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# Mechanistic insights into mode of action of potent natural antagonists of BACE-1 for checking Alzheimer's plaque pathology

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ABSTRACT

Alzheimer's is a neurodegenerative disorder resulting in memory loss and decline in cognitive abilities. Accumulation of extracellular beta amyloidal plaques is one of the major pathology associated with this disease. β-Secretase or BACE-1 performs the initial and rate limiting step of amyloidic pathway in which 37–43 amino acid long peptides are generated which aggregate to form plaques. Inhibition of this enzyme offers a viable prospect to check the growth of these plaques. Numerous efforts have been made in recent years for the generation of BACE-1 inhibitors but many of them failed during the preclinical or clinical trials due to drug related or drug induced toxicity. In the present work, we have used computational methods to screen a large dataset of natural compounds to search for small molecules having BACE-1 inhibitory activity with low toxicity to normal cells. Molecular dynamics simulations were performed to analyze molecular interactions between the screened compounds and the active residues of the enzyme. Herein, we report two natural compounds of inhibitory nature active against β-secretase enzyme of amyloidic pathway and are potent lead molecules against Alzheimer's disease.



## 1. Introduction

Dementia is a term used to describe a variety of diseases in which the normal function of the brain gets impaired due to irreversible loss of neurons ensuing memory loss and decline in cognitive abilities. Over 36 million people worldwide are living with dementia. This number is estimated to double every 20 year with around 115 million people with dementia by 2050 [1]. Alzheimer's disease is the most common type of dementia accounting for nearly 60–80% of the total cases. It is a progressive neurodegenerative disorder in which an individual is not even able to carry out basic bodily functions as walking and swallowing. It is the sixth leading cause of death in United States. One in every eight Americans is suffering from Alzheimer's disease with total number estimated to be 5.4 million [2].

The main cause of Alzheimer's is not known yet but some abnormalities in the brain are the hallmark of this disease. These include depletion of neurotransmitter – acetylcholine at synapses by the over expression of acetylcholinesterase enzyme [3], accumulation of insoluble deposits of beta amyloidal plaques outside the neurons [4] and intracellular neurofibrillary tangles due to hyperphosphorylated tau protein [5]. So far most of the FDA approved drugs for the symptomatic treatment of Alzheimer's belong to the category of acetylcholinesterase inhibitors prominent of which are tacrine, donepezil, galantamine and rivastigmine among others.

The extracellular Aβ plaques found in the brain of Alzheimer's patients are one of the major causes for the progression of Alzheimer's disease. β-Amyloid precursor protein (APP) is a type I transmembrane protein which under normal physiological conditions undergo proteolytic processing with the help of two enzymes – α-secretase and γ-secretase and result in soluble p3 peptides [6]. But in amyloidogenic pathway, 37–43 amino acid long Aβ-peptide is generated by the action of proteolytic enzymes – β secretase and γ-secretase in a sequential manner [7]. These Aβ peptides form insoluble aggregates in the extracellular fluids as plasma and cerebrospinal fluid [8]. A study conducted at MassGeneral Institute for Neurodegenerative Disease predicted a connection between Aβ plaques, calcium accumulation and neurodegenerative mechanism. They showed higher calcium level in the dendrites of mice having Aβ plaques and the structural changes were also comparable to the ones observed in the brains of the patients who died because of Alzheimer's disease. Also the concentration of calcium was almost double in the neurons adjacent to the plaques. It has been reported that excess calcium interferes with neuronal

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communication. Thus blocking the process by which Aβ peptides are generated will help in improving cognition and control the degeneration of dendrites [9].

β-Secretase or β-site APP cleaving enzyme-1 (BACE-1), a membrane bound aspartyl protease, performs the initial and rate limiting step of APP processing during Aβ generation in amyloidic pathway and thus is a potential therapeutic target [10]. It is expressed as a pro-enzyme in endoplasmic reticulum. After post/co-translational modifications it reaches the plasma membrane where it comes in contact with APP for Aβ generation [11]. It is ubiquitously expressed with highest levels in brain and pancreas. BACE-1 has a bilobar structure where the N-terminus lobe (residue 1–150) and the C-terminus lobe (residue 151–385) form a substrate binding cleft which is partially covered by a hairpin loop also known as the flap (residue 67–77) [12]. A catalytic Asp dyad (Asp 32 and Asp 228) has been found crucial for the activity of the enzyme [13]. Not much study has been carried so far to elucidate other physiological functions of BACE-1, but studies on BACE-1 knockout mice did not show any major behavioral, morphological or developmental deficits [14–16]. Thus it can be targeted for inhibition, making it a prime drug target for Alzheimer's treatment.

Much progress has been made in the recent years for the generation of BACE-1 inhibitors [17–19]. A novel BACE-1 inhibitor, LY2811376 was discovered but due to its preclinical toxicity its clinical development had to be discontinued. Likewise, many of the proposed synthetic inhibitors are usually found to be associated with drug related or drug induced toxicity during preclinical and clinical trials and hence natural products which could act as inhibitors are regaining popularity and are of keen interest to the researchers these days [20,21]. The study presented here is an attempt to find natural products that could act as BACE-1 inhibitors and could be used for the treatment of Alzheimer's disease.

## 2. Materials and methods

### 2.1. Protein and dataset preparation

The crystal structure of human BACE-1 was retrieved for Protein Data Bank [PDB ID: 4DH6] [22]. Crystal water molecules and all non-bonded heteroatoms were removed from the protein structure using Accelerys Viewerlite 5.0 [23]. Further protein preparation was done using Schrödinger's protein preparation wizard [24] involving addition and optimization of hydrogen bonds, removal of bad contacts, optimization of bond lengths, creation of disulfide bonds, capping of protein terminals, conversion of selenomethionine to methionine and fixing of missing residues.

A special subset consisting of 1,69,109 natural compounds was downloaded from ZINC database [25]. This dataset was then prepared using LigPrep's ligand preparation protocol which generated different tautomeric, stereochemical and ionization variants of these molecules, followed by energy minimization and flexible filtering. This prepared protein and small molecule dataset was then used further for virtual screening and docking studies.

### 2.2. Prediction of ligand binding site in the protein

The function of any protein depends upon the interactions it makes with other proteins or ligands involving the residues residing in the active cleft of that molecule. There are many computational methods available for the prediction of active pockets in the protein molecule. Q-SiteFinder is one such method that uses energy criteria to identify pockets in the protein. It returns top ten energetically favored pockets in the protein molecule. It has been reported that it gives at least one successful prediction in the top three predicted sites for 90% of the proteins. It also gives an estimated volume of the pockets along with the residues surrounding those sites [26]. The structure of BACE-1 retrieved from the PDB was submitted to the Q-SiteFinder server after removal of all heteroatoms bound to it.

### 2.3. High throughput virtual screening and docking studies

A grid was generated at the active site of the prepared protein structure using the Glide docking module of Schrödinger [27,28]. Prepared dataset of natural compounds was then virtually screened against the prepared protein at desired grid coordinates using Glide's HTVS docking protocol [28]. The compounds above the threshold of 6.00 HTVS docking score were then subjected to Glide's XP docking protocol for score refinement. The top scoring compounds above a cutoff of 11.00 XP docking score were then inspected through MD simulations.

### 2.4. Molecular dynamics simulations of ligand-bound complexes

Desmond Molecular Dynamic System [29] with Optimized Potentials for Liquid Simulations (OPLS) all-atom force field 2005 [30] was used to inspect the top scoring compounds through MD simulations. The protein–ligand complex obtained from Glide's XP docking protocol was prepared using Desmond set-up wizard. Missing residues were corrected manually. The prepared system was solvated in a triclinical periodic box of SPC water and neutralized using an appropriate number of counter-ions. The distance between box wall and protein–ligand complex was set to more than 10Å to avoid direct interaction with its own periodic image. Energy minimization of the prepared system was done up to a maximum 10 steps using steepest descent method or until a gradient threshold (25 kcal/mol/Å) was reached. Default protocol of Desmond was used to equilibrate the system. Further MD simulations were carried out on this equilibrated system for a time period of 10 ns at a constant temperature of 300 K and constant pressure of 1 atm with a time step of 2 fs. During the simulations process, smooth particle Mesh-Ewald method was used to calculate long range electrostatic interactions. A 9 Å radius cut-off was used for coulombic short range interaction cutoff method. Frames of trajectory were captured after every 4.8 ps time step.

The root mean square deviation (RMSD) of the protein–ligand complex was calculated for the entire simulations trajectory with reference to the first frame. The hydrophobic interactions and H-bonds were calculated using Ligplot program [31].

## 3. Results and discussion

### 3.1. Validation of the ligand binding site in the BACE-1 protein

To predict the active pocket in BACE-1, its PDB structure was submitted to the Q-SiteFinder server. The top most site had the maximum volume of 234 cubic Å. The ligand binding site usually lies in the largest pocket. The protein residues within a suitable range were also identified. These included Leu 30, Asp 32, Gly 34, Ser 35, Pro 70, Tyr 71, Thr 72, Gln 73, Gly 74, Lys 107, Phe 108, Ile 110, Trp 115, Ile 118, Asp 228, Gly 230 and Tyr 231. This site contained the two aspartic residues thought to be crucial for the catalytic activity of almost all the proteins belonging to the family of aspartyl peptidases [32]. This pocket was also coinciding with the site in the co-crystallized structure, retrieved from PDB, where another ligand was docked, thereby confirming it to be the active site for BACE-1. For molecular docking studies the grid was generated around Asp residues residing in this active pocket.

### 3.2. Virtual screening of 1,69,109 natural compounds representing a diverse subset of ZINC database

The prepared library of natural compounds was firstly screened against the crystal structure of BACE-1 using HTVS protocol. Several hundred compounds with glide docking score more than −6.00, indicating a considerable binding affinity for the protein molecule, were further analyzed for their binding affinity with more precision using the XP docking protocol. Six compounds showed significant binding affinity possessing Glide XP docking score of more than −9.00. The two top scoring natural compounds: 2-(amino-(2,2,2-triaminoethyl)amino)ethane-1,1,1-triamine (ATE, docking score = −11.90) (Fig. 1A) and [(2R,3S,4R,5R,6R)-6-[(2S)-2-(3,4-dihydroxy-phenyl)-2-hydroxy-ethoxy]-4,5-dihydroxy-2-[[(2S,3R,4S,5R)-3,4,5-trihydroxyoxan-2-yl]oxymethyl]oxan-3-yl](E)-3-(3,4-dihydroxy-phenyl)prop-2-enoate (DHP, docking score = −11.00) (Fig. 1B) were studied for their detailed interactions with BACE-1. XP protocol also calculates the ligand efficiency score which estimates the efficiency with which a ligand binds to a protein. ATE and DHP were found having a significantly high efficiency score for BACE-1, 0.99 and 0.25 respectively. Glide Emodel, another score obtained from Glide docking studies, is a combined function derived from Gscore, Coulombic and Van der Waal energies, and the strain energy of the ligand [33]. It has been reported in the earlier studies that the Glide Emodel score gives a good correlation with the experimental binding energies. The higher Emodel score in magnitude obtained in our studies for both the ligands ATE (66.99) and DHP (82.49) ensured a better binding affinity of these compounds with BACE-1 enzyme.

### 3.3. ATE, a potential natural candidate as BACE-1 inhibitor

BACE-1 facilitates hydrolysis of the peptide bond that results in the processing of APP for the generation of beta amyloidal plaques. It follows the general acid–base reaction mechanism involving two Asp residues that form the catalytic dyad of this enzyme [34]. Asp 32 remains protonated for the reactant and product stage, while Asp 288 gets protonated in the intermediate stage which is in accordance with the general acid–base reaction mechanism [35]. As illustrated in Fig. 2A, ATE formed hydrogen bond interactions with these two critical residues – Asp 32 and Asp 228 along with Thr 231. It was also involved in multiple hydrophobic interactions with the surrounding residues in the binding pocket including Leu 30, Gly 34, Try 71, Ile 118 and Gly 230 (Fig. 2B).

### 3.4. Molecular dynamics simulation of ATE complexed with BACE-1

To study the dynamical behavior of interactions, molecular dynamics simulation was performed for BACE-1 in complex with ATE. The low RMSD value along the trajectory (Fig. 3A) signified that the simulated structure did not deviate much from its initial structure and soon acquired a stable conformation. A shift in the position of ATE was visualized by superimposing the pre and post MD complexes (Fig. 3B). An average structure was computed for the most stable time frame. Analysis of hydrogen bond interactions between BACE-1 and ATE after the 10 ns duration of molecular dynamics simulation showed that the interactions with Asp 32, Asp 228, observed subsequent to docking persisted even after MD simulation where as the hydrogen bond with Thr 231 was lost. Two additional H-bonds were formed with the same residues due to a favorable conformational change in the ligand (Fig. 2C). Post simulation, ATE was found forming hydrophobic interactions with residues Thr 33, Gly 230 and Thr 231 (Fig. 2D). ATE substantially and stably interacted with both the catalytic residues of BACE-1. So in the presence of this compound, the first proteolytic cleavage step of APP, which is the rate limiting step of BACE-1, will be hindered and hence ATE can be strongly suggested to be a potential inhibitor of BACE-1 enzymatic activity.

### 3.5. DHP interacts with residues of BACE-1 involved in intermediate stage of catalysis

DHP showed a slight different docking location involving H-bond interactions with residues Ser 10, Gly 11, Try 72, Thr 231, Arg 235, Pro 308, Lys 321 and Ser 325 (Fig. 4A) along with hydrophobic interactions with Gln12, Tyr 71, Ile 110, Gly 230, Asp 228, Gly 230, Thr 232, Asp 233, Val 309 and Gln 326 (Fig. 4B). The peptide hydrolysis reaction proceeds via an intermediate step where a series of reactions occur resulting in the formation of extended hydrogen bond chains bringing a conformational change in the flap region (67–77 residues) [12] and thus initiating interactions with the substrate. The other residues which help in attaining this transitional state include Asp 32, Ser 35, Tyr 71, Gln 73, Try 76, Asp 228, Thr 231, Thr 232, Arg 235, Arg 307 and Lys 321 [35]. In our docked structure, DHP was found interacting with many of these important residues – Tyr 71, Thr 72, Thr 231, Thr 232, Arg 235 and Lys 321.

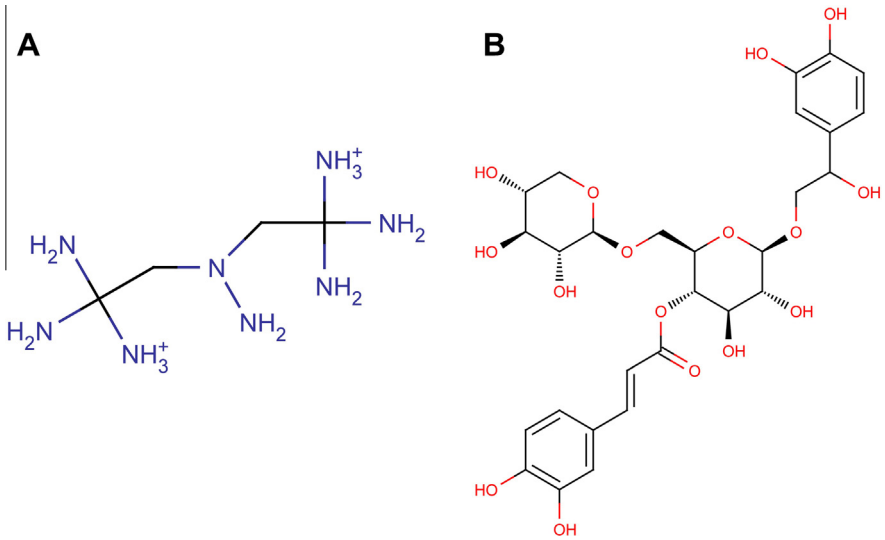


**Fig. 1.** Chemical structure of (A) ATE and (B) DHP.

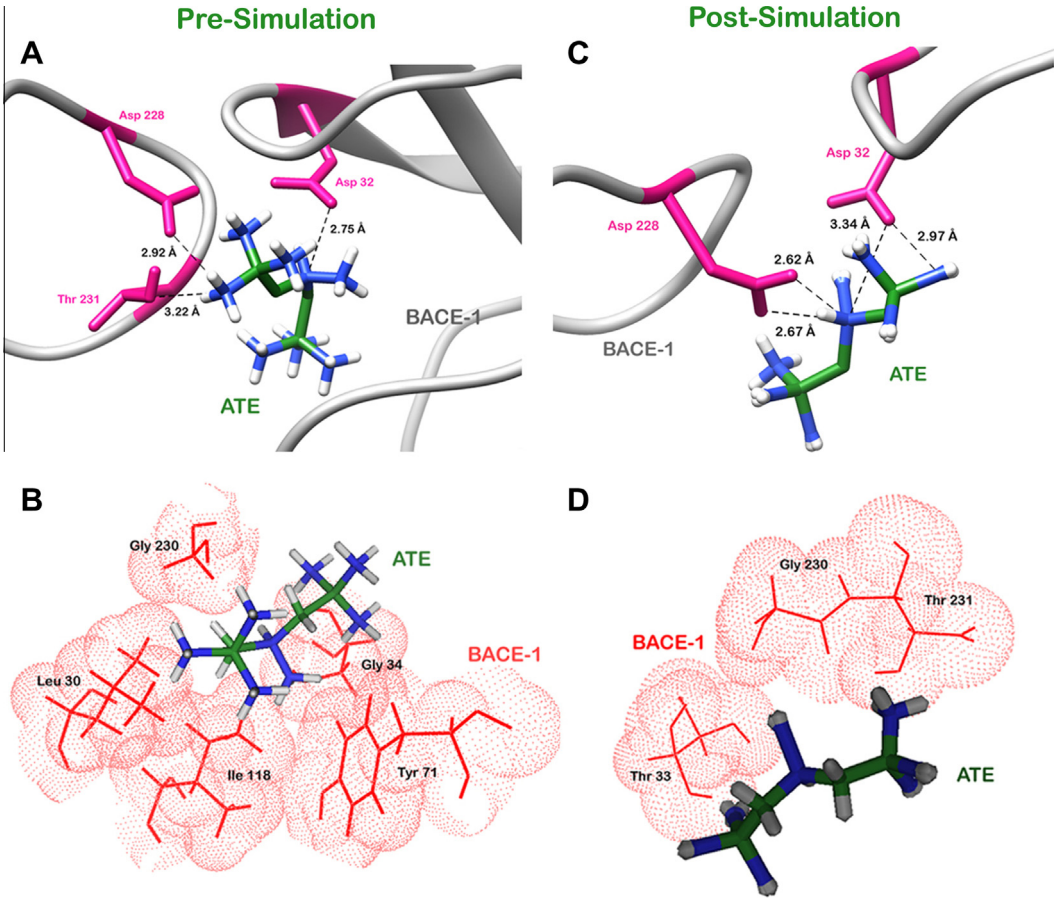


**Fig. 2.** (A) Hydrogen bond and (B) hydrophobic interactions between BACE-1 and ATE after molecular docking. Post simulation (A) hydrogen bond and (D) hydrophobic interaction pattern between ATE and amino acid residues of BACE-1.

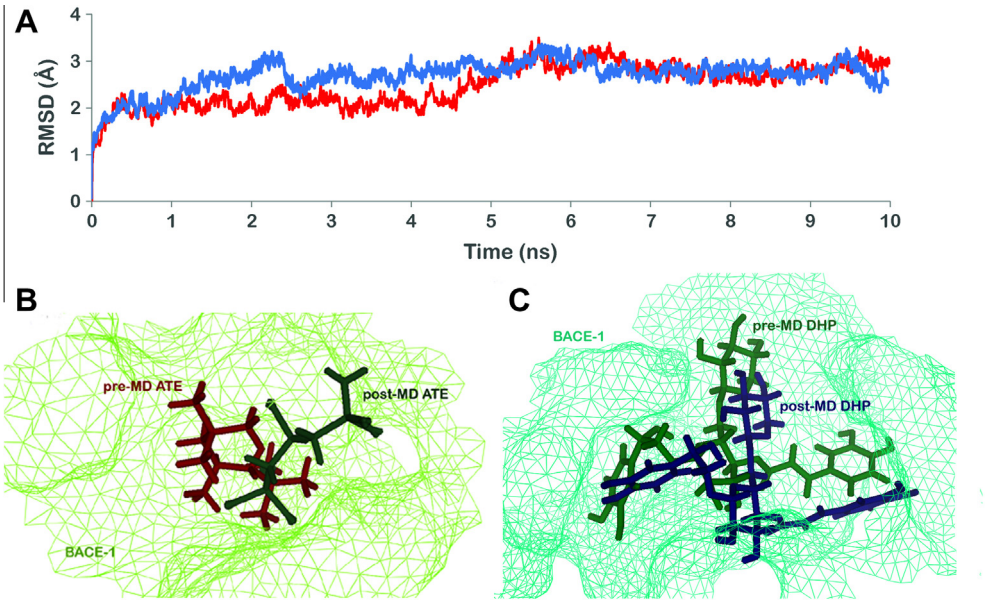


**Fig. 3.** (A) RMSD trajectories of BACE-1 in complex with ATE (red) and DHP (blue) over the 10 ns simulation run. Change in orientation of (B) ATE and (C) DHP after MD simulation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

### *3.6. Molecular dynamics simulation of BACE-1 in complex with DHP*

Again to mimic the bodily conditions, a 10 ns MD simulation was carried out for BACE-1 in complex with DHP. The backbone of complex deviated about 2.4 Å in the first 2.5 ns after which it acquired a stable trajectory. The new acquired conformation was more stable than the initial one and persisted till the end of simulation without any significant change (Fig. 3A). A shift was

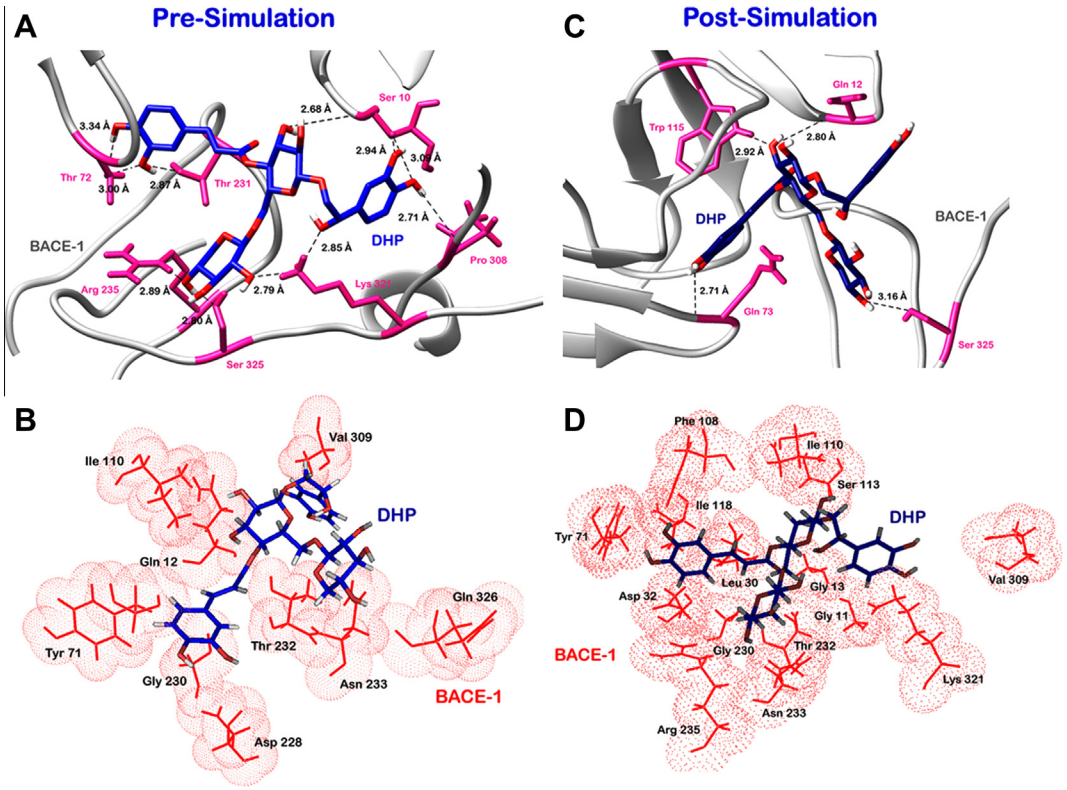


**Fig. 4.** (A) Hydrogen bond and (B) hydrophobic interactions between BACE-1 and DHP after molecular docking. Post simulation (A) hydrogen bond and (D) hydrophobic interaction pattern between DHP and BACE-1 residues.

observed in the position of the docked ligand. The complexes obtained after molecular docking and MD simulation were superimposed to identify the deviation in the conformation of DHP (Fig. 3C). An average structure was computed for the most stable time period (8–10 ns) of the simulation. A significant change in the H-bond interaction pattern was observed during the simulation run. The H-bond formed by DHP with Ser 325 of BACE-1 remained conserved. Three new H-bonds with Gln 12, Gln 73 and Trp 115 also came into existence (Fig. 4C). The residues of BACE-1 which formed hydrophobic interactions with compound 2 were Gly 11, Gly 13, Leu 30, Asp 32, Tyr 71, Phe 108, Ile 110, Ser 113, Ile 118, Gly 230, Thr 232, Asn 233, Arg 235,Val 309 and Lys 321 (Fig. 4D). Some of these residues are part of the flap region and many other are those involved in the formation of intermediate complex. In a previous study, attempts were made to model the changes that occur in the flap region of BACE-1 during aspartic protease catalysis. It was seen that while the structure outside the flap essentially remains the same, the major difference in the inhibitor bound and unbound BACE-1 is a large movement of flap. Towards the N terminal of the flap only 5 residues are involved in the flap movement (68–72) [36]. DHP was showing hydrogen bond interaction with Gly 73, a major residue of the flap region. It was also interacting with other residues involved in flap movement from an open to closed conformation. Interaction with these residues will restrict the flexibility of the flap and down modulate the functionality of the enzyme by deterring the formation of intermediate complex. Hence we propose DHP to be another potential candidate for BACE-1 inhibition.

BACE-1 has been reported previously to be an essential enzyme involved in the processing of β amyloid precursor protein. It performs the initial and rate limiting step of amyloidic pathway. Thus inhibiting this enzyme will help improve cognition and block the process which generates amyloidic plaques. We have screened a large dataset of natural compounds against this target protein. Finally, we reported two natural compounds which display inhibitory activity for BACE-1. The study provides evidence for consideration of these compounds as prospective small ligand molecules in treatment of plaque pathology associated with Alzheimer's disease.

## Competing interests

The authors declare that they have no competing interests.

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