Original Article:

Identification of potent inhibitors for β-secretase through structure based virtual screening and molecular dynamics simulations

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ABSTRACT

Background: Alzheimer's disease (AD) is a neurodegenerative disorder in elderly persons aged above 65 years. The cleavage of amyloid precursor protein (APP) by β-secretase generates peptide fragment Aβ42, the main cause of memory and cognitive defects in AD. Therefore, elevated level of β-secretase results in accumulation of insoluble form of Aβ peptides (senile plaques) representing the protein as an attractive drug target of AD.

Methods: Five recently reported β-secretase antagonist (thiazolidinediones, rosiglitazone, pioglitazone, SC7 and tartaric acid) structural analogs were searched from ligand info database and prepared using LigPrep. The crystal structure of β-secretase was optimized using Maestro v9.2 protein preparation wizard applying optimized potential for liquid simulations (OPLS)-2005 force field. Structure based virtual screening was performed for β-secretase from prepared ligands using virtual screening workflow of Maestro v9.2 and was ranked based on XP Gscore.

Results: Eight lead molecules were identified to have better binding affinity (lower XP Gscore) compared to five existing β-secretase antagonists and appear to have good pharmacological properties. Binding orientations of the lead molecules were in well agreement with existing inhibitors. Lead1 showed lowest XP Gscore (-10.72 Kcal/mol). Molecular dynamics (MD) simulations of β-secretase-lead1 complex was stable in all trajectories.

Conclusion: Lead1 is proposed as potent inhibitor of β-secretase and thus could be considered for rational drug design against AD.

Key words: *Alzheimer's disease, Beta-secretase, Virtual screening, Molecular docking*

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INTRODUCTION

Alzheimer's disease (AD) is a debilitating neurodegenerative disorder in older persons because it relentlessly, progressively and irreversibly destroys the brain. Neurons are the chief cells which are destroyed by disease. It is mostly seen in persons over the age of 65 years and is characterized by progressive cognitive deterioration together with declining activities of daily living, memory and intellectual performance.[1-3] It remains as a global challenge for which no treatment is currently available.

One of the hallmarks of AD is the accumulation of amyloid plaques between nerve cells (neurons) in the brain. The β-amyloid is a fragment of a protein that is snipped from another protein amyloid precursor protein (APP). In a healthy brain, these protein fragments would be broken down and eliminated. In AD these fragments accumulate to form hard, insoluble plaques. The β-secretase is also known as β-site amyloid precursor protein cleaving enzyme (BACE-1) memapsin-2 or aspartyl protease-2. The β-secretase is a type 1 transmembrane aspartic protease with the high activity in neuronal cells in the brain.[4-6] In majority of the cases β-secretase protein is detected in the golgi and in endosomal compartments where APP is located.[7] Recently, soluble Aβ-oligomers of 56 kDa were detected in Tg2576 mice and were shown to contribute to cognitive deficits associated with AD.[8] β-secretase was also observed to be elevated in AD.[9] Therefore, β-secretase

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appears to be an important therapeutic target for treatment of AD.[10,11] Inhibition of β-secretase would stop amyloid plaque formation. Thiazolidinediones, rosiglitazone, pioglitazone and SC7 are the recently reported inhibitors for AD, however, these compounds revealed poor pharmacological properties. In the present study, potent antagonists of β-secretase were identified through structure based virtual screening.

MATERIAL AND METHODS

Protein preparation

The three dimensional structure of human β-secretase in complex with the inhibitor N'-{(1S,2R)-1-(3,5-Difluorobenzyl)-2-[(2R,4S)-4-Ethoxypiperidin-2-YL]-2-Hydroxyethyl}-5-Methyl-N, N-Dipropylisophthalamide [SC7;protein database (PDB) code: 2QP8] was retrieved from the protein data bank.[12] The inhibitor binding site of β-secretase was determined through analysis of 2QP8 using PyMOL, an open viewer molecular software.[13]The protein preparation wizard of Schrodinger 2011 was used to prepare the protein.[14] The protein was preprocessed by deleting inhibitor and the crystallographically observed water molecules (water without H-bonds) beyond 5 Å of the inhibitor. The hydrogen atoms were added and atomic charges were assigned. Optimized potential for liquid simulation-2005 (OPLS-2005) force field was utilized to optimize the geometry and minimize the energy of the protein.

Ligand preparation

Ligand.Info Meta-database tool retrieves structural analogues for the queried small molecule by implementing 2D geometry search techniques from eight renowned small molecule databases such as Havard's ChemBank, ChemPDB, Kyoto encyclopedia of genes and genomes (KEGG) Ligand, Druglikeliness National Cancer Institute (NCI), Anti-HIV NCI, Unannotated NCI, AkoS GmhB, Asinex Ltd etc.[15] Five recently reported β-secretase inhibitors (thiazolidinediones, rosiglitazone, pioglitazone, SC7 and tartaric acid) were searched for structural analogs from Ligand.Info Meta-database tool.[16-22] Maximum of 50 structural analogues of each β-secretase inhibitors were retrieved from each of eight structural databases of Ligand.Info.[16-22] Consequently, an in-house library of structural analogues of β-secretase inhibitors was compiled.

LigPrep[23] is an application tool in Schrödinger software suite that combines tools for generating three-dimensional (3D) structures from one-dimensional (1D) (SMILES) and two-dimensional (2D) (SDF) representation, ionization states using Epik[24] and searching for tautomers and steric isomers to generate broad chemical and structural diversity compounds from a single input structure. The structural analogs of five human β-secretase inhibitors such as thiazolidinediones, rosiglitazone, pioglitazone, SC7 and tartaric acid were prepared using LigPrep. The ligands with poor pharmacological properties and reactive functional groups were discarded by employing Lipinski's filter and reactive filter.[25] A customized library of non-redundant pharmacologically preferred conformations were prepared.

Virtual screening

Structure based virtual screening is one of the proficient method of potent lead discovery. A grid (20 × 20 × 20 Å) was generated centered on the inhibitor binding site of β-secretase.[26] Virtual screening was performed from the in house ligand library by using virtual screening workflow (VSW) module of the Schrödinger software suite. VSW uses glide docking protocol to rank the best compounds which utilizes the multi-step workflow, Glide high-throughput virtual screening (HTVS), standard precision (SP) and extra precision (XP) to retain lead molecules with better binding affinity in a good binding orientation without steric classes.[26,27] The molecules which are docked most favorably were ranked based on XP Gscore.

Molecular dynamics simulations

The simulations of β-secretase-lead1 docking complex was carried out by using Desmond v3.0.[28-30] The system was embedded with simple point

charge (SPC) water model and neutralized by replacing solvent molecules with Na^+ ions. Forcefield parameters for the protein-ligand systems were assigned using the OPLS-2005 forcefield. The system was stipulated with periodic boundary conditions, the particle mesh Ewald (PME) [31] method for electrostatics, a 10 Å cutoff for Lennard-Jones interactions and SHAKE algorithm[32] for restricting motion of all covalent bonds involving hydrogen atoms. The final system was simulated through a multistep protocol devised in Maestro v9.2. In brief, this included energy minimization using hybrid method of steepest descent and the limited-memory Broyden-Fletcher-Goldfarb-Shanno (LBFGS) algorithm[28-30,33] with a maximum of 2000 steps including the following: initial 10 steps of steepest descent with solute restrained; similar energy minimization for 2000 steps without solute restraints, 12 ps simulation in number of atoms, volume and temperature constant (NVT) ensemble (temperature 10K) restraining nonhydrogen solute atoms; 12 ps simulation in the number of atoms, pressure and temperature constant (NPT) ensemble (temperature 10K) restraining nonhydrogen solute atoms; 24-ps simulation in the NPT ensemble restrained with solute nonhydrogen atoms (temperature 300K); and 24-ps simulation in the NPT ensemble (temperature 300K) with no restraints. The temperatures and pressures in the short initial simulations were controlled using Berendsen thermostats and barostats, respectively. The equilibrated system was simulated for 10 ns with a time step of 2 fs, NPT ensemble using a Nose´-Hoover thermostat at 300K and Martyna-Tobias-Klein barostat at 1.01325 bar pressure.

RESULTS

A high resolution (1.50 Å) crystallographic structure of human β-secretase in complex with SC7 was reported by Iserloh *et al.*[12] The 3D structure was retrieved from the PDB and analyzed inhibitor binding site residues. The residues such as Gly72, Gln73, Leu91, Asp93, Gly95, Ser96, Pro131, Tyr132, Thr133, Gln134, Gly135, Lys168, Phe169, Ile171, Trp176, Tyr259, Ile287, Asp289, Gly291, Thr292, Thr293 and Arg295 were observed to be present within 4Å region of SC7 (Figure 1). Therefore, these residues were proposed to constitute β-secretase inhibitor binding site. The residues such as Asp93, Gly95, Thr133, Gln134, Asp289, Gly291, Thr292 and Thr293 were forming intermolecular hydrogen bonds with SC7, hence, were considered as important residues for β-secretase inhibition.

The β-secretase structure was optimized adding hydrogen atoms and removing water molecules beyond 5 Å. The structure was energy minimized applying OPLS-2005 force field to remove bad atomic contacts in the 3D structure and to obtain a structure with lower energetic state. The ligand SC7 was removed from β-secretase prior to grid generation, and a grid was placed centered on the inhibitor binding site.

After structural analog search for five existing β-secretase inhibitors from ligandinfo database, 1834 ligands were obtained. These ligands were optimized and converted to 3D form in LigPrep. 9655 protonation and tautomeric states of 1834 ligands were generated during ligand preparation. The higher energetic conformations were discarded during Post LigPrep evaluations to reduce the dataset to 5715 compounds. 4291 compounds were passed Lipinski's filter. In order to find ligands with zero reactive functional group, reactive filter was applied. 3968 compounds were identified to have no reactive functional group; hence, these compounds were selected as ligand dataset for structure based virtual screening.

The grid was generated for β-secretase and ligand dataset was given as input in three stages of Glide docking. The 3968 compounds were docked in subsequent HTVS docking, SP docking and XP docking. Nineteen ligand molecules with good binding affinity were obtained and ranked based on XP Gscore. Analysis of XP Gscore revealed that eight lead molecules were having lower XP Gscore compared to five existing inhibitors (Figure 2A). Therefore, the eight lead molecules were proposed as potential β-secretase inhibitors (Fig-

ure 2B). The pharmacological properties of lead molecules were well within the parameters of 95% of existing drug molecules (Tables 1A and 1B).

Lead1 showed lowest XP Gscore (-10.72 Kcal/mol) and strong intermolecular hydrogen bond network (seven hydrogen bonds) compared to other proposed leads and published β-secretase inhibitors (Figures 2A, 2B, and 2C), hence, represents the highest binding affinity towards β-secretase. The molecular interactions of docking complex of β-secretase - lead1 showed that the residues such as Asp93, Gly95, Pro131, Thr133, Tyr259 and Asp289 were involved in intermolecular hydrogen bonding; Leu91, Asp93, Gly95, Ser96, Val130, Pro131, Tyr132, Thr133, Gln134, Phe169, Ile171, Trp176, Ile179, Ile187, Arg189, Trp258, Tyr259, Ile287, Asp289, Gly291 and Val393 were involved in good van der Waal contacts (Figure 2D).

The interactions obtained through molecular dynamics simulations were more convincing compared to docking complexes. The energy plot of β-secretase-lead1 complex was stable throughout the simulations (Figure 3A). Root mean square deviation (RMSD) of the protein and lead1 was stable and within the limit of 2.5Å (Figure 3B). Root mean square fluctuation (RMSF) for backbone and side chains of all the residues of β-secretase were within the limit of 3.0Å during entire period of MD simulation run (Figure 3C). The

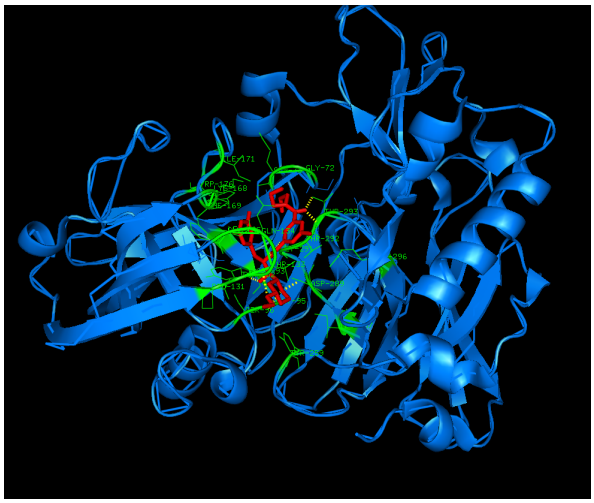

Figure 1: Three dimensional structure of human β-secretase in complex with SC7

hydrogen bonds observed in the docking complex were monitored in all trajectories. It was observed that the binding interactions observed in docking complex (Figure 2C) were reproduced after MD simulations (Figure 3D). The amino acid residues such as Asp93, Gly95, Pro131, Thr133, Tyr259 and Asp289 were involved in seven intermolecular hydrogen bonds in the docking complex. These hydrogen bonds were monitored in all 2084 trajectories recorded during 10ns MD simulations. The result revealed that Asp289 formed at least one hydrogen bond with Lead1 in ~99.5 % trajectories and two hydrogen bonds in ~80% trajectories (Figure 4A). Similarly, Tyr259 was observed to form hydrogen bond with lead1 in ~90% trajectories (Figure 4B). The intermolecular hydrogen bond between lead1 and residues Asp93, Gly95, Thr133 were consistent in more than ~60% trajectories (Supplementary Figure 1A; Supplementary Figure 1B; Supplementary Figure 1C). An additional hydrogen bond between Gly291 and lead1was observed in ~50% of the trajectories though MD simulations (Supplementary Figure 1D). Three to eleven water bridges between β-secretase and lead1 were identified in all 2084 trajectories (Figure 4C).

DISCUSSION

Virtual screening from small molecule database is an extremely efficient method of identifying drug molecules for a particular disease. Virtual screening method predicts binding affinities between drug target and ligands through molecular docking and ranks them in decreasing order. Along with binding affinity it also predicts accurate binding modes and molecular interactions between protein and ligand, hence, became immensely important to carry out initial steps of drug discovery prior to experimental validation.

Accurate binding affinity prediction between protein and ligand through molecular docking requires careful optimization of their 3D structures. Therefore, the protein was optimized in protein preparation wizard applying OPLS-2005 force field. The ligand preparation in LigPrep ensured all ligands

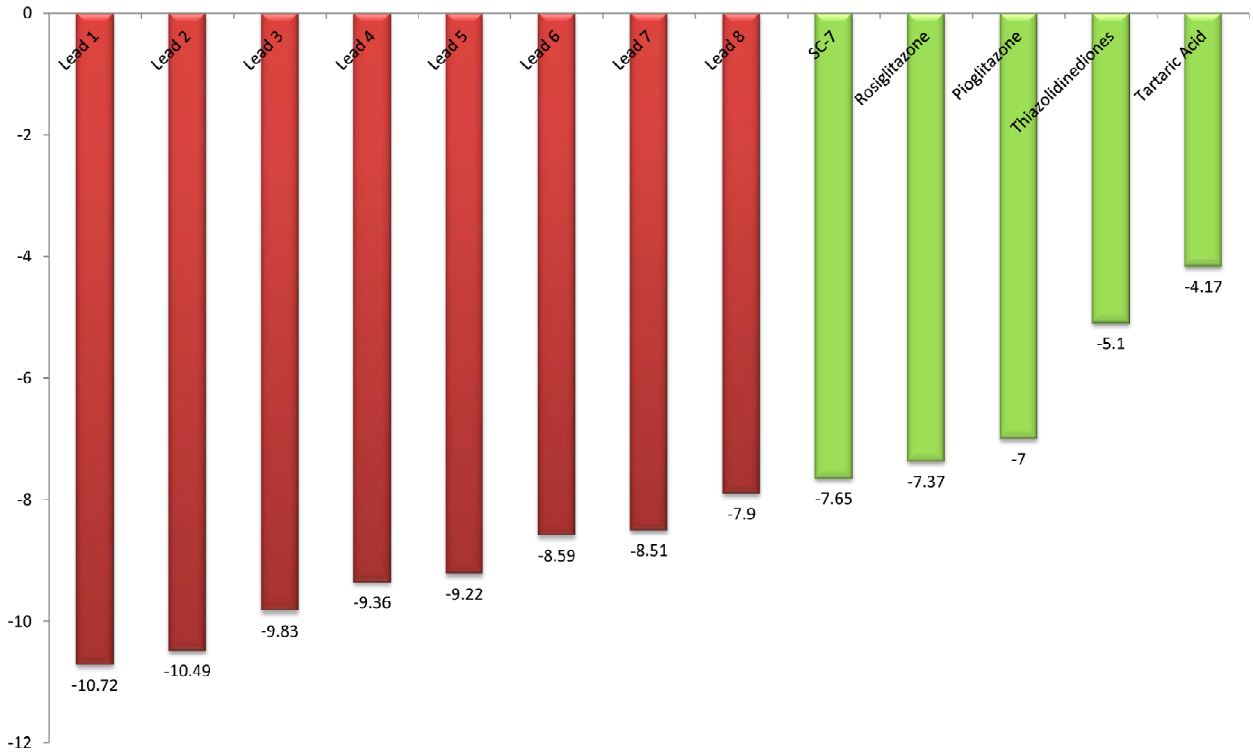

Figure 2A: Comparative analysis plot of XP Gscore of lead molecules and published β-secretase inhibitors
XP = extra precision

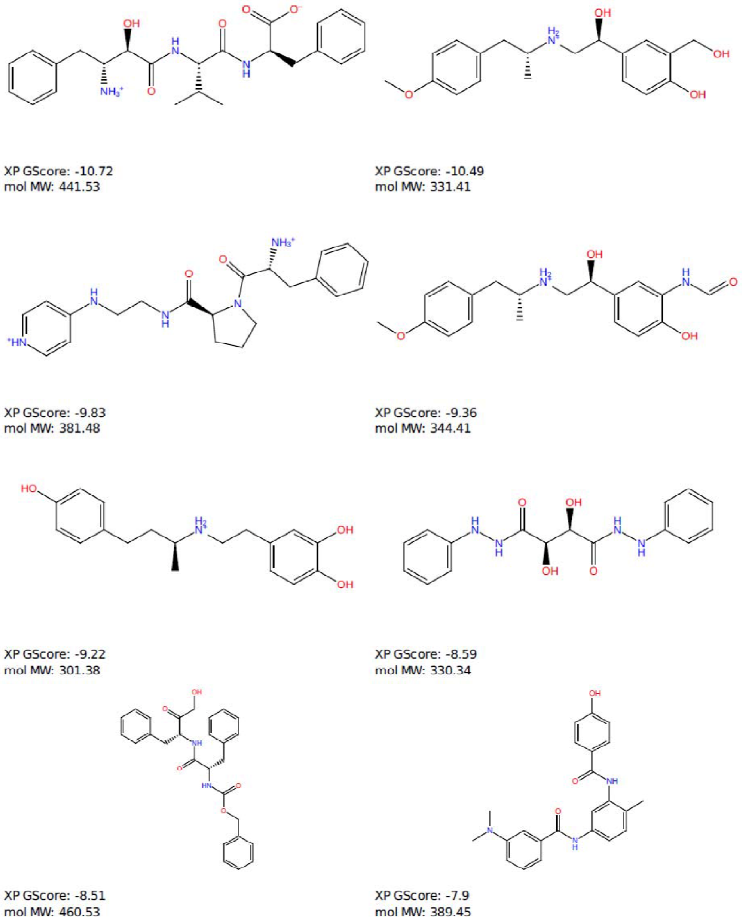

Figure 2B: Structures of eight proposed potential β-secretase inhibitors

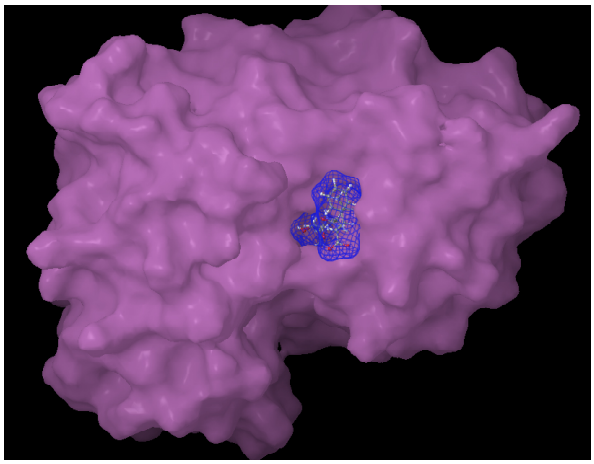

Figure 2C: β-secretase-lead docking complex

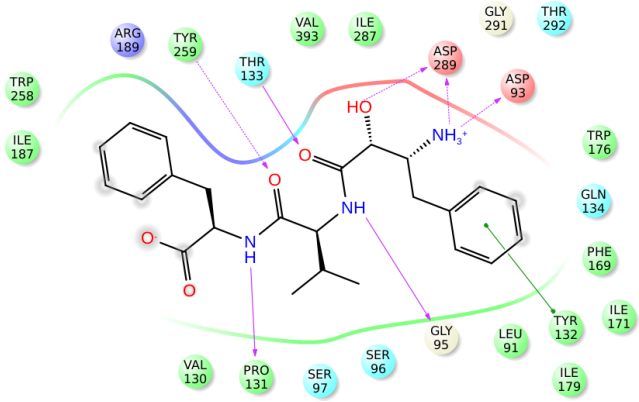

Figure 2D: 2D interaction plot of lead1 with 4 Å region of the β-secretase inhibitor binding site

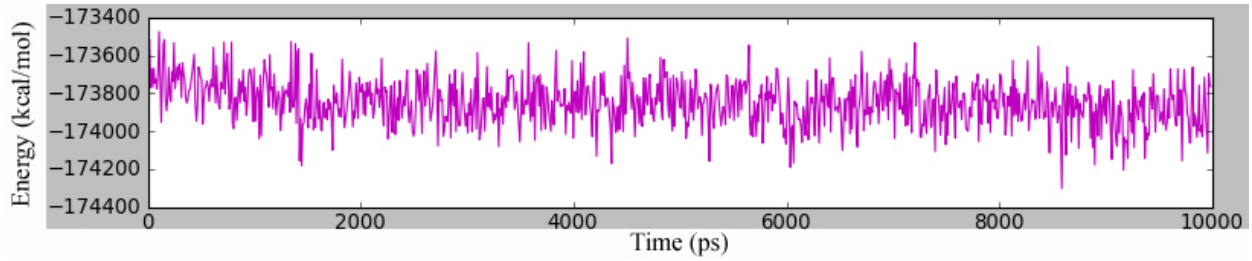

Figure 3A: Molecular dynamics simulations of β-secretase-lead1 docking complex A) Energy plot

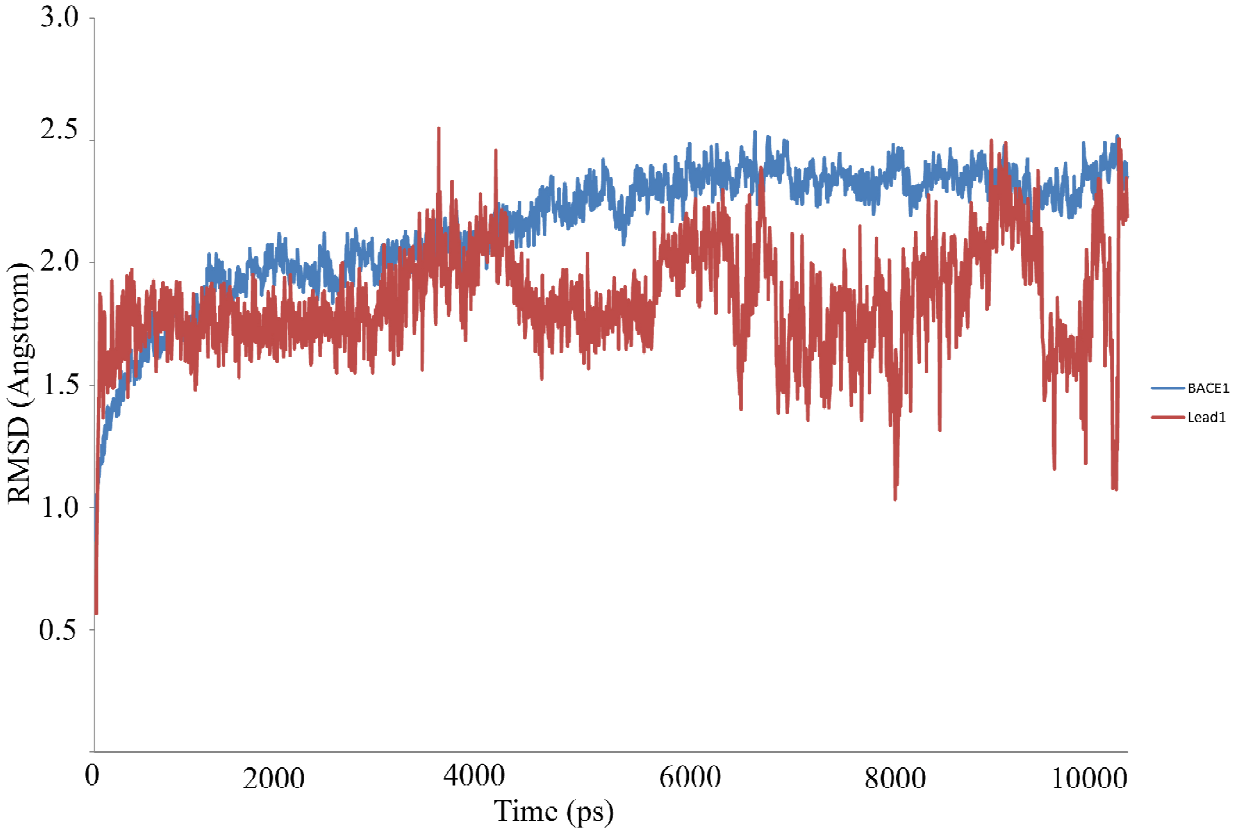

Figure 3B: RMSD plot
RMSD = Root mean square deviation

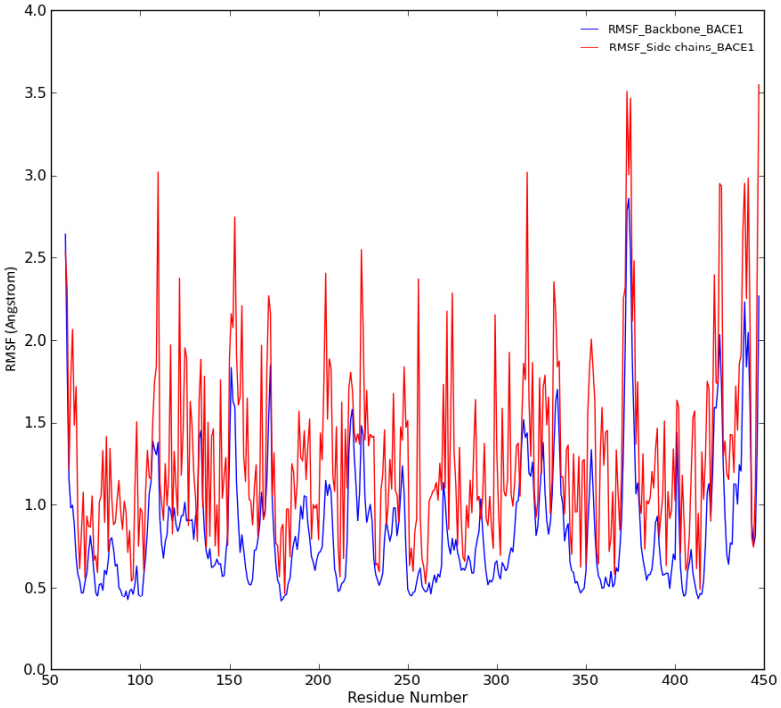

Figure 3C: RMSF plot
RMSF = Root mean square fluctuation

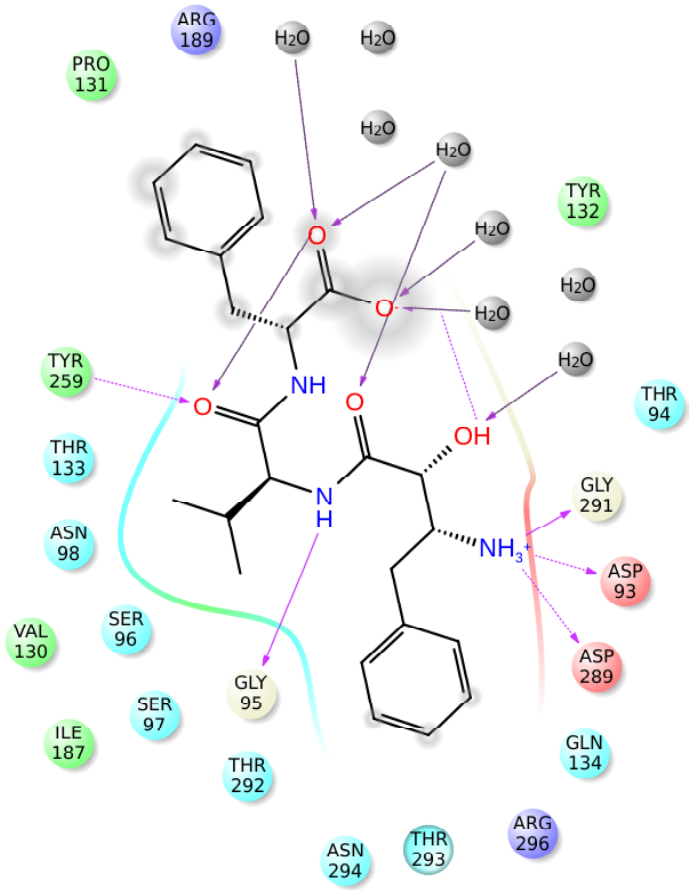

Figure 3D: 2D interaction plot of lead1 with ?-secretase at 2084th trajectory (after 10 ns MD simulations)
MD = molecular dynamics

Table 1A: Pharmacological properties of eight proposed inhibitors of β-secretase

Variable	MW	Rotor	Dipole	SASA Å^2	FOSA Å^2	FISA Å^2	PISA Å^2	WPSA Å^2	PSA Å^2	Volume Å^3	donorHB	accptHB	IP (eV)	EA (eV)	glob
Reference range[33] Lead no.	130.0/725.0	0.0/15.0	1.0/12.5	300.0/1000.0	0.0/750.0	7.0/330.0	0.0/450.0	0.0/175.0	7.0/200.0	500.0/2000.0	0.0/6.0	2.0/20.0	7.9/10.5	0.9/1.7	0.75/0.95
1	441.52	13	4.63	741.61	244.03	142.22	355.34	0	142.36	1388.13	3.5	7.20	9.50	–0.189	0.81
2	331.41	11	3.58	643.04	285.37	137.97	219.69	0	82.59	1131.73	4.0	6.40	8.87	–0.116	0.81
3	344.41	10	4.18	629.92	238.19	189.20	202.52	0	110.13	1128.94	4.0	7.20	9.02	0.491	0.83
4	301.38	10	4.46	629.41	195.37	167.09	266.94	0	79.32	1072.49	4.0	3.75	8.81	–0.153	0.80
5	381.47	9	10.61	706.56	236.25	129.50	340.80	0	108.35	1277.81	4.0	9.00	8.37	–0.366	0.80
6	330.34	9	6.83	641.28	30.89	192.94	417.44	0	139.69	1084.08	4.0	8.40	8.34	0.025	0.79
7	460.52	12	10.72	828.85	138.83	146.87	543.13	0	123.18	1508.64	1.5	7.20	9.39	0.095	0.76
8	389.45	6	7.54	737.58	223.12	137.43	377.03	0	94.68	1289.29	3.0	6.75	8.18	0.269	0.77

MW = molecular weight; rotor = no. of rotatable bonds; dipole = dipole moment; SASA = total solvent accessible surface area; FOSA = hydrophobic solvent accessible surface area; FISA = hydrophilic solvent accessible surface area; PISA = carbon pi solvent accessible surface area; WPSA = weakly polar solvent accessible surface area; PSA = vdW polar surface area; volume= molecular volume; donorHB = donor hydrogen bonds; acceptHB = acceptor hydrogen bonds; IP (eV) = ionization potential; EA (eV) = electron affinity; glob= globularity

Table 1B: Pharmacological properties of eight proposed inhibtors of β-secretase

Variable	logP o/w	logS	CIlogS	logBB	logKp	logKhsa	PMDCK	PCaco	OA	Rule of five	Rule of three
Reference range[33] Lead no.	–2.0/6.5	–6.5/0.5	–5/0.5	–3.0/1.2	Kp in cm/hr	–1.5/1.5	maximum is 4	maximum is 3	<25% is poor	<25 poor, >500 great	<25 poor, >500 great
1	0.896	–2.926	–3.527	–1.261	–3.703	–0.494	14.613	13.545	39.491	1	2
2	2.248	–2.600	–2.937	-1.080	–4.295	–0.152	56.045	121.441	77.411	0	0
3	1.369	–2.216	–2.920	–1.461	–5.396	–0.203	16.727	39.676	63.572	0	0
4	2.424	–2.965	–3.191	–1.360	–4.761	0.081	28.189	64.304	73.502	0	0
5	0.904	–2.091	–1.763	–0.909	–3.904	–0.740	68.457	81.327	66.426	0	1
6	1.393	–3.518	–3.335	–2.010	–2.741	–0.467	62.100	146.616	60.913	1	0
7	4.647	–6.266	–6.112	–1.822	–1.161	0.425	184.199	322.027	100.000	0	1
8	3.713	–6.063	–5.410	–1.317	–2.148	0.434	230.177	492.694	96.878	0	1

ogP o/w = log P for octanol/water; logS = log S for aqueous solubility; CIlogS = log S - conformation independent; logBB = log BB for brain/blood; log Kp = log Kp for skin permeability; log Khsa = log K hsa serum protein binding; Rule of fove=Lipinski Rule of five violations; Rule of three=Jorgensen rule of three violations; OA % human oral absorption in GI (+-20%); PCaco=apparent Caco-2 permeability (nm/sec); PMDCK=apparent Madin-Darby canine kidney permeability (nm/sec)

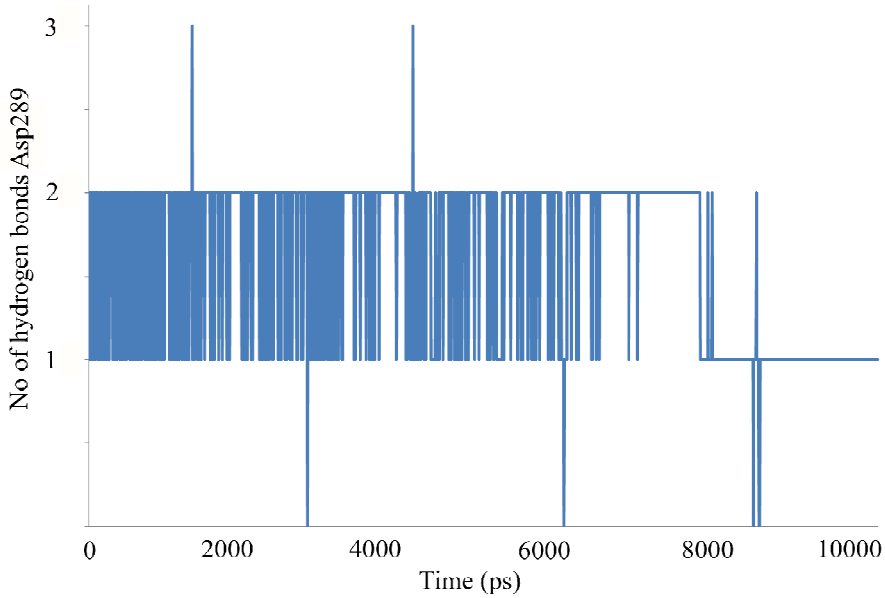

Figure 4A: Hydrogen bond monitoring in all 2084 trajectories A) Asp289 and lead1

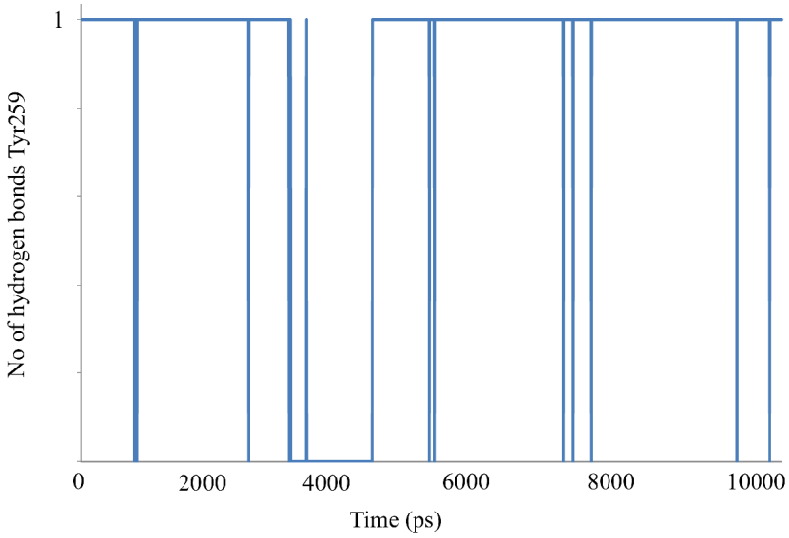

Figure 4B: Tyr259 and lead1

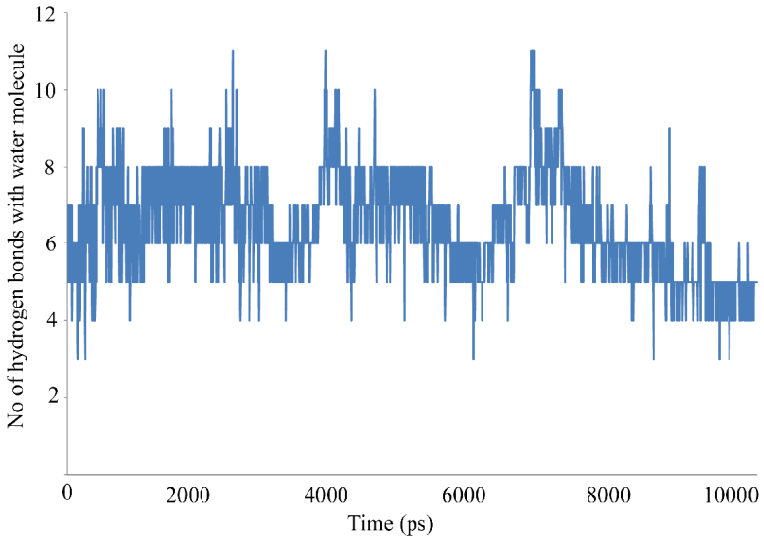

Figure 4C: Water molecules and lead1

at their lower energetic conformation with good pharmacological properties. The three mode of virtual screening was used subsequently for fast screening of small molecules. The XP modes of docking is highly accurate and penalizes highly for minor steric classes, hence, the compounds predicted to have good binding affinity were ranked based on XP Gscore. Lower XP Gscore represents higher binding affinity of the ligand towards protein. Eight lead molecules with lower XP Gscore compared to five published inhibitors were proposed as potential β-secretase antagonist. The results revealed that binding interactions of lead1 was well in agreement with binding interactions of SC7. Therefore, lead1 would be encouraging to start experimental analysis for designing drug molecule against AD.

The energy plot obtained after MD simulation had revealed that the system was energetically stable. The low RMSD and RMSF reflected conformational stability of the system. Hydrogen bond monitoring and correlation with the docking results also revealed that the interactions were stable in physiological environmental condition. The 10ns MD simulations were also deciphered new interactions between β-secretase and lead1 through water bridges as well as hydrogen bond. Overall, the binding interactions between β-secretase and lead1 were stable during MD simulations hence lead1 could be considered as potent inhibitor.

β-amyloid is central to the pathophysiology of AD and plays an early role in this intractable neurodegenerative disorder. β-secretase initiates the formation of β-amyloid and elevated β-secretase levels were observed in AD provide direct and compelling reasons to consider β-secretase as drug target of AD. Computational docking approaches implemented in the present study to develop therapies directed at β-secretase inhibition, thus reducing β-amyloid and its associated toxicities. Eight lead molecules were proposed as potential β-secretase inhibitors based on XP Gscore. The binding orientations of these lead molecules were compared favorably with published inhibitors. The proposed inhibitors also revealed good pharmacological properties. The 'lead 1' showed the lowest XP Gscore (-10.72 kcal/mol), favorable binding mode and good pharmacological properties, hence, the β-secretase -lead1 complex was evaluated for its binding stability through 10ns MD simulations. The result revealed that binding between β-secretase and lead1 was stable. Thus, lead1 was proposed to develop therapeutic strategies for Alzheimer's disease through in *vitro* and in *vivo* evaluation.

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