary file). Also, binding interactions between standard BACE1 inhibitors and catalytic amino residues was explored and 2D molecular interaction diagram given in Figure S2 (Supplementary file). It is illustrated that participation of interactions with two catalytic dyad amino acid residues Asp32 and Asp228 are important for the exhibiting inhibition of BACE1[47]. From Table S1 and Figure S2, it can be observed that AZD3293 interacted with Asp32, and CNP520 form binding interactions with both Asp32 and Asp228. Moreover, AZD3293 was also formed an additional interaction with Gly230. AZD3839 and RG7129 were found to form a single interaction with Gly35 and Phe108, respectively. Remaining molecules were failed to form any molecular binding interaction with BACE1. The binding energy of AZD3293 and CNP520 was found to be -9.00 and -7.00 Kcal/mol, respectively. Therefore obtained data clearly suggested that AZD3293 and CNP520 were the most active standard molecules among all studied nine compounds. The IC_{50} value of AZD3293 and CNP520 was reported as 0.60 and 11.00 nM, respectively. Hence, AZD3293 was selected as a control compound in the current study and binding energy -9.00 kcal/mol used as a threshold value to reduce the chemical space after molecular docking. The virtual screening method was accompanied by multiple molecular docking steps using AutoDock

Vina, and Glide - HTVS, SP and XP docking protocol following different algorithms. The flow diagram of the work is given in Figure 1.

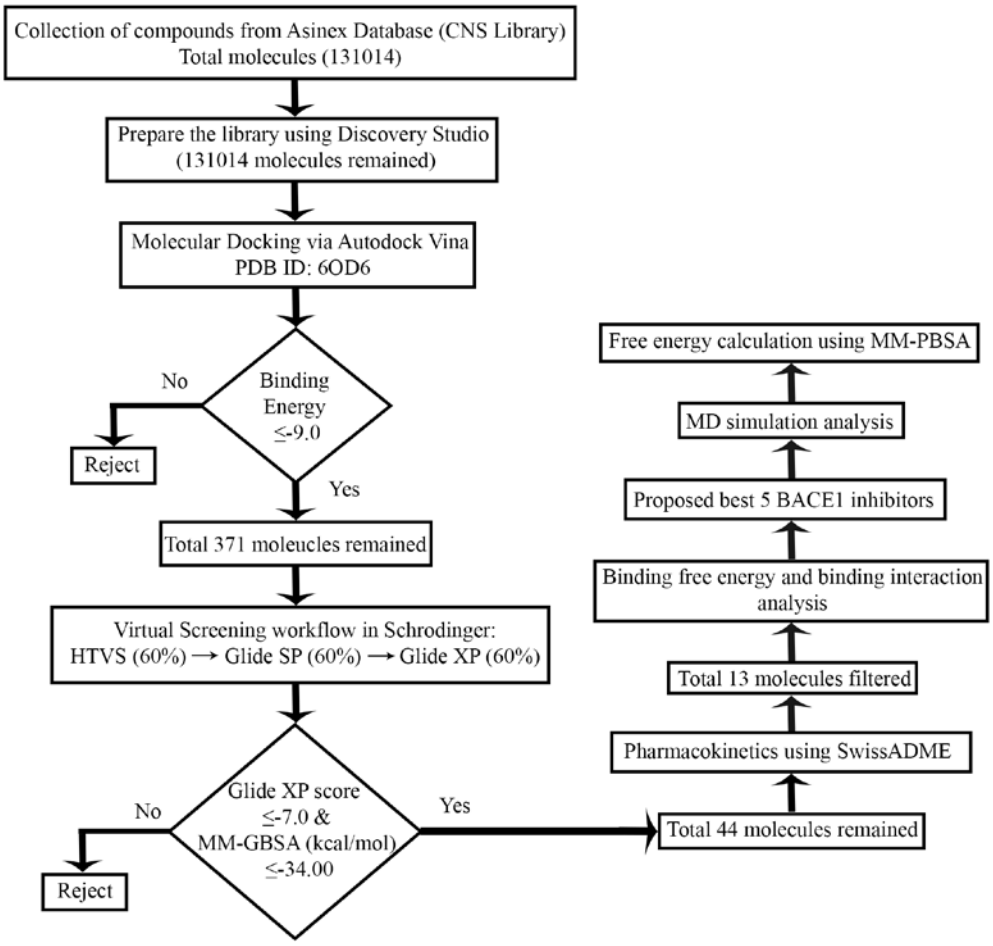


Figure 1. Flow diagram of virtual screening of BACE1 inhibitors

Out of the 131014 CNS biased chemical agents were docked against the BACE1 using AutoDock Vina, only 371 hits initially selected based on the threshold binding affinity scores of the compounds. Precisely, the selection was made by user-defined binding affinity score i.e. -9.00 Kcal/mol, as the cut-off value of the standard compound. The binding energy distribution of all 131014 compounds are given in Figure S3 (Supplementary file). Further, in order to narrow down the chemical space and filter out the inactive compounds, the VSW utility was adopted where the docking of 371 compounds performed hierarchically using the Glide based different–docking programs. In each docking step, almost 60% compounds were filtered out, and hence, the final docking step (Glide-XP) was listed as the top 75 compounds. In successive steps during the execution of VSW in Schrodinger suite, another highly

rigorous and important parameter such as the contribution of energy properties or ligand binding free energies for all 75 compounds were calculated using Prime MM-GBSA method. Therefore, the VSW screening in turn provided the XP-GlideScore and MM-GBSA score for each compound as an output. For the standard compound AZD3293, also the XP-GlideScore and MM-GBSA energy calculation was carried out, which revealed the following values *viz.* -3.71 and -35.08 Kcal/mol, respectively. In order to further screen out the most active and potential compounds for BACE1 inhibitor, the XP-GlideScore and MM-GBSA values of AZD3293 were considered as the cut-off scores. Therefore, from the 75 compounds, 44 compounds were further identified which follows both the scoring criteria and demonstrated the best docking poses. However, for the final selection of best compounds, *in-silico* pharmacokinetic studies were performed for all 44 compounds discussed in the next section.

A number of pharmacokinetics and drug-likeness parameters were explored for the compounds found in the previous step. The analyses revealed that a total of 13 compounds were found to show good absorption, distribution, metabolism excretion and drug-likeness characteristics. Further, in detailed the binding interactions and binding energies were analyzed. Interactions association with the catalytic dyad was given priority for further screening and selection process and finally, five molecules (B1, B2, B3, B4 and B5) were identified to be most promising BACE1 inhibitors based on the following criteria. 2D representation of all proposed molecules are given in Figure 2.

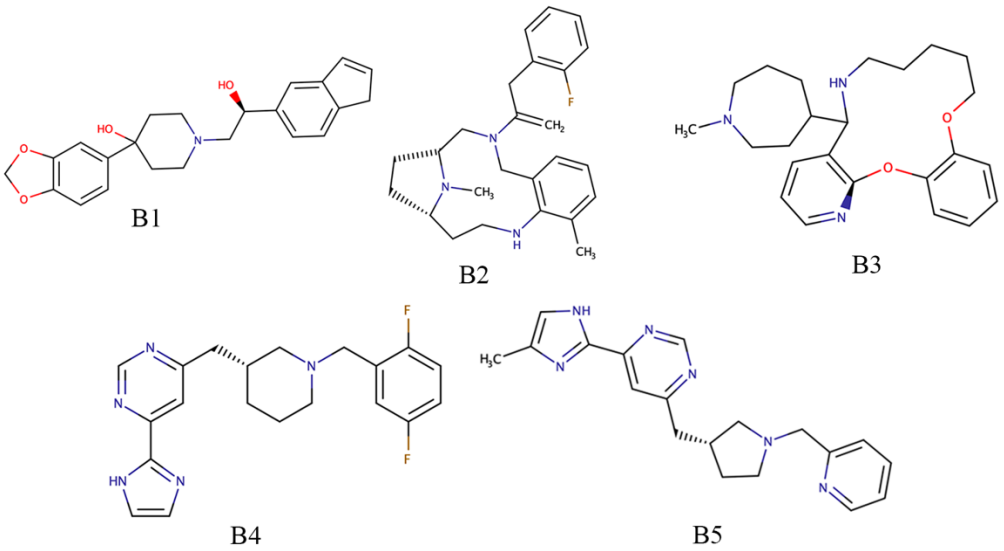


Figure 2. Two-dimensional representation of finally proposed BACE1 inhibitors

4.2.Molecular docking analysis

Analysis of molecular docking based interaction profiles of the selected compounds (B1, B2, B3, B4, and B5) including AZD3293 are portrayed in Figure 3. Molecular docking analyses revealed that compound B1 was formed hydrogen bond (H-bond), hydrophobic, π-stacking and salt-bridge interactions with several amino acid residues of BACE1. In particular, residues Asp32, Gly34, Lys224 and Arg235 and Thr329 were formed H-bond interactions with ligand B1. Two hydrophobic interactions were observed with Try71 residue of BACE1. Apart from that, two other types of molecular interactions profile were generated via π-stacking interaction with residue Phe108, and salt-bridge interactions with catalytic dyad residues Asp32 and Asp228. Compound B2 was found to be involved in H-bond interactions with residue Tyr198. Hydrophobic interactions were noticed with BACE1 residues Tyr71, Arg128 and Val332. Amino acid residue Tyr71 not only participated in hydrophobic interaction but also formed π-stacking interaction with compound B2. A significant number of H-bond and salt-bridge interactions were observed between BACE1 and compound B3 (Figure 3). Precisely, amino acid residues Gln73, Gly230 and Thr231 were formed H-bond interactions, whereas BACE1 catalytic dyad residues Asp32 and Asp228 participated to form salt-bridge interactions with compound B3. With a high GlideScore value (-6.58 Kcal/mol), compound B4 was participated to form 4 types of molecular interactions. Two consecutive amino acid residues Thr231 and Thr232 created halogen and H-bond interactions, respectively with B4. Few other residues Tyr71, Phe108, Ile110 and Trp115 of BACE1 were participated in hydrophobic interaction with compound B4. It was also observed that one catalytically active amino acid residue Asp32 was formed a salt-bridge interaction with compound B4. Although, B5 was generated the highest GlideScore (-6.66 Kcal/mol) in docking analysis, interestingly, only two H-bond interactions were observed between compound B5 and amino acid residue Gly230 of BACE1.

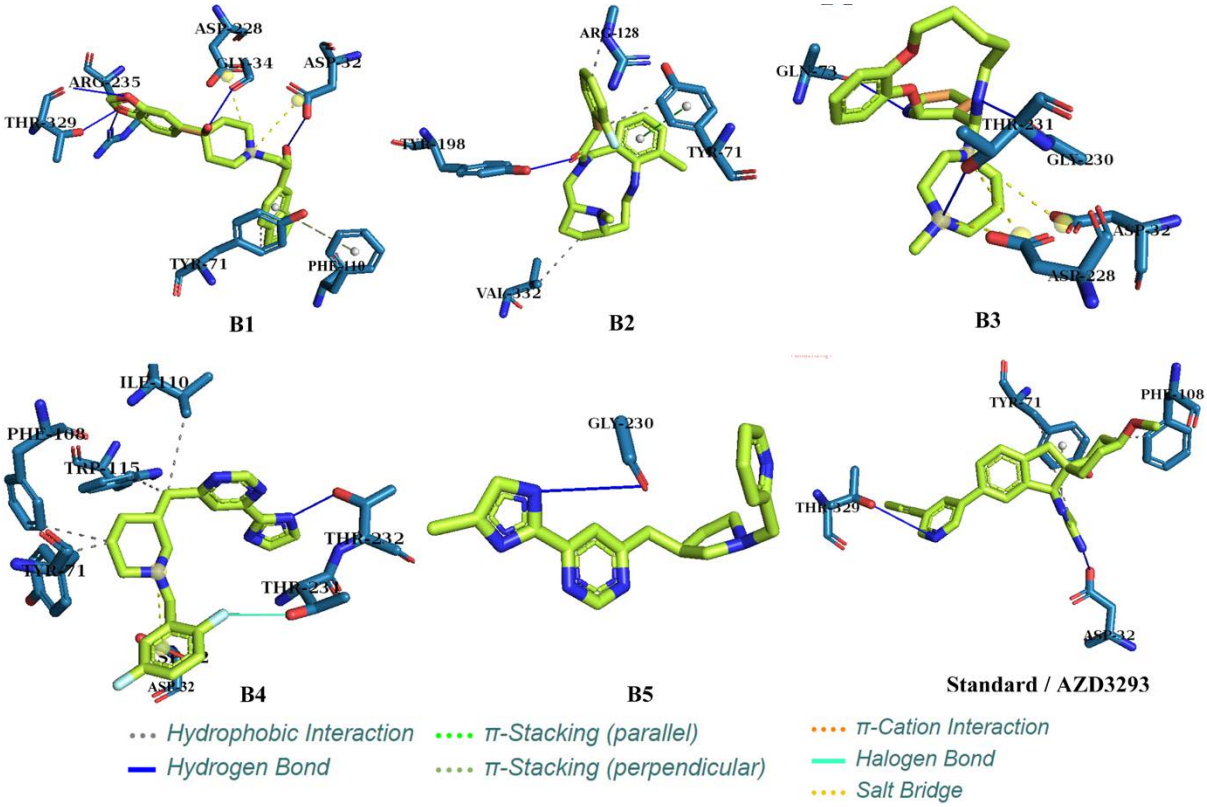


Figure 3. Binding interactions mode of proposed inhibitors with BACE1

The standard compound, AZD3293 interacted with BACE1 by forming three types of molecular interactions profiles viz. H-bond, hydrophobic and π-stacking interactions. Docked complex of protein BACE1 and standard compound revealed that Asp32 and Thr329 involved in H-bond interactions. The hydrophobic interactions were noticed with residues Tyr71 and Phe108 of BACE1. The same residue Tyr71 also formed salt-bridge interaction with the standard compound. Overall, the docking study revealed that standard compound showed relatively much lower GlideScore value (-3.71 Kcal/mol) than the selected five compounds and therefore considered for subsequent molecular modeling purposes. The 3D surface view orientation of each molecule inside the BACE1 active site cavity was checked closely and given in Figure 4. Figure 4 clearly indicate that all identified molecules were perfectly fitted inside the active site cavity of the BACE1.

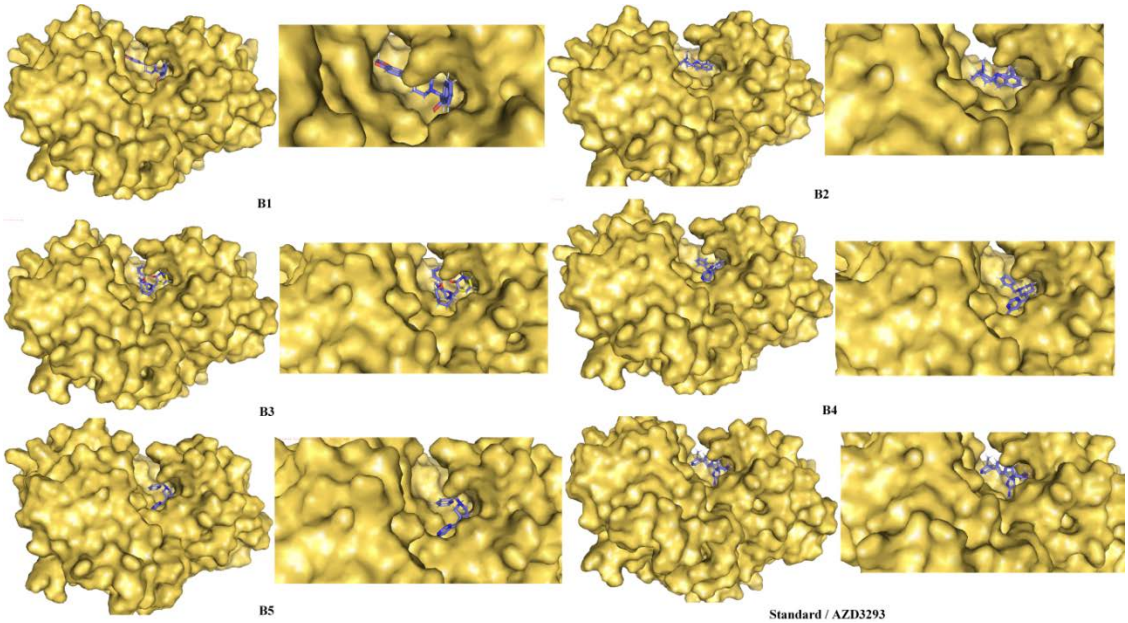


Figure 4. The binding pose in 3D space of proposed molecules and AZD3293

4.3.Comparative analysis of molecular interaction profiles

Earlier, several studies reported a number of small molecule BACE1 inhibitors using *in-silico* methods in the drug-designing aspect. In this study, the docking based molecular interactions maps revealed for the five selected compounds against BACE1 were demonstrated selective inhibition mechanism and specificity for ligand binding interactions. For instance, one of the catalytic dyad residues Asp32 was found to be a prominent interacting residue for interacting with all identified compounds except B2 and B5. However, the types of molecular interactions observed were not the same for all the compounds. In particular, Asp32 participated through mainly two types of molecular interactions such as H-bond and salt-bridge interactions. Another catalytic residue Asp228 also found to form salt-bridge interactions with compounds B1 and B3. Not only the Asp-catalytic dyad residues of BACE1 binds with the selected identified compounds, but also few important non-Asp binders (Gly34, Tyr71, Gln73, Phe108, Ile110, Trp115, Arg128, Lys224, Gly230, Thr231, Thr232, Arg235, Thr329, Val332) found to be interacted with the selected compounds. A similar observation was noticed earlier which suggested that few non-Asp binders residues (Leu30, Tyr71, Phe108, Ile110, Trp115, and Ile118) participated in π-stacking and hydrophobic interactions with their identified compounds [48]. According to the study of Yechun Xu et al., the flexibility of the flap in the active site of BACE1 was revealed by crystal structures and molecular dynamics simulations study demonstrated that residues Gly34, Gln73, Tyr198, Gly230, Thr232 can frequently form hydrogen bonds with the peptides and other small organic ligands. They have also reported that few other residues Tyr71, Thr72, Gln73,

Phe108, Ile110, Trp115, Ile118, Ile126, Tyr198, Ile226 and Gly230 often contributed to form hydrophobic interactions profile with the ligand [49]. Most importantly, the study demonstrated that residues Tyr71 and Gln73 showed the highest tendency to interact with the ligand, hydrophobically [49]. The present study findings also reveal a similar interaction map for the residues Tyr71 and Gln73. Precisely, at N-terminal region, the Asp catalytic dyad of BACE1 is usually covered with 11 amino acid residues (Val67- Glu77) fragment of antiparallel hairpin loop which long been known as a flap. It was proposed that by acid-base mechanism of hydrolysis, BACE1 catalyzes the cleavage of peptide bonds of the substrate with the help of Asp catalytic dyad. It was found that the Tyr71 residue of flap interacts with APP and facilitated the movement of the flap, which in turn provides the entry of substrate into the BACE1 active site and organizes the correct conformation in the catalytic site [50]. In another study, ensemble based docking and MM-GBSA residue decomposition analysis identified few potential BACE1 inhibitors for AD suggested that residues Asp32, Asp228, and Tyr71 as the main contributors for showing crucial binding mechanisms [51].

4.4. Pharmacokinetics assessment

It is crucial to examine the physicochemical properties and pharmacokinetics of a molecule to discuss the potentiality of the molecule in depth. Propose BACE1 inhibitors (Figure 2) were taken into account to study the ADME profile and other properties such as physicochemical properties, lipophilicity, water-solubility, drug-likeness, and medicinal chemistry. The above characteristics were obtained from online tool, SwissADME (http://www.swissadme.ch/) and given in Table 1.

The molecular weight was measured to be less than 404 g/mol of all the molecules. The prediction of transport properties of drugs can be explained using the polar surface area and the values was measured to be 74.35, 63.17, 70.67, 67.23 and 76.58 $Å^2$ for B1, B2, B3, B4 and B5, respectively. For an orally active molecule, the value of topological polar surface area (TPSA) should be less than 130$Å^2$, therefore values of all proposed molecules indicated to be orally active. The solubility class describes that all molecules were soluble in nature. The absorption of the molecule in the intestine is explained by the gastro-intestinal (GI) parameter, here it is reported as high which means all the molecules are highly absorbed in the intestine. The BBB parameter of all molecules was explored and found that B2, B3 and B4 capable to penetrates in the brain. The synthetic accessibility (SA) describes the ease of synthesis of the molecule. The low SA value of all proposed molecules indicated that not a single molecule is difficult to synthesis.

Table 1. Physiochemical parameters proposed BACE1 inhibitors

Parameters	B1	B2	B3	B4	B5
Formula	$C_{21}H_{23}N_5O_2$	$C_{21}H_{23}N_5O$	$C_{22}H_{30}FN_3O_3$	$C_{19}H_{21}FN_4O_2$	$C_{22}H_{26}N_4O_3$
[1]MW (g/mol)	377.44	361.44	403.49	356.39	394.47
[2]NHA	28	27	29	26	29
[3]NAHA	16	18	6	11	12
[4]NRB	3	6	5	6	4
[5]MR	114.62	108.64	118.86	97.52	115.44
[6]TPSA (Å^2)	74.35	63.17	70.67	67.23	76.58
[7]LogS	-2.76	-3.96	-3.87	-2.53	-2.98
[8]SC	Soluble	Soluble	Soluble	Soluble	Soluble
[9]GI	High	High	High	High	High
[10]BBB	No	Yes	Yes	Yes	No
[11]vROF	0	0	0	0	0
[12]vGhose	0	0	0	0	0
[13]vVeber	0	0	0	0	0
[14]BS	0.55	0.55	0.55	0.55	0.55
[15]SA	3.24	3.53	4.73	3.8	3.75
iLOGp	2.91	3.03	3.45	2.58	3.28

[1]Molecular weight; [2]No.of heavy atoms; [3]No.of aromatic heavy atoms; [4]No. of rotatable bonds; [5]Molar refractivity; [6]Topological polar surface area; [7]Solubility; [8]Solubility class; [9]Gastrointestinal absorption; [10]Blood Brain Barrier Penetration; [11]Violation of Lipinski's rule of five; [12]Violation of Ghose rule; [13]Violation of Veber rule; [14]Bioavailability Score; [15]Synthetic accessibility

The bioavailability radar plot was obtained from the SwissADME and shown in Figure 5. The pink area is represented as the different features such as unsaturation (INSATU), insolubility (INSOLU), hydrophobicity (LIPO), rotatable bonds (FLEXI), molecular weight (SIZE) and polar surface area (POLAR). The optimum range of LIPOPHILICITY i.e. XLOGP3 is between -0.7 to +5.0, SIZE i.e. MW should be less than 500 g/mol, POLARITY :TPSA should lie between 20 to 130 Å^2, INSOLU should lie between 0 to 6, INSATU should be in between 0.25 and 1, FLEX should not have more than 9 rotatable bonds. The radar plot of B1, B2, B3, B4 and B5 molecules represents that they have adequate drug-likeness properties.

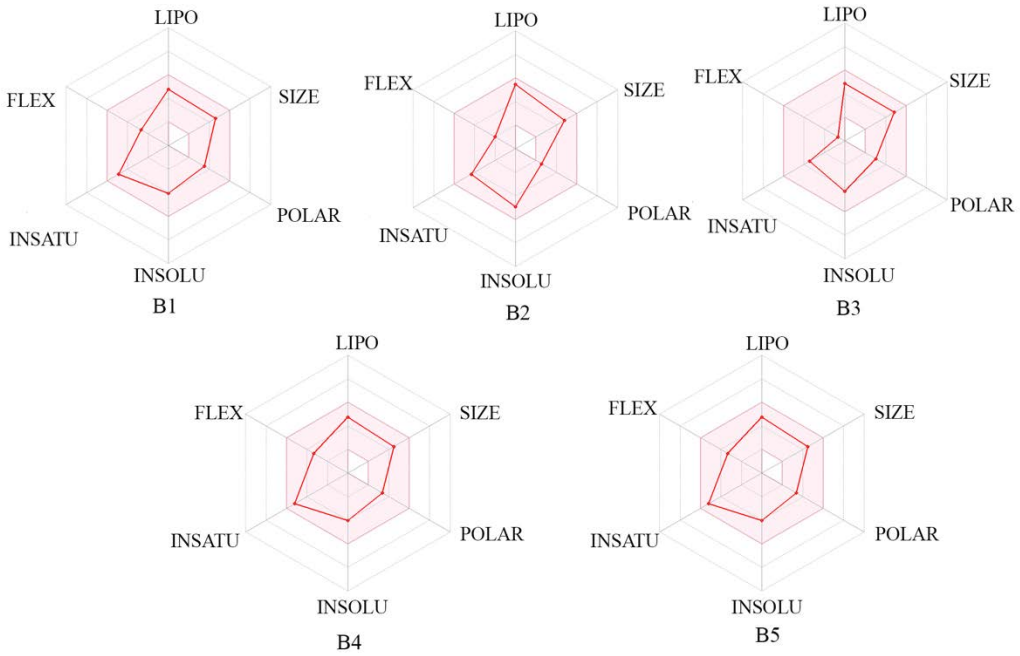


Figure 5. The bioavailability radar plot of proposed BACE1 inhibitors

The boiled-egg figure was obtained which depicts the two important aspects such as human intestinal absorption (HIA) and BBB as shown in Figure 6. The yellow part (yolk) represents the BBB permeation and the white part (albumin) represents the HIA absorption. The albumin and yolk regions are independent of each other. The boiled-egg model also represents the prediction of substrates (PGP+) and non-substrates (PGP-) of the permeability glycoprotein (PGP) as shown in Figure 6. Blue dots represent the PGP+ molecules that are predicted to be effluated from the CNS by the P-glycoprotein whereas red dots represent the PGP- molecules that are predicted not to be effluated from the CNS by P-glycoprotein. All BACE1 inhibitors were PGP+, therefore they belong to the substrate. From the above analysis of the physicochemical properties and pharmacokinetics, it is consequentially justified that all the BACE1 inhibitors are potential enough to show drug-like nature.

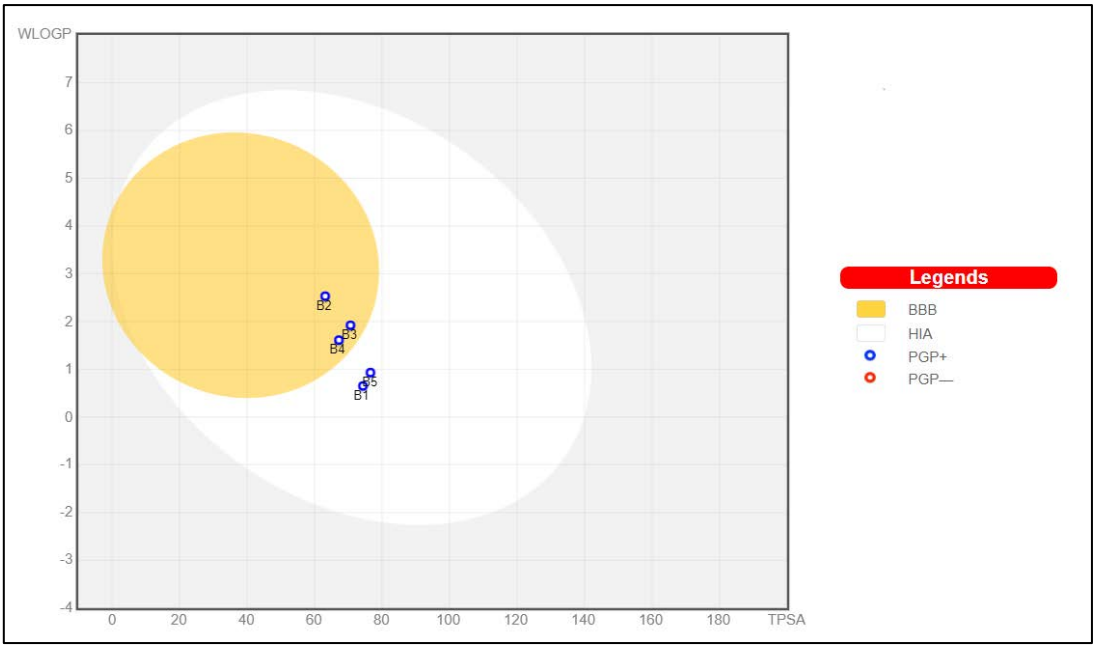


Figure 6. Egg-boiled model of final proposed BACE1 inhibitors

4.5.Quality Assessment

To assess the quality of the molecules various parameters were calculated which include ligand efficiency (LE), ligand efficiency scale (LE_scale), fit quality (FQ), and LE-dependent lipophilicity (LELP), shown in Table 2. The important drug-likeness parameter, LE was proposed by Hopkins *et al*.[52] and can be calculated using the equation (1). The LE is the negative ratio between binding energy and the number of heavy atoms. The value of LE <= 0.4 specifies the lead-like nature. In this case, B1, B2, B3, B4 and B5 were having a value of 0.350, 0.344, 0.317, 0.365 and 0.331 respectively which states all the compounds as a lead-like molecule.

$$LE = \frac{-BE}{NHA} \qquad (1)$$

The second parameter is proposed by Reynolds *et al*. [53] known as LE_Scale and can be evaluated using equation (2). The LE_Scale depicts the size-dependent comparison of the small molecule. As shown in Table 2, the LE_Scale value of B1, B2, B3, B4 and B5 was found to be 0.358, 0.369, 0.347, 0.380 and 0.347 respectively which indicates the potentiality of the molecule.

$$LE\_Scale = 0.873 \times e^{-0.026 \times NHA} - 0.064 \qquad (2)$$

Table 2: Bioactivity and efficiency parameters of BACE1 inhibitors

Molecule	[1]BE	[2]LE	[3]LE_Scale	[4]FQ	[5]LELP
B1	-9.8	0.350	0.358	0.979	8.314
B2	-9.3	0.344	0.369	0.934	8.797
B3	-9.2	0.317	0.347	0.915	10.875
B4	-9.5	0.365	0.380	0.961	7.061
B5	-9.6	0.331	0.347	0.955	9.908

[1]Binding energy; [2]Ligand efficiency; [3]Ligand efficiency scale; [4]Fit quality; [5]Ligand-efficiency-dependent lipophilicity

The fit quality score can be evaluated by using equation (3) and expressed for a good binding molecule in the receptor. The FQ value should be about 1. The FQ value of B1, B2, B3, B4 and B5 are 0.979, 0.934, 0.915, 0.961, and 0.955 respectively as given in the Table 2. Therefore, all molecules have a strong binding capability to the BACE1.

$$FQ = \frac{LE}{LE\_Scale} \tag{3}$$

The LELP value can be calculated by using equation (4). LELP is the ratio between logP and LE and proposed by Keseru and Makara[54]. The LELP value should be more than 3. The LELP value of B1, B2, B3, B4 and B5 was found to be 8.314, 8.797, 10.875, 7.061 and 9.908 respectively. Hence, the LELP values from the above table show that all the molecules have drug-like properties.

$$LELP = \frac{\log p}{LE} \tag{4}$$

4.6. Molecular dynamics simulation

The stability and dynamic behavior of the protein-ligand complex was explored using all-atom MD simulation study. Application of classical MD simulation approach on the structure-based discovery of molecules become an excellent approach to explore conformational analysis and binding pattern of molecule inside the receptor cavity [55]. In order to check the retaining conformational comforts of the proposed BACE1 inhibitors and AZD3293 in dynamic state of both small molecule and protein, the 100 ns time span of MD simulation was performed. A number of parameters were calculated including RMSD, RMSF and Rg from MD simulation trajectories to evaluate the dynamic charateristics of each complex.

From the MD simulation trajectory, the RMSD values of the protein backbone of each complex was calculated and given in Figure 7. The average, maximum and minimum RMSD values are given in Table 3. The RMSD plot portrayed in Figure 7 clearly revealed that RMSD values of protein backbone atoms bound with compounds B1, B2, B3, B4, B5 and AZD3293 within the range of 0 to 0.3 nm, which is no-doubt an acceptable range to judge the protein's conformational stability with respect to the bound ligand. Deep observation on RMSD values revealed that protein backbone bound with compound B1 attained a bit higher RMSD in comparison to others. BACE1 backbone bound with B2 was also found to exhibit a similar type of fluctuation profile throughout the MD simulation, howevere the RMSD values remained below 0.27 nm. Taken together, analysis of MD simulated trajectories explained that the backbone of BACE1 was achieved enough stability with bound proposed BACE1 inhibitors.

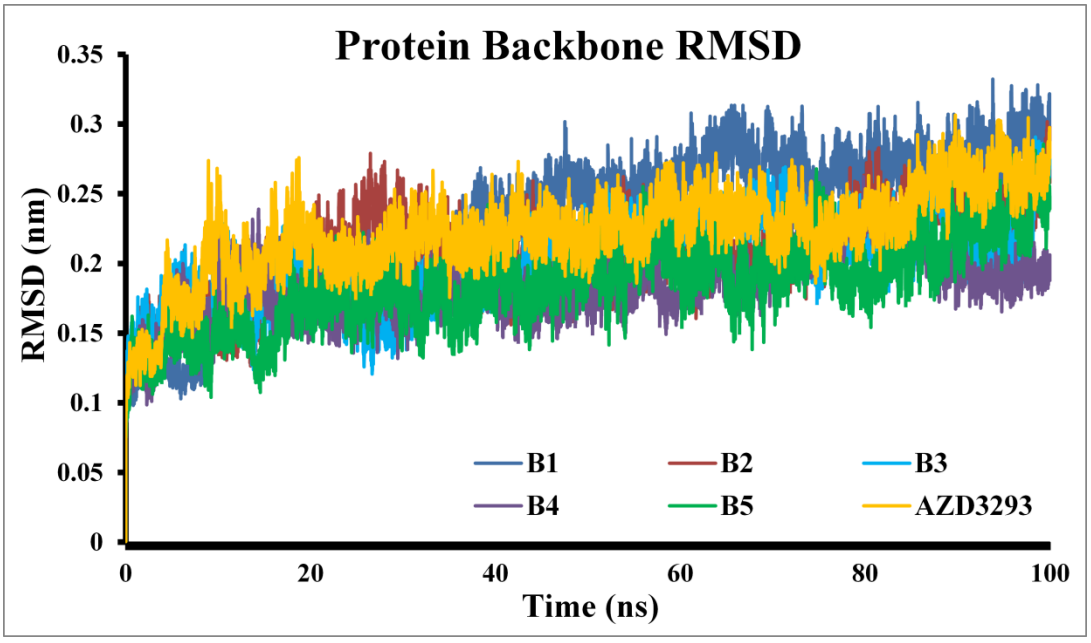


Figure 7. BACE1 protein backbone RMSD values over time obtained from MD simulated complexes

Table 3. Average, maximum and minimum RMSD, RMSF and Rg values of proposed BACE1 inhibitors and AZD3293

	B1	B2	B3	B4	B5	AZD3293
RMSD						
Min	0.001	0.001	0.001	0.001	0.001	0.000
Max	0.332	0.302	0.291	0.262	0.286	0.307
Average	0.231	0.206	0.201	0.184	0.186	0.222
RMSF						
Min	0.052	0.048	0.046	0.047	0.047	0.051
Max	0.557	0.512	0.869	0.623	0.537	0.495
Average	0.143	0.135	0.128	0.133	0.123	0.138
Radius of gyration (Rg)						
Min	2.108	2.108	2.108	2.105	2.105	2.109
Max	2.230	2.213	2.209	2.198	2.220	2.223
Average	2.177	2.167	2.162	2.160	2.172	2.183

The average RMSD value of the protein backbone can explain the deviation from the initial native form of crystal structure during the MD simulation. The average RMSD value of the protein backbone was found to be 0.231, 0.206, 0.201, 0.184, 0.186 and 0.222 nm when bound with B1, B2, B3, B4, B5 and AZD3293, respectively. The above low mean value undoubtedly explained that any weird deviation or instability of the BACE1 was not found during the conformational changes throughout the MD simulation run period.

The fluctuation of individual amino residue plays a critical role in the stability of protein-ligand complexes. The RMSF values for the backbone of BACE1 were calculated separately bound with B1, B2, B3, B4, B5 and AZD3293 and presented in Figure 8. The RMSF plot shows a similar pattern with little exemption in fluctuation of amino residues for BACE1 bound with proposed and standard molecules. Moreover, it was revealed that more or less in each complex the amino acid residues around Arg128, Pro129, Asp130 and Asp131; Gly158, Phe159, Pro160, Leu161, Asn162, Glu163, Ser164, Glu165, Val166, Leu167 and Ala168; Ser253, Thr254, Glu255 and Lys256; and, Val312, Ala313 and Thr314 were found to fluctuate more in comparison to rest of the amino acid residues. It was also noted that amino residues around Gly158, Phe159, Pro160, Leu161, Asn162, Glu163, Ser164, Glu165, Val166, Leu167 and Ala168 fluctuated in large scale when BACE1 bound with B3. Such above fluctuation may be due to open up the protein molecules or lack of binding interactions

with the ligand. But it is also worth to note that not a single amino residues was fluctuated beyond 0.869 nm. The average, maximum and minimum RMSF values were found to be 0.143, 0.557 and 0.052; 0.135, 0.512 and 0.048; 0.128, 0.867 and 0.56; 0.133, 0.623 and 0.047; 0.123, 0.537 and 0.047; and, 0.138, 0.495 and 0.051 for B1, B2, B3, B4, B5 and AZD3293 respectively. Hence, the above observation of RMSF analysis can conclude that BACE1 was undergoing some structural changes to form an active conformation when bound with the identified ligands.

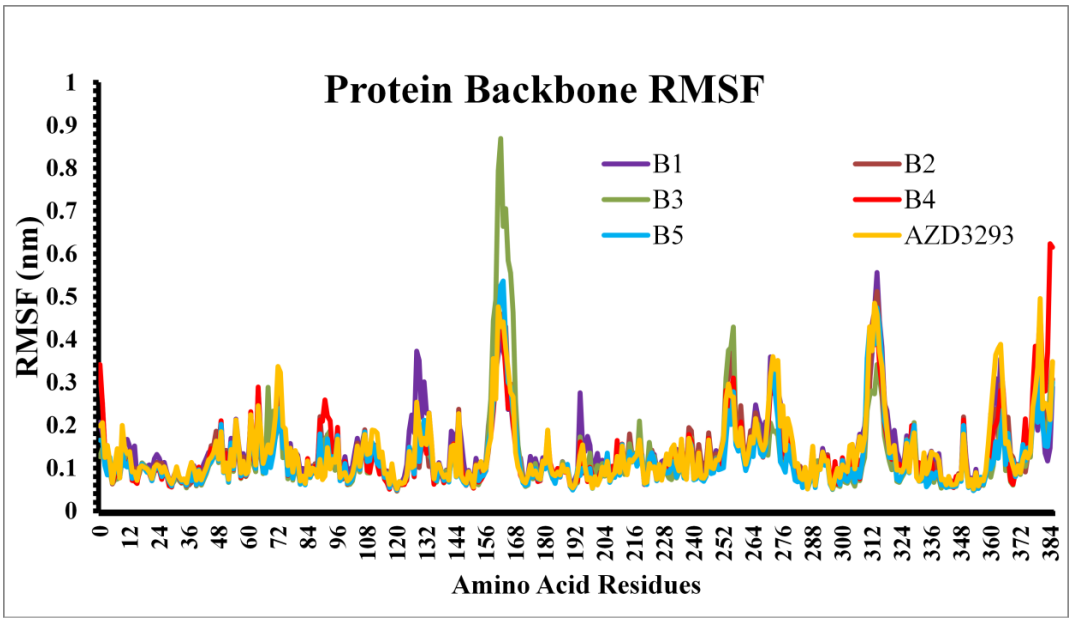


Figure 8. RMSF vs residue number of BACE1 when bound to final screened BACE1 inhibitors and AZD3293

The rigidity of the system can be explained through a detailed analysis of Rg of the protein-ligand system during the MD simulation. The Rg value of each frame was calculated and plotted against the time span of MD simulation, presented in Figure 9. The trajectories obtained from the MD simulation undoubtedly explained that all complexes were remained rigid during the entire simulation. The average, maximum and minimum Rg values were calculated and given in Table 3. The difference between maximum and average, and, average and minimum can give an idea about the deviation of the system throughout the simulation. The difference between maximum and average, and, average and minimum was found as 0.053 and 0.069; 0.046 and 0.059; 0.046 and 0.054; 0.038 and 0.055; 0.048 and 0.067; and, 0.040 and 0.073 nm for the protein-ligand system bound with B1, B2, B3, B4, B5 and AZD3293, respectively. The low value of the above parameters clearly indicated without any doubt that all systems retained the rigidity throughout the simulation. Therefore, the above

analysis of MD simulation trajectories of proposed BACE1 inhibitors with protein suggested that B1, B2, B3, B4 and B5 might be promising molecules and retained binding with the catalytic amino residues in static and dynamic states.

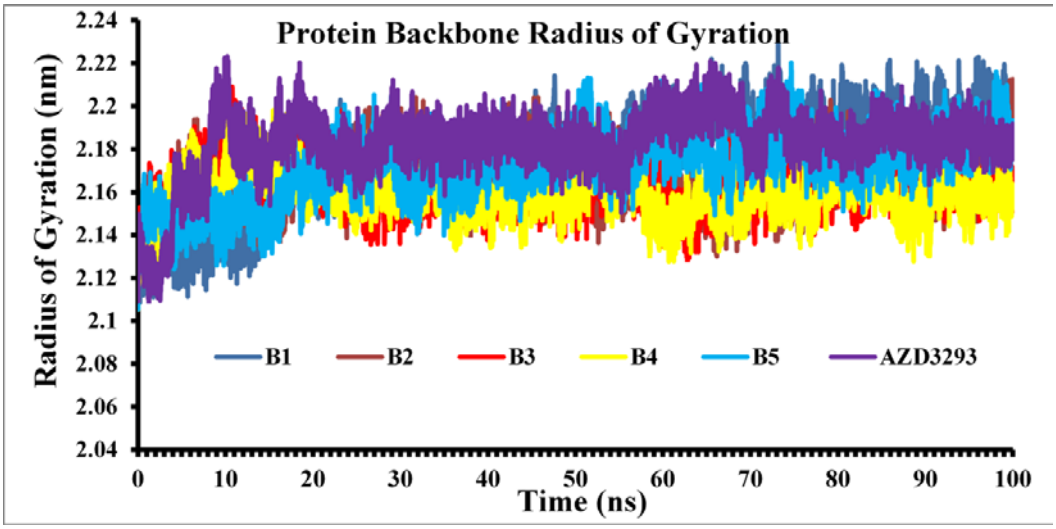


Figure 9. Radius of gyration vs time during MD simulation for all proposed and standard compound

4.7. Binding free energy using MM-PBSA approach

MD simulated entire trajectory frames were used to calculate the binding free energy for all proposed BACE1 inhibitors including AZD3293 using the MM-PBSA approach. In this method, the binding free energy was calculated from each of the frames and can be considered more accurate in comparison to binding energy calculated from any other static frame such as molecular docking. For details analysis, the maximum, minimum and average binding free energy was calculated and given in Table 4. Binding free energy against frame numbers plotted and displayed in Figure 10.

Table 4. Binding free energies of proposed BACE1 inhibitors and AZD3293 calculated using MM-PBSA approach

	B1	**B2**	**B3**	**B4**	**B5**	**AZD3293**
Min	-1225.380	-1033.990	-1195.000	-1084.640	-1174.100	-357.776
Max	-727.861	-155.436	-604.601	-650.001	-650.215	-83.617
Average	-994.978	-561.562	-961.925	-851.570	-844.707	-247.313
Std. dev.	68.116	184.440	111.581	49.876	68.561	34.399

It was observed that standard BACE1 inhibitor, AZD3293 was showed the lowest binding free energy (-247.313 KJ/mol) in comparison to other proposed molecules. Highest binding free energy was found to be -994.978 kJ/mol for compound B1 followed by other compounds B3, B4, B5 and B2 of -961.925, -851.570, -844.707 and -561.562 kJ/mol, respectively. The above data explained without any doubt that all proposed molecules were shown a strong affinity towards BACE1 in comparison to standard compound AZD3293.

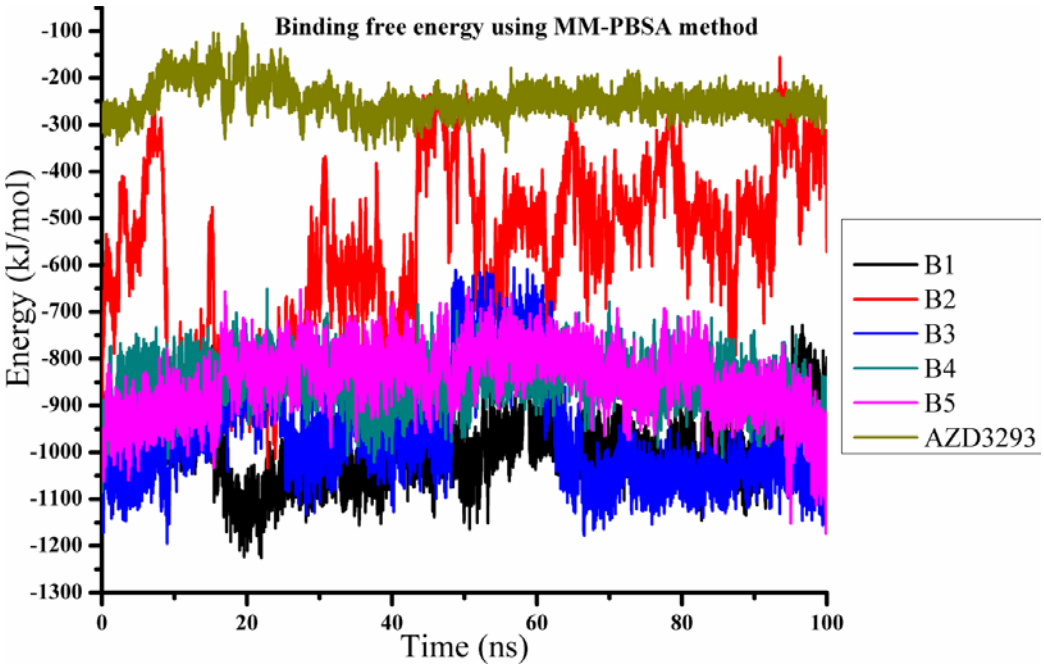


Figure 10. Binding free energy of proposed BACE1 inhibitors and AZD3293

Figure 10 explained that binding energy of all frames (~100000) consisted throughout the simulation except B2. The binding energy of different frames in the case of B2 fluctuated much but not a single frame was found to have a value greater than -155.436 KJ/mol. Moreover, it was observed that the Coulomb or electrostatic interaction ($\Delta G_{Coulomb}$) and van der Waals interaction energy (ΔG_{vdW}) majorly contributed to achieve higher ΔG_{bind} values. Hence, from the above data and discussion, it can be clearly seen that all proposed BACE1 molecules bind in the receptor and possess strong competency to inhibit the BACE1.

4.8. Future prospects

Despite gigantuous application of pharamcoinformatics in drug discovery research, there is a need to check the potentiality of the proposed BACE1 inhibitors through a number of experimental validation approaches. The affinity of the molecules can also be checked

through the thermal shift assay approach. The binding and unbinding mechanism through kinetic study can be assessed which is a crucial approach to explore the stability of the molecules in dynamic states. Further optimization may be required based on experimental assessments to improve the therapeutic efficacy of the molecules.

5. Conclusion

Structure-based virtual screening of large molecular database becomes the pivotal and crucial paradigm to identify potential molecules for the specific target. Encouraged to find out promising chemical therapeutic agents for managing AD, the pharmacoinformatics based screening of small molecular databases was explored. Multiple molecular docking strategies were applied on more than 100 thousands molecules obtained from the CNS subset of Asinex database. Based on binding energy, binding interactions pattern and ADME profile analysis finally five promising BACE1 inhibitors were proposed. A number of binding interactions were observed between proposed molecules and catalytic amino residues of BACE1 which clearly suggested that proposed molecules possess a number of important chemical functionalities that actively participated in bond formations. The important catalytic dyad residues were also found to interact with proposed molecules which indicated that proposed molecules efficient enough for successful inhibition of BACE1. A number of drug-likeness characteristics calculated from proposed BACE1 inhibitors favor as drug-like candidates. The ADME profile analysis also clearly explained that each molecule consists of good absorption, distribution, metabolism and excretion characteristics. Several parameters from 100ns MD simulation study revealed that BACE1 retained stability after the binding of proposed molecules. The binding energy calculated using MM-GBSA from the MD simulation trajectories strongly suggested that all molecules possess a strong affinity towards BACE1. Therefore, the pharmacoinformatics approach directed that proposed BACE1 molecules consist of all characteristics for successful inhibition of BACE1 and might be crucial for the control and management of the AD.

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Conflict of interest

Authors declare that there is no competing interest.

CRediT authorship contribution statement

Shruti Gupta: Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **Devendra Parihar:** Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **Mokshada Shah:** Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **Shivali Yadav:** Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **Husain Managori:** Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **Shovonlal Bhowmick:** Conceptualization, Supervision, Writing - original draft. **Preeti Chunarkar Patil:** Writing - review & editing. **Siham A. Alissa:** Conceptualization, Writing - review & editing. **Saikh Mohammad Wabaidur:** Conceptualization, Writing - review & editing. **Md Ataul Islam:** Conceptualization, Investigation, Supervision, Data curation, Writing - original draft.

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