In Silico Discovery of Novel BACE-1 Inhibitors for Alzheimer's disease using Pharmacophore and Molecular Docking-based Virtual Screening followed by ADMET studies.

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Abstract

BACE-1, a type 1 membrane-anchored aspartyl protease responsible for the earliest stage of the proteolysis of APP, is one of the intriguing targets for the therapy of AD. This approach resulted in potent BACE-1 inhibitors. Due to the high rate of failure of lead treatment options that targeted BACE-1, the goal of this study was to find new lead compounds that can treat and maybe halt the disease's progression. Verubecestat, Lanabecestat, and Atabecestat were used in this study as input ligands to obtain pharmacophores from which 100 Hits were obtained by pharmacophore-based virtual screening and studied for their capacity to dock on the BACE-1 protein using multiple docking technique (SBDD) as well as for their toxicities in order to find the most effective and safe ones that can be suitable for use (ADMET prediction). The findings indicated that hits with the molecular formulas C15H15N3O2, C16H17N3O2, C15H16N4O2, and C16H17N3O3 were identified as potential candidates to be BACE-1 inhibitors because they demonstrated promising potential with least binding energy, better pharmacokinetics, good drug likeliness, and low toxicity when compared to the reference molecules verubecestat, lanabecestat, and atabecestat. Since these molecules were druggable, novel Alzheimer's disease treatments may be created using them.

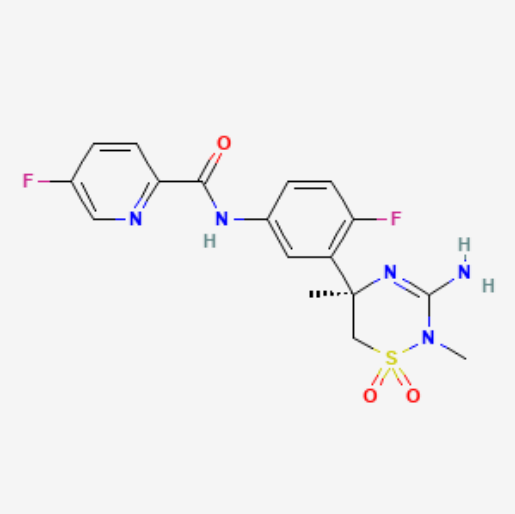
Keywords: *BACE-1, Verubecestat, Lanabecestat, Atabecestat, Pharmacophore-based virtual screening, Molecular docking, Structure-based drug discovery (SBDD), ADMET prediction.*

1. Introduction

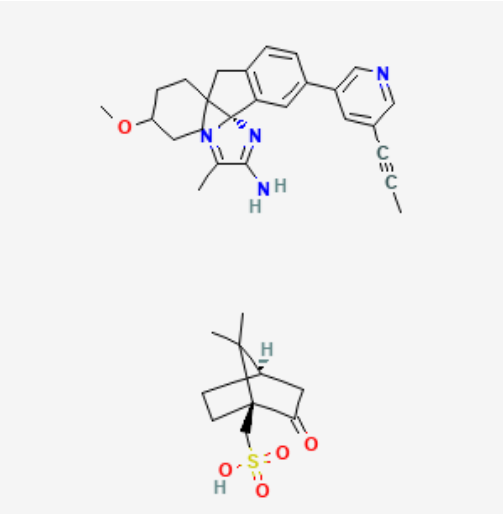
Alzheimer's disease, the primary cause of dementia, is rapidly shifting to the top of the list of the most costly, fatal, and debilitating diseases of the twenty-first century [1]. Alzheimer's disease (AD) is by far the most prevalent dementia condition, accounting for up to 80% of dementia diagnoses [2]. According to statistics from 2020 regarding the disease, the number of Americans with Alzheimer's (age 65 and older) may increase from 5.8 million to 13.8 million by the year 2050. Between 2000 and 2018, AD death tolls increased by 146.2% in America's senior population, making it the fifth-leading cause of mortality [3]. With all of these considerations, new medications that may be inexpensive and easily accessible are preferred. It has thus been crucial to identifying a neuroprotective chemical that has few adverse effects but several desirable benefits on the brain.

Amyloid plaques and neurofibrillary tangles (aggregates of hyperphosphorylated tau protein) are the dominant elements of Alzheimer's pathology. Furthermore, the coexistence of neuropil treads, dystrophic neurites, concomitant astrogliosis, and microglial activation is common. These pathogenic processes' aftereffects include neurodegeneration with synaptic and neuronal loss that causes macroscopic atrophy. The four acetylcholinesterase inhibitors AChEi), tacrine (1993 approval), donepezil (1996 approval), rivastigmine (1998), and galantamine (2000) (approved in 2001) are the medications authorized to treat AD currently, but these drugs only relieve symptoms, while not being able to impact the progression of the disease [4].

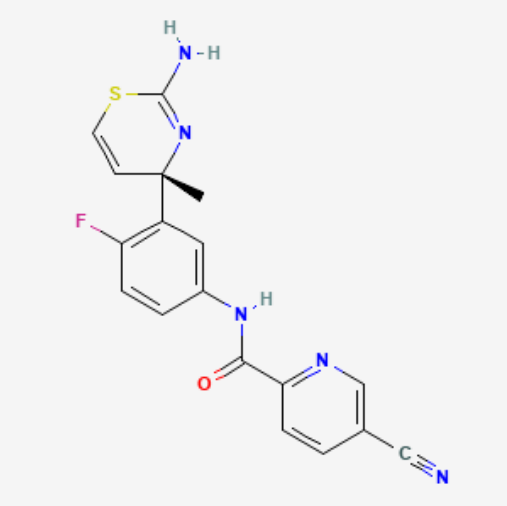
BACE-1, a type-1 membrane-anchored aspartyl protease discovered in 1999, is in charge of the initial stage of the proteolysis of APP [4]. Therefore, one of the promising targets for the therapy of AD is BACE-1. This strategy resulted in the creation of strong BACE-1 inhibitors, many of which had passed to the final stages of clinical trials. However, the high level of failure of lead treatment candidates that targeted BACE-1 led this study to focus on finding new lead molecules that can treat and possibly stop the progression of the disease. Some of such BACE-1 inhibitors that failed to pass the clinical trials include, Verubecestat (Adverse effects) [5][6], Lanabecestat (low efficacy) [7][8], Atabecestat (elevation of liver enzymes) [7][9]. These three drugs have been shown to have higher adverse effects and lower efficacy (being P-gp substrates with an effect on liver enzymes leading to drug toxicity and drug-drug interactions). In this study, these drugs are used as input ligands to obtain pharmacophores using which 100 Hits were obtained and studied for their docking ability on the BACE-1 protein and their toxicities were also studied to find the most effective and safe ones which can be suitable for further studies on their efficacy in vivo.



Lanabecestat



Verubecestat



Anabecestat

Figure 1. Structures of Reference Drugs
Source: https://pubchem.ncbi.nlm.nih.gov/

2. Design of the study

The current study was divided into three sections.

As input ligands, Verubucestat, Lanabecestat, and Atabecestat (obtained in 3D SDF format, from PubChem-NIH) were used in the first section to identify potential pharmacophore candidates (using PharmaGist, a free webserver). Then, ZincPharmer was used to conduct pharmacophore-based virtual screening and generate about 100 hits (ZINC Compounds).

The second section involved using the molecular docking technique (software used: iGEMDOCKv2.1 for multiple docking) to identify the hits that are most effectively docked to the BACE-1 protein (PDB ID- 3K5G) based on their binding energy (as reference molecules

i.e., the above-mentioned BACE-1 Inhibitors had docking energy from 120-140, this range was used to select best-bound hits). This method is known as structure-based drug discovery (SBDD).

The best-bound hits from molecular docking were studied for their ADMET properties in the third and final section using freely accessible web servers pkCSM- Biosig Lab and Swiss ADME (ADMET prediction).

3. Methods

Pharmacophore-based virtual ligand screening:

The structural organization of the crucial molecular components of an interaction between a ligand and its receptor is described by a pharmacophore [10][11][12]. A consensus pharmacophore even of a set of up to 32 ligands can be found quickly using PharmaGist, a free web server [13]. The three reference molecules were combined into one mol2 file using OpenBabel-2.4.1 (A collection of drug-like molecular structures known to bind to the receptor serve as the input) and inputted into the input section in PharmaGist with a number of output pharmacophores set at 5. The result, after running the program, was a list of potential pharmacophores, presented as numerous alignments for each subset size of the input ligands, and sorted according to the number of aligned molecules. Out of these, a pharmacophore with the highest aligned molecules was selected for the study. Extracted pharmacophore was then opened in ZincPharmer, a comprehensive library of physiologically significant, commercially available chemicals that are appropriate for screening [14][15]. Hits were generated using the filter and pharmacophore query editor with settings like H-donor<5, H-acceptor<10, MW=100-300, Rotatable bonds, 3-6, max hits per conformations=1, max total hits=100. Hits after generation were saved as a folder which was then opened in the DataWarrior application and downloaded in mol2 format individually. All the obtained hits were subjected to docking in the next phase of the study.

Structure-based Drug Discovery (SBDD)- molecular docking:

Docking is the process of using computers to simulate the shape, size, and conformation of medicines, enzymes, and proteins in an effort to discover the best possible match between various molecules [16]. The target protein, BACE-1, was likewise uploaded in pdb format after each hit was inputted into iGEMDOCKv2.1 after individual extraction of all the hits in mol2 format from DataWarrior. Docking was then initiated using Population size-200, generations-70, number of solutions- 2, and standard docking with default settings. Once the docking is done, results were obtained out of which hits with the least binding energy were determined (as reference molecules had docking energy from 120-140, this range was used to select best-bound hits).

ADMET prediction:

As a less expensive alternative to the costly experimental evaluation of ADMET profiles, the in silico ADMET properties of the chosen molecules were determined. Free online webservers, SwissADME (for physicochemical characteristics, pharmacokinetics, and

druglikelyness) and pkCSM-Biosig lab (for toxicity prediction) were used to forecast the ADMET Properties of the short-listed hits.

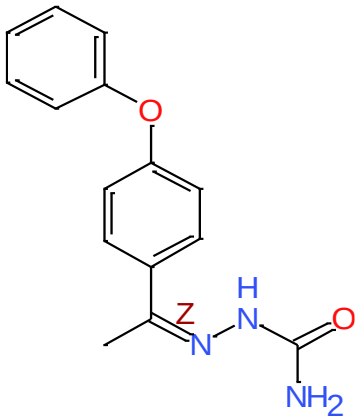
4. Results

Results from Pharmacophore-based virtual ligand screening:

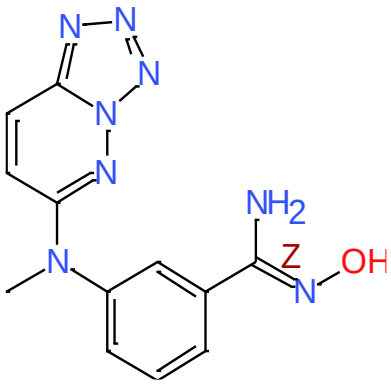
As previously indicated, the three reference compounds were entered into PharmaGist's input section, which produced a list of candidate pharmacophores from which the pharmacophore with the highest alignment of molecules was chosen for the study. The extracted pharmacophore is opened in ZincPharmer, and using the predefined settings, after which 100 hits were produced.

Results from Structure-based Drug Discovery (SBDD)- molecular docking:

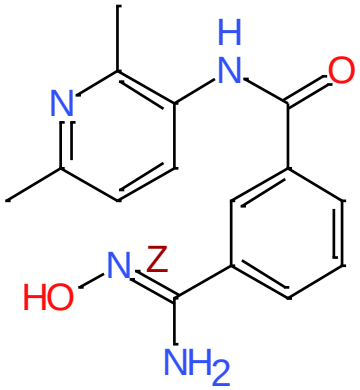
Molecular docking was used to assess binding affinities and comprehend potential interactions between ligands and proteins. Table 1. lists the binding energies, Vander walls forces and H-bond of the hits that were of the least binding energies.



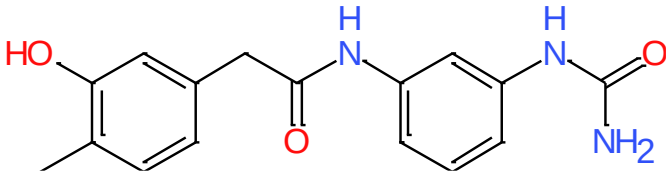
C15H15N3O2



C16H17N3O2



C15H16N4O2



C16H17N3O3

Figure 2. Structures of Short-listed Hits with their Molecular Formula

Table 1. Binding Energy, VDW, H-bond

S. No.	Molecular Formula	Binding energy	VDW	H-Bond
1	C17H17F2N5O3S (Verubecestat)	-122.27	-102.606	-20.387
2	C26H28N4O (Lanabecestat)	-141.74	-133.165	-8.5794
3	C18H14FN5OS (Atabecestat)	-122.99	-104.794	-17.475
4	C15H15N3O2	-120.63	-97.96	-22.67
5	C16H17N3O2	-124.11	-95.0959	-9.847
6	C15H16N4O2	-125.05	-109.497	-22.910
7	C16H17N3O3	-125.22	-146.614	-9.500

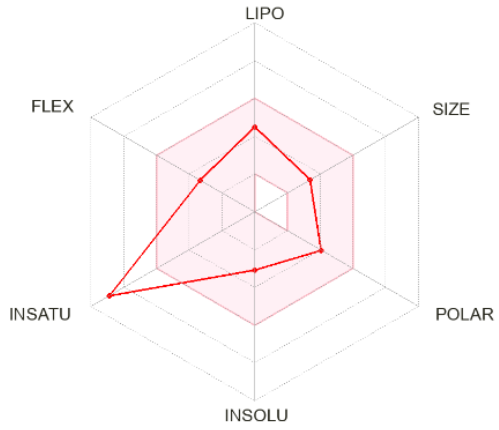
Results from ADMET prediction:

The hits that made the cut were further studied for their physicochemical properties and lipophilicity in table 2, table 3 lists the pharmacokinetics, table 4 lists the drug-likeliness using swiss ADME website while toxicity was listed in table 5 (studied using free access website pkCSM) compared to the results of reference molecules obtained by the same methods. Bioavailability radars of the selected hits are shown in figure 3.

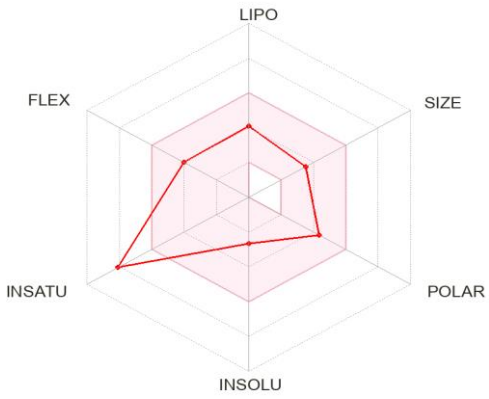
Table 2. Physicochemical Properties, and Lipophilicity of Short-listed Molecules

S. No.	Molecular Formula	Molecular Weight (g/mol)	No. of Rotatable bonds	No. of H-Bond acceptors	No. of H-Bond donors	Lipophilicity (Consensus Log $P_{o/w}$)
1	C17H17F2N5O3S (Verubecestat)	409.41	4	7	2	1.59
2	C26H28N4O (Lanabecestat)	412.53	2	4	1	3.97
3	C18H14FN5OS (Atabecestat)	367.40	4	5	2	2.23
4	C15H15N3O2	269.30	5	3	2	2.33
5	C16H17N3O2	283.33	6	2	3	2.02
6	C15H16N4O2	284.31	4	4	3	1.77

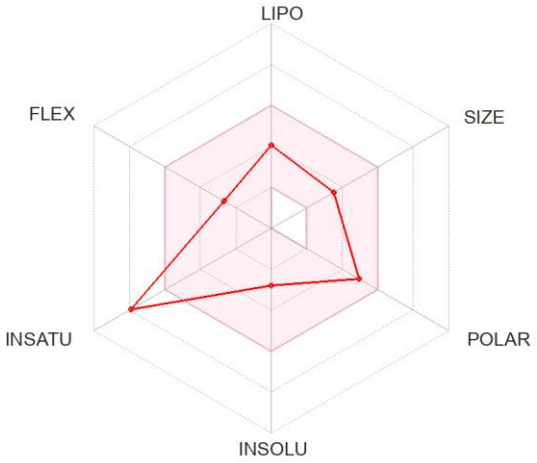
7	C16H17N3O3	299.32	6	3	4	1.53



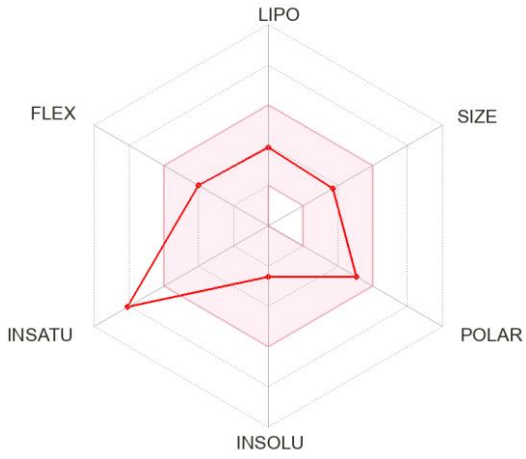
C16H17N3O2



C15H15N3O2



C15H16N4O2



C15H16N4O2

Figure 3. Bioavailability Radars of Hits with Molecular Formula

Table 3: Pharmacokinetics of Short-listed Molecules

S. No.	Molecular Formula	GI Absorption	BBB Penetration	P-gp Substrate	CYP-enzyme inhibition	Log K_p (cm/s)
1	C17H17F2N5O3S (Verubecestat)	High	No	Yes	No	-8.38
2	C26H28N4O (Lanabecestat)	High	Yes	Yes	Yes	-6.70
3	C18H14FN5OS (Atabecestat)	High	No	No	Yes	-7.14
4	C15H15N3O2	High	Yes	No	No	-6.27
5	C16H17N3O2	High	No	No	No	-6.85
6	C15H16N4O2	High	No	No	No	-6.88
7	C16H17N3O3	High	No	No	No	-7.20

Table 4: Drug-likeliness of the Short-listed Molecules

S. No.	Molecular Formula	Lipinski rule	Ghose filter	Veber (GSK) filter	Egan filter	Muegge filter	Bioavailability score
1	C17H17F2N5O3S (Verubecestat)	Yes	Yes	Yes	Yes	Yes	0.55
2	C26H28N4O (Lanabecestat)	Yes	No	Yes	Yes	Yes	0.55
3	C18H14FN5OS (Atabecestat)	Yes	Yes	Yes	Yes	Yes	0.55
4	C15H15N3O2	Yes	Yes	Yes	Yes	Yes	0.55
5	C16H17N3O2	Yes	Yes	Yes	Yes	Yes	0.55
6	C15H16N4O2	Yes	Yes	Yes	Yes	Yes	0.55
7	C16H17N3O3	Yes	Yes	Yes	Yes	Yes	0.55

Table 5: Toxicity

S. No.	Molecular Formula	AMES Toxicity	hERG-I Inhibition	hERG-II Inhibition	Hepatotoxicity	LD 50 (mol/kg)	LOAEL (log mol/kg_bw/day)
1	C17H17F2N5O3S (Verubecestat)	No	No	No	Yes	1.773	2.122
2	C26H28N4O (Lanabecestat)	No	No	Yes	Yes	2.626	1.046
3	C18H14FN5OS (Atabecestat)	No	No	No	No	2.389	0.527
4	C15H15N3O2	No	No	No	No	2.169	2.342
5	C16H17N3O2	No	No	No	No	2.205	1.725
6	C15H16N4O2	No	No	No	No	2.658	1.48
7	C16H17N3O3	No	No	No	No	2.189	2.216

5. Discussion

The first phase of the proteolysis of APP is carried out by BACE-1, a type 1 membrane-anchored aspartyl protease that was identified in 1999. The cleavage of APP in the amyloid cascade results in the production of two peptide fragments, A40 and A42. According to current theories, A42 is the primary species among two peptide fragments responsible for the neurotoxicity and amyloid plaque development that led to memory and cognitive issues in Alzheimer's disease (AD) [4]. Inhibitors of BACE-1 prevent the rate-limiting step in the synthesis of Aβ. Only five BACE-1 inhibitors—verubecestat, lanabecestat, atabecestat, umibecestat, and elenbecestat—have advanced to phase III clinical trials despite years of development. All five of these medications effectively lowered the levels of Aβ levels in AD patients' cerebrospinal fluid (CSF). However, due to a lack of cognitive or functional improvement and many adverse effects, these five substances were terminated [7]. Thereby, an effort has been undertaken in the current study to obtain and identify prospective BACE-1 inhibitors using pharmacophores of the existing BACE-1 inhibitors that target the BACE-1 enzyme by Pharmacophore-based virtual ligand screening.

The conformational sampling of (a) known ligands to be used for pharmacophore extraction in so-called ligand-based approaches and (b) of all the candidate compounds from the electronic

database that need to be confronted with the pharmacophore hypothesis during the virtual screening is an important step in pharmacophore modeling [17]. 100 hits were acquired after a 3D query was created using the acquired pharmacophore model to screen the ZINC buyable database. The target protein BACE-1, PDB ID 3K5G, was then docked with the generated hits. This showed that hits with molecular formulas C15H15N3O2, C16H17N3O2, C15H16N4O2, C16H17N3O3 were found to be showing the least binding energy as N Kondapalli, K Sruthi, 2020 [18], mentioned in their study that when a molecule binds with the least amount of binding energy, it is said to be powerful. and the values obtained for van der-walls forces and hydrogen bonding also indicate a strong binding of hit molecules to the target protein.

The physicochemical characteristics, lipophilicity, pharmacokinetics, and drug-likeliness of the aforementioned short-listed hits were further investigated using the Swiss ADME website, while toxicity was investigated using the free platform pkCSM. Table 2 indicated that the results procured from the ADME predictions show that all four hits obey Lipinski's rule of five, with its components being, <5 hydrogen bond donors, <10 hydrogen bond acceptors, molecular weight less than 500 daltons, and Consensus Log $P_{o/w}$ <5 showing that the hits can be orally active [19]. Table 3 shows the pharmacokinetics of the hits whose values indicate that all the hits have high GI absorption while C15H15N3O2 is the only one that shows blood-brain barrier penetration implying the use of it to treat brain diseases effectively. All four hits are predicted to not inhibit the CYP- enzymes and not to be P-gp substrates indicating minimal chances of drug-drug interactions, toxicity, or inefficacy (as mentioned in a study by ML Amin, 2013 [20], P-gp plays an important role in the excretion of drugs, inhibition of it may lead to accumulation of the drug in the body while excitation of it may lead to premature excretion of the drug leading to the inefficacy of drugs). Log K_p (cm/s) values of the selected hits show similar skin permeability as of the reference molecules due to the values being close but according to A Daina et al., 2017 [21], the higher the negative Log K_p (cm/s) value is, lower will be the permeability and the ideal Log K_p (cm/s) value being in between -4.901 to 3.526 as mentioned by CP_Chen et al., 2018 [22], in their study, it can be claimed that the chosen hit molecules are not skin permeable. Table 4 indicated that all four hit molecules obey the Lipinski rule, Ghose filter, Veber filter, Egan filter, and Muegge filter, and with their bioavailability scores being 0.55, it can be said that the hits are suitable for oral administration as predicted to have good oral absorption. For a quick assessment of drug-likeness, bioavailability radars are displayed in figure 3. Lipophilicity, size, polarity, solubility, flexibility, and saturation are six physicochemical characteristics that are taken into consideration. While the selected hits had almost all the parameters in the required range, insaturation was seen to be slightly out of range.

The toxicity of the hit molecules was predicted using a free web server, pkCSM. From table 5, it can be seen that the selected hits were predicted to not show AMES toxicity and hepatotoxicity, at the same time it is estimated that the four hits do not inhibit hERG-I and II receptors thus not leading to Torsade de Pointes. The computation of LD50, or the dose that will kill 50% of an animal species, can be used to estimate a drug's acute toxicity. The LD50 values of selected hits are mentioned in table 5 which can be useful for preclinical studies on the hit molecules. The lowest dose at which the effects seen in the treated group imply a negative effect for the subject is known as the lowest observed adverse effect level (LOAEL) GS King, 2002 [23], and the dose values are as mentioned in the table.

6. Conclusion

From the results, it can be concluded that hits with molecular formula C15H15N3O2, C16H17N3O2, C15H16N4O2, C16H17N3O3, are potential candidates to be considered as BACE-1 inhibitors as they show promising potential with least binding energy, better pharmacokinetics, good drug likeliness, and low toxicity when compared to the reference molecules, verubecestat, lanabecestat and atabecestat. Considering that these molecules are druggable, new medications for the treatment of Alzheimer's disease may be created using them.

Acknowledgment

The authors are grateful to the faculty of G. Pulla Reddy College of Pharmacy in Hyderabad, India for funding the research and supplying the necessary materials.

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