Research Article

To Design Novel Lead Molecules for the Enzyme, AChE Associated with Alzheimer's Disease

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ABSTRACT

Alzheimer's disease (AD) is a progressive degenerative disease of the brain marked by Gradual and irreversible declines in cognitive functions such as language deficits, depression and behavioral problems including agitation, mood disturbances and psychosis. Alzheimer's dementia is presently treated exclusively by inhibitors of the enzyme Acetylcholinesterase (AChE), following the cholinergic hypothesis. One of the most promising approaches for treating this disease is to enhance the acetylcholine level in the brain using Acetylcholinesterase (AChE) inhibitors. Acetylcholinesterase plays a biological role in the termination of nerve impulse transmissions at cholinergic synapses by rapid hydrolysis of acetylcholine. Deficit in acetylcholine leads to poor nerve impulse transmission. Thus, the cholinesterase inhibitors are proved o be the most potential compounds to restore the acetylcholine levels and eventually reverse the memory impairments characteristic of the disease. In the present work, 26 Ligands were docked into active site of Acetyl cholinesterase using the docking programs, Autodockvina. Among these docked compounds, Galantamine showed the best binding activity (-9.1). These ligands were further submitted to zinc database by collecting analogues for Galantamine. Among these docked compounds, 44281452 (-11.0) and 14947228 (-10.7) have given higher fitness scores. From our observations on the interaction and visualization aspects using PyMol**,** it was evident that these inhibitor compounds also satisfied the OSIRIS and PASS results by inhibiting the activity of Acetylcholinesterase. The type of interaction they exhibit and the residues with which they interact conveyed that both the compounds are good inhibitors of Acetylcholinesterase as they exhibit drug like activity.

Keywords: Alzheimer's disease (AD), Acetylcholinesterase**,** Galantamine analogues, lead molecules.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia in adults, which is characterized by senile plaques and cholinergic deficits. The disease affects approximately 10% of the world population with 65years of age and 50% of individuals with more than 85 years of age. It is associated with the progressive loss of cognitive capacity as well as deterioration of memory and learning capacity. The progress of the symptoms of the illness is associated with structural modifications of cholinergic synapses and, consequently with the reduction of cholinergic neurotransmission potential. Improvement of cholinergic neurotransmission is the basis of some drugs currently used in the treatment of Alzheimer's disease. Besides the neuropathologic hallmarks of this disease, namely NeuroFibrillary Tangles and neuritic plaques, it is characterized neurochemically by a consistent deficit in cholinergic neurotransmission, particularly affecting cholinergic neurons in the basal forebrain[1,2]. Proper treatment is achieved by Acetyl cholinesterase (AChE) inhibition, the enzyme responsible for acetylcholine hydrolysis[3, 4]**.** The evidence stems from several reports which demonstrated reduction in the activity of Enzymes involved in the synthesis of acetylcholine, i.e. choline acetyl transferase or excess degradation of ACh by AChE[5-7]. The first AChE inhibitors (AChEIs) specifically approved for the treatment of AD was introduced in 1993 as 1, 2, 3, 4-tetrahydro-9-aminoacridine (tacrine).[8]

Currently, several AChE inhibitors, such as donepezil[9], Galantamine[10] and rivastigmine[11] are available for the symptomatic treatment of patients with mild to moderate AD. The 3D structure of AChE and the structural details of a number of AChE–inhibitor complexes are largely known[12-17]**.** Galantamine is a recently developed AChE inhibitor[18] that may offer some clinical benefit[19-22]. The alkaloid Galantamine was isolated from the Caucasian snow drop Galanthus woronowi[23] and was subsequently determined to occur naturally in other plants of the Amaryllidaceous family. Galantamine enhances brain cholinergic activity via a dual mode of action. Besides being a brain-selective, reversible, competitive AChE inhibitor[24]**,** Galantamine is an allosterically potentiating Ligand (APL) that amplifies the action of the neurotransmitter at the nicotinic AChR[25-27]**.** In clinical studies, long-term treatment with galantamine significantly improves cognitive, functional and behavioral symptoms of patients with mild to moderate AD[28-30]**.**

In view of the above earlier findings, the present study is an attempt to design new lead molecules with larger selectivity and anti-cholinesterase activity to treat Alzheimer's disease.

MATERIALS AND METHODS

Ligand & Protein Selection

The Protein Data Bank (PDB) is the single worldwide archive of structural data of Biological macromolecules established in Brookhaven National Laboratories in

1971[31]. It contains structural information of the macromolecules determined by x-ray Crystallography and NMR methods. The PDB database is operated by the Research collaborator for structural Bioinformatics (RCSB), as on Aug 10, 2010, PDB has 67131 macromolecular structures data Entries. Crystal structure of human Acetylcholinesterase with (PDB ID 1B41) having Structure molecular weight 66664. 94 were retrieved from PDB. It has two chains, A and B and the total numbers of amino acids are 539.Additional Ligands which are present along with the structure were removed using Swiss PDBViewer which is software for analysis of protein and loaded for docking studies. The PubChem Compound database contains validated chemical depiction information that is provided to describe substances in PubChem Substance. Structures stored within PubChem Compound are pre-clustered and cross-referenced by identity and similarity groups. Additionally, calculated properties and descriptors are available for searching and filtering of chemical structures. The structure of the Galantamine with compound ID 13096748 is retrieved from PubChem Compound Database using keyword search.

Table 1: Tools and software were employed for analyses of physical properties

Name of the parameter	Tool/database employed
Collection of sequences and information	1. NCBI 2. PDB 3. PubChem
Energy minimization	Argus lab
Active site prediction	CAST-p
Collection of Ligand molecules	1. PubChem 2. Zinc Database
Protein Ligand docking	Auto Dock Vina (Pyrex)
Interaction and Visualization	PyMol
Biochemical test of lead molecule	1. OSIRIS 2. PASS Prediction

Identification of Ligands for 1B41

We have taken 26 inhibitors for AChE protein from CRIPS (vol. 11; No.1 January-March 2010). The structural information was collected from the Pub Chem data base. All these ligand molecules were subjected to docking studies to know the best binding affinity. After completion of docking studies, the best binding affinity Ligand for Galantamine molecule was subjected to Zinc database. After submission, we collected 2,323 compounds based on the similarity search and then these Ligands were used for docking studies. Finally we selected the best ligand molecule based on the binding affinity.

RESULTS

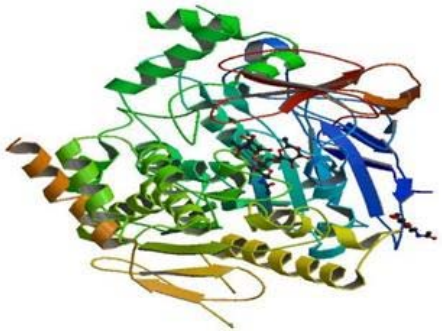
Sequence of the selected protein 1b41 retrieved from NCBI-Fasta format

>gi|13096478|pdb|1B41|A Chain-A, Human Acetyl Cholinesterase Complexed With Fasciculine-I, Glycosylated Protein.

DAELLVTVRGGRLRGIRLKTPGGPVSAFLGIPFAEPPMGPRRFLPPEPKQ
PWSGVVDATTFQSVCYQYVDTLYPGFEGTEMWNPNRELSEDCLYLNV
WTPYPRPTSPTPVLVWIYGGGFYSGASSLDVYDGRFLVQAERTVLVSM
NYRVGAFGFLALPGSREAPGNVGLLDQRLALQWVQENVAAFGGDPTS
VTLFGESAGAASVGMHLLSPPSRGLFHRAVLQSGAPNGPWATVGMG
EARRRATQLAHLVGCPPGGTGGNDTELVACLRTRPAQVLVNHEWHVL
PQESVFRFSFVPVVDGDFLSDTPEALINAGDFHGLQVLVGVVKDEGSYF
LVYGAPGFSKDNESLISRAEFLAGVRVGVPQVSDLAAEAVVLHYTDWL
HPEDPARLREALSDVVGDHNVVCPVAQLAGRLAAQGARVYAYVFEHR
ASTLSWPLWMGVPHGYEIEFIFGIPLDPSRNYTAEEFAQRLMRYWANF
ARTGDPNEPRDPKAPQWPPYTAGAQQYVSLDLRPLEVRRGLRAQACA
FWNRFLPKLLSAT

Protein structural information

The human acetyl cholinesterase and its E202Q mutant as complexes with fasciculein-I, a three finer's polypeptide toxin purified from the venom of the eastern green mamba (Dendroa spis angusticeps) are reported the structure of the complex of thewild type enzyme was solved to 2.8A resolution by molecular replacement starting from the structure of the complex of Torpedocalifornica Acetyl cholinesterase with Fasciculin-II and verified by starting from a similar complex with mouse acetyl cholinesterase.



PDB 3D protein structure for the 1B41

Ligands selected for docking studies

Docking studies using Auto Dock Vina software on these 26 selected Ligand molecules (table 2) for the enzyme, Acetyl cholinesterase (1B41) showed the following structural details.

Zinc data base

The galantamine Analogues (table 3) were collected different ratios from Zinc Database by using key word search. From the data generated from Zinc Database, about 2323 analogues were collected. Then these analogues were again subjected to against IB41 by using Auto Dock Vina (Pyrex). Among these, only 6 compounds showed high binding affinity with 1B41. Hence, these Galantamine analogues were used to study the binding interactions with AChE and bioactivity test.

Table 2: Ligands selected for docking studies

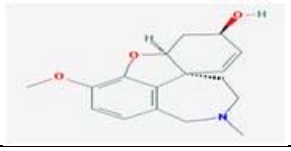
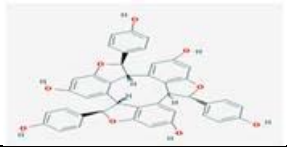
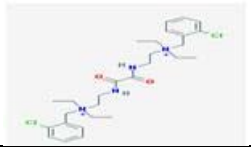
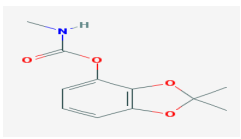
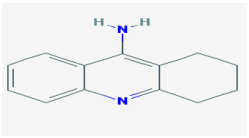
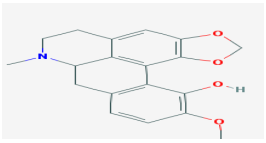
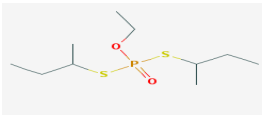
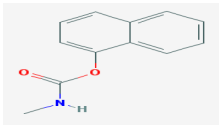
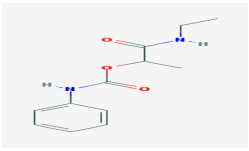
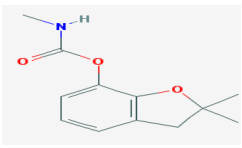
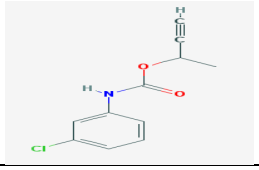
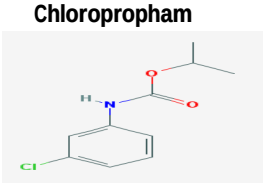
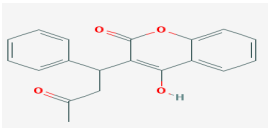
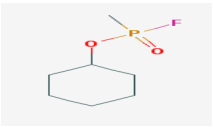
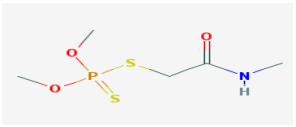
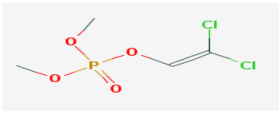
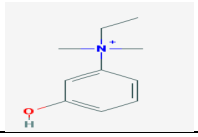
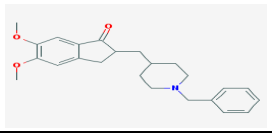
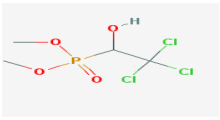
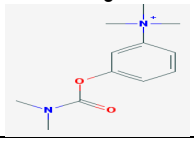
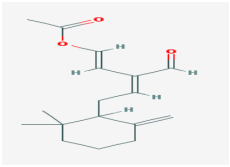
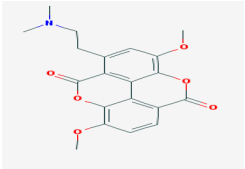
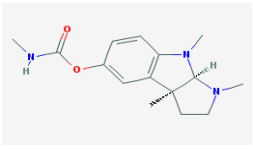
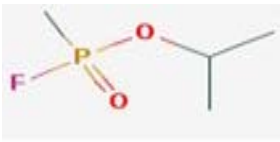
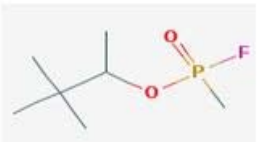
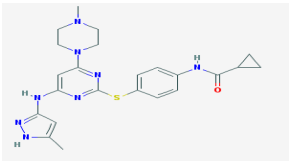
Galantamine 	Alpha-vinifinin 	Ambenonium 	Bendiocarb 
Tacrine 	Bulbocapanine 	Cadusfos 	Carbaryl 
Carbetamide 	Carbofuran 	Chlobufam 	Chloropropham 
Coumarins 	Cyclosarin 	Dimethonate 	Dochlorvos 
Edrphonium 	Onepezil 	Metrifonate 	Neostigmin 
Onchidol 	Paspine 	Physostigmine 	Sarin 
Soman 	Vx-680 		

Table 3: Galantamine zinc analogue structures

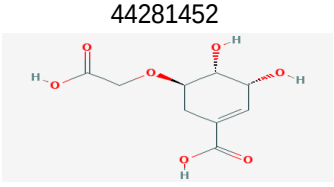
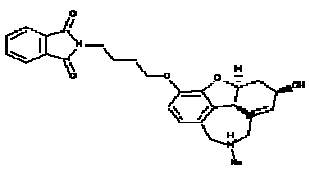
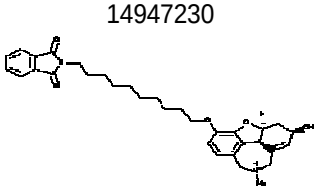
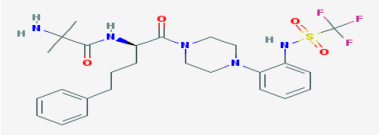
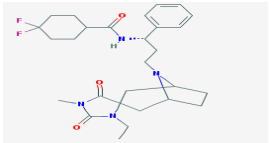
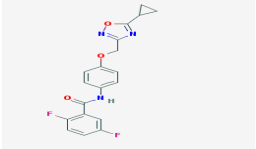
44281452 	14947228 	14947230 
44281448 	59827700 	60285168 

Table 4: Interaction studies on protein (1b41) - ligand through PyMol.

Zinc id	Hydrogen bonding			Distance	Binding affinity
	Residues	Atom	Ligand		
(44281452)	Tyr-72	O	C	3.5	-11.0
	Tyr-337	O	O	2.9	
	Tyr-124	O	O	2.8	
	Ser-125	O	O	3.1	
(14947228)	Tyr-124	O	O	2.9	-10.7
	Tyr-124	O	O	2.2	
	Tyr-124	O	O	2.8	
	Tyr-337	O	O	2.6	
	Tyr-341	O	O	3.0	
(14947230)	Tyr-124	O	O	3.2	-10.2
	Ser-125	O	O	3.2	
	Trp-286	O	H	2.4	
(44281448)	Ser-125	O	O	3.2	-10.1
	Ser-124	O	O	2.7	
	Tyr-337	O	O	3.3	
(59827700)	His-287	N	O	2.9	-9.4
	Tyr-72	O	O	2.9	
	Tyr-124	O	O	3.2	

Figure 1: Picture showing interaction between 1b41 and galantamine zinc anologues

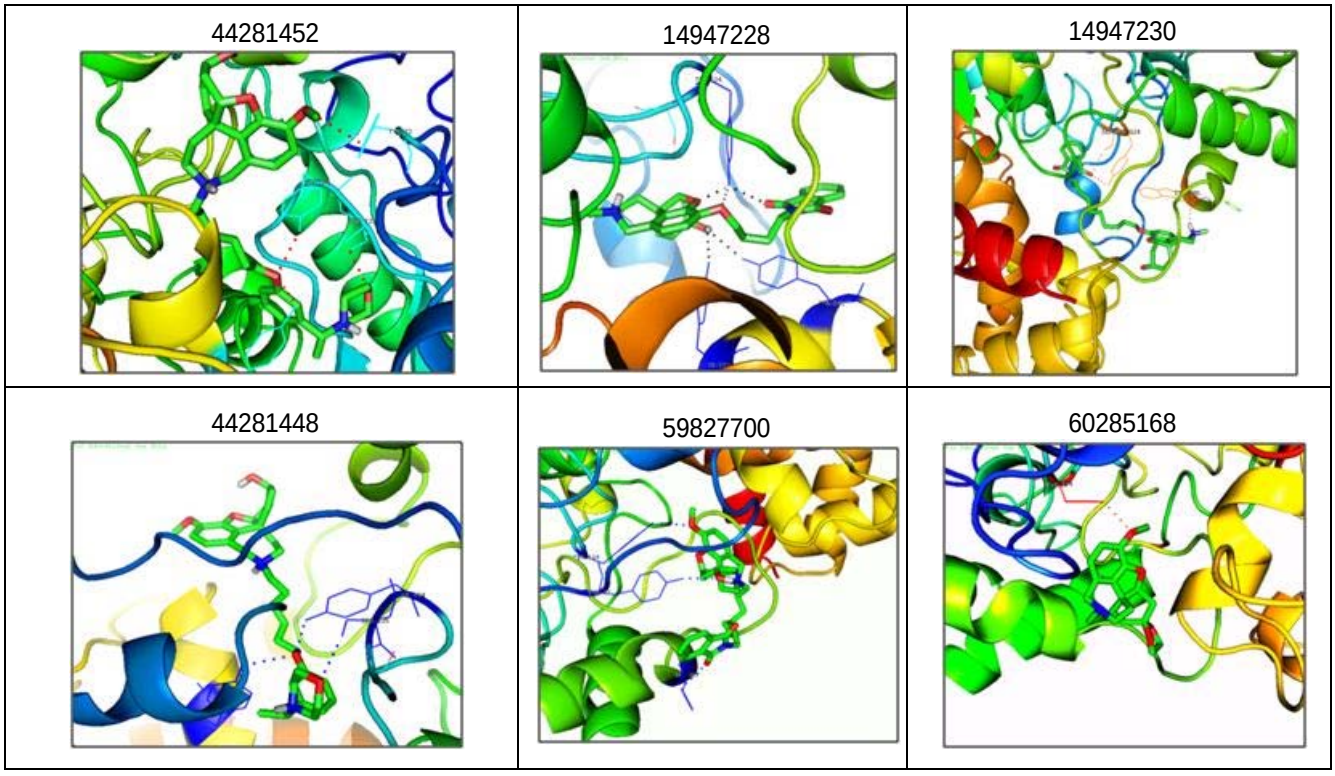


Table 5: Data generated on biological activity properties for selected analogues by Osiris.

S.no	Drug name	C log p	Solubility	Molecular weight	Druglik Eliness	Drug score
1.	44281452	-0.13	-0.88	172.0	1.07	0.68
2.	14947228	3.79	-4.35	492.0	-9.01	0.13
3.	14947230	6.51	-6.18	516.0	-13.53	0.06
4.	44281448	1.61	-2.98	411.0	4.42	0.08
5.	59827700	3.45	-4.3	530	5.2	0.26
6.	60285168	3.31	-3.26	387.0	1.12	0.00

Table 6: Data generated for selected analogues to predict the biological activity through pass.

S.No	Pa	Pi	Activity
1.	0,938	0,003	Prolyl amino peptide inhibitor
2.	0,926	0,004	Alkenyl glycerophospho choline hydrolase inhibitor
3.	0,919	0,005	Methylenetetrahydrofolate reductase (NADPH) inhibitor
4.	0,918	0,004	Sugar-phosphate inhibitor
5.	0,905	0,003	Pullulanase inhibitor
6.	0,888	0,005	Antieczematic
7.	0,880	0,003	Exoribonuclease II inhibitor
8.	0,878	0,007	Chlordecone reductase inhibitor
9.	0,871	0,005	Non mutagenic, Salmonella
10.	0,864	0,002	Fructan beta-fructosidase inhibitor
11.	0,860	0,004	Ribulose-phosphate 3-epimerase inhibitor
12.	0,860	0,009	Benzoate-CoA ligase inhibitor
13.	0,861	0,012	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
14.	0,844	0,005	Membrane permeability inhibitor

Active site prediction by cast-p

From the Cast-p results, we predicted that 1B41 was having the following residues which are present at the protein pocket binding site.

TYR72, TYR-337, TYR-124, SER-125,TYR-124, TYR-337, TYR-341, SER-125, TRP-286, SER-125, SER-124, HIS-287, GLN-71, TYR-72, VAL-74, ASP-74, GLY-88, THR-83, TRP-86, ASN-87, PRO-88, GLY-120, GLY-121, GLU-202, SER-203, LEU-289, SER-293, VAL-294, GLN-396, PRO-368, ARG-296, SER-293, PRO-290, LEU-289, ARG-247, TYR-465,ARG-463, GLU-452, TYR-449, SER-438, LEU-743, THR-436, ALA-434, ASP-131,LEU-130, MET-85, GLY-82, GLU-81, TYR-449, GLY-448, HIS-447, TRO439, PHE-338, ARG-296, PHE-295, VAL-294, SER-293, LEU-289, TRP-286, ALA-204.

Docking studies

In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. 1B41 protein was used to dock with lead molecules by using Auto Dock vina for the fast calculation of interaction between the selected protein and its Ligands. In our study, 6 Ligand molecules showed best binding affinity with 1B41. Among these, 44281452 and 14947228 showed the best binding affinity (-11.0 and -10.7) and hence were suggested as the best lead molecules for inhibition of AChE activity.

Protein Ligand Interaction and Visualization by PyMol

After prediction of docking results, some residues showing interaction with 1B41 protein viz. Tyr-72, Tyr-337, Tyr-124, Tyr-125, Tyr-341, Ser-125, Trp-86, Ser-125, Ser-124, His-287 were identified. Based on the present study, we suggested that these molecules have high interaction with the, protein 1B41 and hence these residues are useful for inhibition of AChE activity.

Bioactivity analysis for Ligands

From the above data, it was obvious that these protein inhibits showed the best binding affinity with AChE protein. The ligand-drug related properties, predicted by using Osiris in this program 44281452 and 14947228 were showing more drug likeness, drugscore, less solubility and no mutagenic properties. Hence, these can act as the best AChE inhibitors.

The output of Pass prediction revealed that Galantamine showed the best biological inhibitory activities on the enzyme, AChE Kinases based on the Structure of the lead molecule.

DISCUSSION

Alzheimer's disease is a multifunctional syndrome with proteins contributing to its etiology. AChE plays an essential role in cholinergic transmission at both the central and peripheral nervous system. Several evidences showed that AChE inhibitors can interface with the progression of Alzheimer's disease. On the other hand, many earlier research findings suggested aggregation of Amyloid beta into amyloid fibrils during the progression of AD. The AChE inhibitors can act through the cholinergic pathway that would cause muscarinic and nicotinic receptor stimulation. Galantamine, a long acting, selective, reversible and competitive AChE inhibitor, is considered to be more effective in treatment of AD and to have fewer limitations. The drug, Galantamine already has a proven efficacy in rodent models of memory impairment[34].

In this present study we developed novel lead molecules of Galantamine derivatives that can potentially interfere with the function of AChE activity. Recent investigations [32, 33], have shown that this enzyme is not only responsible for cleaving the neurotransmitter acetylcholine, but also for β-amyloid plaque formation by promoting the aggregation of this peptide at the peripheral anionic site. This finding makes Galantamine a most valuable drug for

Alzheimer treatment as this interaction with protein enhances the signal transmission of acetylcholine production.

In view of the above, we studied the structure activity relationship aspects also and found out that Galantamine analogs bind to the acetylcholine esterase binding pocket. It was evident that the amino acids present on the Acetylcholine esterase binding pocket are, Tyr-72, Tyr-337, Tyr-124, Tyr-125, Tyr-341, Ser-125, Trp-286, Ser-125, Scr-124, His-287.

CONCLUSION

From our observations on docking studies to predict the binding orientation of small molecule drug candidates to their protein targets, it was proposed that Galantamine is an excellent target for AChE inhibition. In the present study, we concluded that two new potential Galantamine Analogues viz. 44281452 and 14947228 are best inhibitors of AChE in Alzheimer's disease.

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