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In Silico Docking Analysis on Alzheimer's Beta Secretase (BACE1) with Putative Drug from Brahmi Extracts, Bacopasaponins

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Abstract:

BACE1 which is a beta secretase enzyme initiates the Alzheimer's disease (AD) pathogenesis by amyloid β (Aβ) peptide production. Thus it can serve as a potential therapeutic target for AD treatment. Earlier experimental studies showed that bacopasides, which are key component of Bacopamonniera (Brahmi), are effective against treatment for AD. In our study, we used the molecular modeling approach to study the inhibitory action of Bacopasides on BACE1 activity. For the study, we selected the nine bacopasides and performed the molecular docking studies to study its interaction with the BACE1 key active site residues, which is essential for inhibiting the activity of BACE1. The compounds were selected on the basis of its interaction with the active site residues for the further study of their ADMET properties which are used to evaluate its drug like behavior. Our findings show that bacopasides C, B, N2 and A can serve as potential inhibitors.

Key words: Beta Secretase, BACE1, Bacopasides, Docking, Drug targeting, Schrodinger, Glide.

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Introduction-

Alzheimer's disease is an age-related neurodegenerative ailment which is characterized by gradual loss of memory as well as destruction of higher cognitive functions and is often observed in persons of more than 65 years of age[1].This disorder is clinically characterized by memory loss, cognitive decline and pathologically by occurrence of extracellular insoluble senile plaques, intracellular neuro fibrillary tangles (NFT). Senile plaques, mainly consisting of the amyloid beta (Aß) peptide, get accumulated in the form of harmful oligomers and initiate the series of events including NFT formation, neuronal deterioration resulting in AD dementia[2,3].

The particular Aß peptide is produced from the significant Type1 integral membrane Amyloid Precursor Protein(APP), through sequential proteolytic cleavage of the ß-secretase and γ-secretase enzyme complex[4,5]. Two fragments, ß-cleaved soluble APP(APPsß) and carboxy-terminal fragment (CTF, C99) are formed by cleavage of the protein by ß-secretase at Asp1.γ secretase generates a variable length Aß monomer (Aß1−40, Aß1−42) by cleaving the C99[6,7].This kind of processing through ß-secretase along with γ-secretase is known as amyloidogenic whereas α secretase another protease cleaves APP at Leu[17](occurs within the Aß sequence, non-amyloidogenic) resulting in formation of two fragments APPsα and CTF (C83) followed by cleavage of C83 by γ-secretase producing a smaller peptide (Aß17–40, Aß17–42). Hence inhibitions of ß-secretase and/or γ-secretase are known to have an important role in AD treatment. Apart from the processing of APP, γ-Secretase also processes some important proteins associated with developmental as well as physiological functions of the body. Therefore target based toxicity is caused by inhibition of this enzyme. On the other hand ß-secretase knockout mice proved to be viable as well as fertile even in

absence of Aß production indicating ß-secretase to be a prime therapeutic target for the development of AD therapy[4,8,9].

In 1999, several groups working independently identified two proteins matching with ß secretase's activity, expression and localization. These proteins were named as BACE1 (Beta-site APP Cleaving Enzyme1) or Asp2 (aspartyl protease 2) or Memapsin2 (membrane associated aspartic protease 2) and BACE2 or Asp1 (aspartyl protease 1) or memapsin1 (membrane associated aspartic protease1). BACE2 shows 64% sequence homology with BACE1.But specific physiological function of BACE2 seemed to be ambiguous as well as its cleavage specificity for APP was different. Consequently BACE1 is unequivocally verified as the ß-secretase candidate associated with AD pathogenesis[7,10,11].

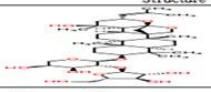
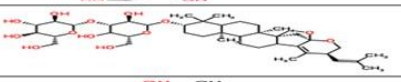
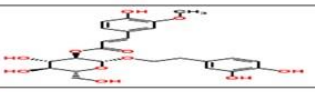
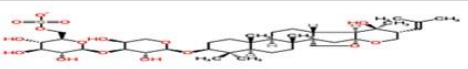
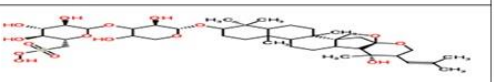
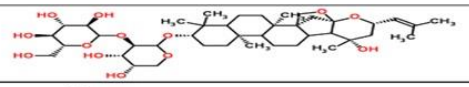
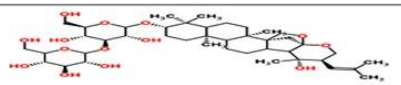
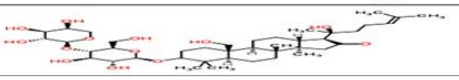
Natural products and their derivatives are being utilized as conventional medication for thousands of years for treatment of various disorders. According to a report more than 60 percent of the total drugs being represented by natural product derived drugs from 1981–2006[12,13].*Bacopa monniera* (also known as Brahmi) is a bitter tasting plant which is mostly found in damp and marshy areas. It is being utilized in ayurvedic treatment for treatment of various disorders like epilepsy, insomnia, asthma and rheumatism. The compounds which are responsible for pharmacological properties of Brahmi are alkaloids, saponins and sterols. Brahmi is utilized to strengthen the memory power and cognitive function[14]. It also inhibits cholinergic degeneration and showed cognition-enhancing effect in rat model of AD[15]. Brahmi suppresses cellular acetyl cholinesterase action and thus it provides protection to neurons from beta amyloid induced cell death[16].A number of researches have reported that Brahmi extract act as potential agent for memory enhancing and neuro protection in Alzheimer's disease[17]. Oral administration of Bacopasides side A and bacopaside B in mice resulted in improved learning and memory in mice[18].Although role of bacopasaponins as anti-

alzheimer therapy have been evaluated by numerous research studies but effect of bacopasaponins on BACE1 inhibition have not been reported and documented yet. To evaluate this, we have studied interaction properties such as binding energy and binding stability of protein-ligand complex by performing *in silico* docking of BACE 1 with bacopasaponins. Results from the study indicated that bacopasaponins might be a potential compound for using it as BACE 1 inhibitor.

Methodology-

Selection of compounds and preparation -

The set of bacopasaponin molecules were collected from Brahmi plant based on pharmacological activity, especially neuro protective behavior[19]. The list of bacopasaponins include Bacopasaponin G, bacopasaponin H, bacopaside B, bacopaside I, bacopaside II, bacopaside III, bacopaside VI, bacopaside VII, bacopaside X, bacopaside C, bacopaside N2, bacopaside A, bacopaside A3. Chemspider is a chemical database which contains structure of millions of unique molecule which are pre clustered and cross referenced based on their identity and similarity[20]. The 2D structure of selected bacopasaponins retrieved from Chemspider database. The co-crystallized BACE1 inhibitor SC7 was used as a control ligand. These compounds were energy minimized using Ligprep tool available in Schrodinger[21]. The list of two Dimensional structures of bacopaside molecules along with their Chemspider id are mentioned in Figure 1.

LIGAND	Chemspider ID	Structure
Bacopasaponin G	8780390	
Bacopasaponin H	28284153	
Bacopaside B	9288874	
Bacopaside III	552715	
Bacopaside VI	28284045	
Bacopaside X	8804917	
Bacopaside C	93210333	
Bacopaside N2	28283147	
Bacoside A	10228105	

Preparation of BACE1 and molecular interaction studies

Protein Data Bank (PDB) is the worldwide repository for structural data of large biomolecules obtained by X-ray crystallography and NMR spectroscopy. The Research Collaboratory for Structural Bioinformatics (RCSB) is maintaining PDB since 1998[22]. The three-dimensional structure of BACE1, co-crystallized with inhibitor SC7, was obtained from the protein databank, PDBID: 2QP8 with a resolution of 1.50Å (as shown in Figure 2)[23].

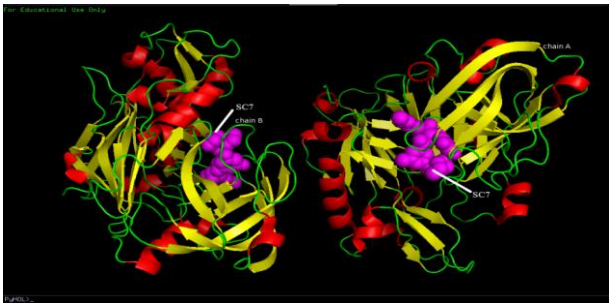


Figure 2- BACE1 co-crystallized with inhibitor

For docking studies, the SC7 inhibitor was extracted from the BACE1 and considered as without ligand. Hydrogen atoms are added and water molecules are removed. The region where SC7 bound in the active site of BACE1 in 2QP8 is considered to be the most favorable binding region [23]. More specifically, two aspartic acid residues (ASP93andASP289) of BACE1(shown in Figure 3) are essential for substrate binding and mutation of these residues make the enzyme to be inactive[6,24]. These active sites are considered to be the most favorable region for docking simulations.

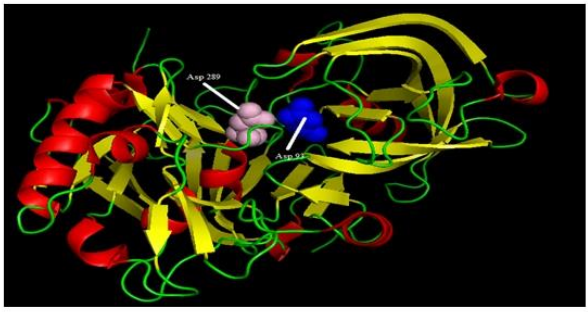


Figure 3- Asp 93 and Asp 289 residues of BACE1

Molecular docking is an important tool in the field of structural molecular biology as well as computer-assisted drug design. The purpose of ligand-protein docking is to predict the prevalent

binding mode(s) of a ligand with a protein of known three-dimensional structure as well as to predict the stability of the complex formed[25]. Schrodinger LLC. , is a company which develops software for use in biotechnology and pharmaceutical applications. It consists of more than 25 standalone analysis applications[26].

Schrödinger's Protein Preparation Wizard is used for correcting common problems such as incomplete side chains and loops, missing hydrogen atoms, flipped residues and ambiguous protonation states[27]. Beta secretase enzyme (BACE1) has chain A and chain B, are sequence unique with same binding pocket, due to which only one chain is used for docking. Chain B, ligand other than SC7, water removed using protein preparation wizard. For predicting protein-ligand binding modes, Schrödinger's flexible ligand docking program, glide is used. Using receptor grid generation, receptor grid is generated by removing the SC7 inhibitor from the prepared protein. Ligand receptor docking is done by using XP (extra precision) mode where elimination of false positives is accomplished by more extensive sampling and advanced scoring[28]. In Schrödinger binding pocket characteristics and protein-ligand interactions are identified and communicated with automatically-generated 2D ligand interaction diagrams.

ADMET descriptors prediction –

Nearly 40% of drug candidates fail in clinical trials due to poor ADME (absorption, distribution, metabolism, and excretion) properties resulting in escalating cost of new drug development. These properties calculated for bacopasaponins molecules using Qikprop tool encoded by Schrödinger program(http://www.schrodinger. com). The molecules are neutralized before being used to the Qikprop tool as neutralized compounds can separately generate both physico- chemical

descriptors and pharmacokinetically relevant properties in the normal mode.

QikProp predicts various properties - octanol/water and water/gas log Ps, log S, log BB, log K_{hsa} for human serum albumin binding, so that decisions about a molecule's suitability can be made based on a thorough analysis It also predicts the accessibility of Lipinski's rule-of-five violations[29].

Results-

Molecular docking is utilized for the precise prediction of protein–ligand interaction geometries at molecular level. This was performed for BACE1with selected nine bacopasaponin molecules and SC7 as the control ligand for validation in Glide. The specific active site residues were kept flexible, along with the ligand. The interaction of each bacopasaponins with the specific active site residue is listed in Table1.

On the basis of ability of forming bond with the active site residues, bacopasaponins were screened and the docked complex of screened bacopasaponins with BACE 1 is shown in Figure 4.

BACE1withBacopaside C -

Bacopaside C interacts with BACE1 residues of Asp289, Thr133, Gly291, Ile 187 and Thr292 with a binding affinity of −11.09 kcal/ mol (shown in Fig 4 (I)). The potential binding orientation of the BACE1 important active site Asp289 residue forms one hydrogen bond with bacopaside C. Similarly, the Thr 292, Ile 187, Gly 291 residues were involved in one hydrogen bond interaction while the Thr 133residue with C involved one hydrogen bond between the Thr 133 N group and -OH group of C of bacopaside C. In addition, hydrophobic interactions were formed byTyr132, Gln134, Gly 95,and Phe169 residues, strengthening the protein–ligand interaction.

Table 1-Molecular interaction results of BACE1 active sites with known inhibitor SC7 and Bacopasaponins

S.no.	Compounds	Asp 93	Asp 289
1	SC7	✓	✓
2	Bacopaside B	-	✓
3	Bacopaside C	-	✓
4	Bacopaside N2	-	✓
5	Bacopaside III	-	-
6	Bacoside A	-	✓
7	Bacopasaponin H	-	-
8	Bacopaside X	-	-
9	Bacopaside VI	-	-
10	Bacopasaponin G	-	-

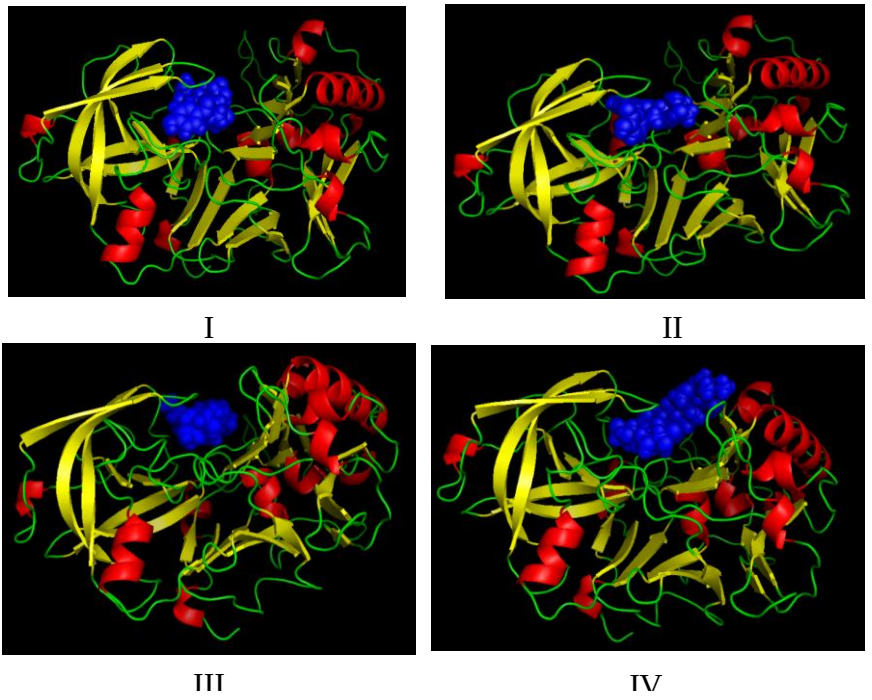


Figure 4-Docked complex of BACE 1 with (I) Bacopaside C (II) Bacopaside B (III) BacosideA (IV) Bacopaside N2

BACE1 with Bacoside A -

Interaction of Bacoside A with many residues of BACE1 with a binding affinity of −8.01 kcal/ mol is shown in Fig 4(II). The potential binding orientation of the BACE1 important active site Asp 289 residue forms one hydrogen bond with bacoside A. Similarly, the Gly 95 residue was involved in one hydrogen bond interaction while the Thr 133 and Gln 73 residues with C involved one hydrogen bond between the N group of the residues and OH group of C of bacoside A. Hydrophobic interactions were formed by Tyr132, Gln134 and Thr 293 residues which further strengthened the protein–ligand interaction.

BACE1 with Bacopaside B-

Bacopaside B interacts with various residues of BACE1 as shown in Fig. 4(III)with a binding affinity of −12.91 kcal/ mol. The potential binding orientation of the BACE1 important active site Asp 289 residue formed hydrophobic interaction with O group of C of Bacopaside B. Phe 169, Pro 131, Thr 390, Ile 187 residues with carbon were involved in one hydrogen bond formation between the hydrogen bonds between the Thr 292 O group and OH group of C of bacoside A. Arg 189 residue shows interaction with the ligand by forming two hydrogen bonds between the residue's N group and OH group of C of ligand while the Thr 133 involved in two hydrogen bond formations by forming one hydrogen form between residue's N group and OH group of C of ligand and second bond by forming bond between residue's O group and OH group of C of ligand. In addition, hydrophobic interactions were formed by Tyr132, Gly 291,Gly 95 residues resulting in strengthening of the protein–ligand interaction.

BACE1 with Bacopaside N2 -

The obtained Bacopaside N2 docking interaction with BACE1 is shown in Fig. 4(IV) with hydrogen bonds and hydrophobic

interactions with various residues with a binding affinity of −9.72 kcal/ mol. The potential binding orientation of the BACE1 important active site Asp 289 residue formed one hydrophobic interaction with the ligand. TheGly 95, Phe 169, Thr 292 and Gly 291 residues were involved in one hydrogen bond interaction by forming bond between residue's O group and OH group of C of ligand. In addition, hydrophobic interactions were formed by Tyr132, Gln134 and Thr 293 residues, strengthening the protein–ligand interaction.

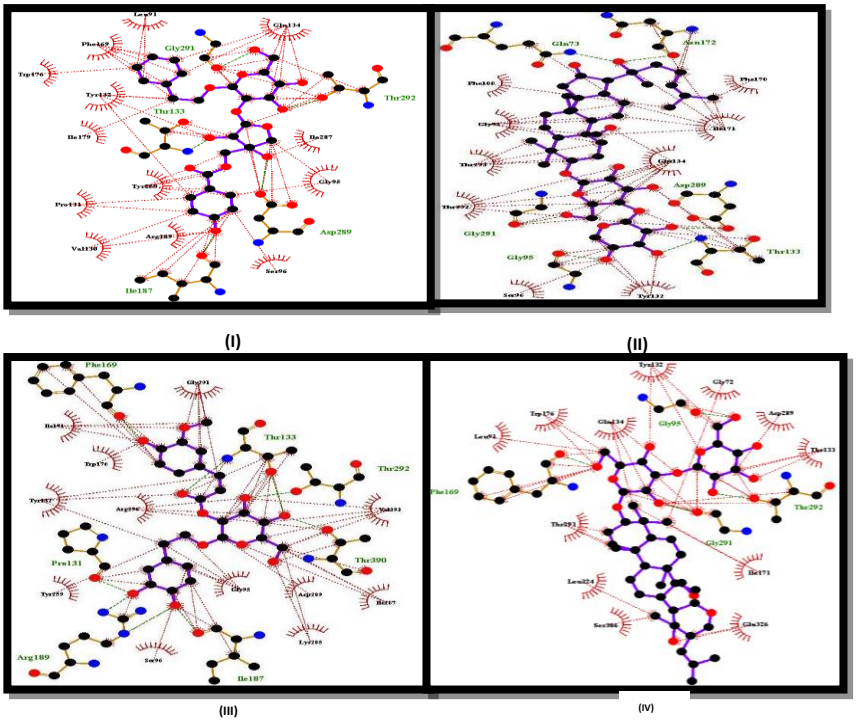


Figure 5- Docking interactions (hydrogen and hydrophobic interactions) of BACE 1 with bacopasaponins (I) Bacopaside C (II) BacosideA(III) Bacopaside B (IV) Bacopaside N2

The interaction energies of the BACE 1 complexes along with the screened bacopasaponins is calculated with the help of Glide in Schrodinger software. Glide score along with the interaction energies of the BACE1 complexes with bacopasaponins is shown in Table 2-

Table 2- Interaction energies and glide score of selected bacopasaponins

Ligand	Gscore	Emodel	vdW	Coul	H Bond	CvdW
Bacopaside B	-12.91	-95.8	-37.7	-28.8	-7	-66.4
Baopaside C	-11.09	-95.3	-48.2	-19.6	-4.5	-67.8
Bacopaside N2	-9.72	-73.4	-32.4	-23.6	-5.7	-56
Bacoside A	-8.01	-57.9	-41.4	-17.4	-5	-58.8

G score – Glide score, Emodel- combination of Gscore and CvdW, VdW - van der Waals interaction energy, Coul - Coulomb interaction energy, HBond - Hydrogen-bonding term, CvdW- Coul + vdW.

ADMET descriptors prediction –

For studying drug like behavior the bacopasaponins compounds were further evaluated. Using Qikprop tool available in Schrodinger, their physico-chemical and pharameceutical properties were calculated. Among all of the properties, the BBB (QPlogBB: acceptable range −3.0 to1.2) is a very important parameter, indicating the ability of the molecule to pass through the blood brain barrier, which is mandatory for Alzheimer's treatment[30,31]. The other properties such as molecular weight, human oral absorption in gastrointestinal tract(QP(%):acceptable range: o25% is poor and 480% is high),Serum protein binding (QP log Khsa: acceptable range −1.5to1.5), octanol/water partition coefficient (QP logPo/w: acceptable range −0.2 to6.5) were measured for selected bacopasaponins molecules. The detailed results of predicted ADMET values of each bacopasaponins with acceptable range as described in Table 3. Finally, after these screening filters were identified four molecules such as bacopasaponins have the

ability to cross the BBB and act as potential agents of AD treatment.

Table 3 – ADMET results of selected bacopasaponins with their pharmacokinetic

Bacopasaponin	**C**	**A**	**B**	**N2**
Mw	536.532	768.98	492.479	796.991
QP log BB	-3.403	-4.315	-3.716	-3.572
QP log Khsa	-1.044	-0.338	-0.761	-0.328
QP log Po/w	0.051	1.686	0.66	1.563
QP(%)	13	15	28	21
Lipinski violation	3	3	2	3

Discussion & Conclusion

Alzheimer's disease is the most common neurodegenerative disease in older populations which still has no cure. Bacopasides, the most active bacopasaponins, contribute to the pharmacological action of Bacopamonniera (Brahmi). Recent studies show that bacopasides have a beneficial effect on both stimulation and protection for brain and CNS-related diseased conditions specially Alzheimer's disease. Though they are being used for centuries but their effect at a molecular level is still uncertain. BACE1 which is one of the most potential therapeutic targets of AD, and the nine bacopasides which shows neuro protective behavior were selected for this study.

We studied the molecular interaction using in silico docking to reveal the orientation of the ligand (bacopasides) in the protein (BACE1) and the strength of the interaction. The strength of the interaction was estimated by a docking score or the binding free energy of the protein–ligand complex. The molecular docking results for bacopasides G, H, III, VI and X suggests that there is no direct interaction was observed in the key residues of BACE1, which are crucial for inhibition. Comparatively, bacopasides C, B, N2 and A interacted with BACE1 in either of the active site residues of BACE1. C formed

five hydrogen bonds, one of which was with the Asp289 residue. Bacopaside A formed 6 hydrogen bonds, one of which was with the Asp 289 residues. B formed seven hydrogen bonds and N2 formed four hydrogen bond, both of them formed hydrophobic interaction with Asp 289 residue. In addition to the active site residues many residues like Thr259, Tyr 292 etc. were also involved, which further enhanced the stability of the interactions. The interaction was validated with known inhibitor SC7. The SC7 was docked with BACE1, and its docking behavior showed interaction with both Asp93 and Asp 289. Based on the molecular docking studies, we selected bacopasides C, B, N2 and A, which showed strong interactions with Asp289 for further evaluation.

The compounds to be considered in the development of therapeutics of AD must cross the blood brain barrier (BBB) and should also possess drug like properties such as safety. Bacopasides showing positive interactions in docking simulations were screened for ADMET and BBB. All the bacopasides have QP log BB value closer to the acceptable range. These results suggest that bacopasides B, C, N2 and A should be considered for further evaluation. Based on obtained results of docking, ADMET screening confirmed the binding orientation of BACE1–bacopasides interactions in order to identify the lead molecules from Bacopasaponins.

From the results obtained it is clear that Bacopasaponins do have great potential to be potent drug molecules for curing AD. Some of the properties of drug likeliness are fulfilled but some are on border line, which could be looked into. Some specific modifications in the basic molecule can further enhance its activity and be more effective drug, which is one perspective area for future research. New compounds can be generated from Bacopasaponins as lead molecule with enhanced drug likeliness properties, and thus being more focused in targeting the BACE1, the most potential target for the disease.

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