



DRUG DESIGNING FOR β -SECRETASE: DEVELOPMENT OF A POTENTIAL THERAPEUTIC INHIBITOR FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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Abstract

One of the leading causes of neurodegeneration and memory loss in older people is Alzheimer's disease. In USA alone, it has affected 5.3 million Americans and is the seventh leading cause of death there. In this disease, the brain cells progressively atrophy leading to loss of memory, and a significant change in personality of the affected individual. Sadly, till date, there is no definitive cure for this disease, as it is genetic in nature. Hence, the key to the treatment of this disease is through the means of gene therapy and Recombinant DNA technology. Several studies have taken place in using this approach and as a result, the genetic pathways and associated enzymes leading to the formation of faulty proteins have been identified to a great extent. One such group of enzymes is the secretase group. It has been observed that the β -secretase enzyme belonging to this category is primarily responsible for the formation of malicious protein aggregates in the brain leading to degeneration. In this work, bioinformatical tools have been used to attempt to develop a potential inhibitor for one of the β -Secretase leading to this disease.

Keywords : Alzheimer's disease, Drug Design, Ligand, Inhibitor

Introduction

Alzheimer's is a disease which is neurodegenerative in nature. It usually occurs in old age, typically around 65 years. It causes dementia, which has affected approximately 24 million people globally. Some common symptoms are: cognitive function disorder, significantly altered behavior, impairment of memory which is progressive in nature, paranoia, delusions etc. (Awada *et al.*, 2010; Dey *et al.*, 2017; Anand *et al.*, 2017a 2017b).

One of the defining features of Alzheimer's disease is the formation of plaques, extracellular in nature, in brain. These plaques result in senility, and are formed as a result of β -amyloid peptide aggregation (Sharma *et al.*, 2017; Rani *et al.*, 2017). This β -amyloid has an important function in the disease pathogenesis of Alzheimer's. Protein aggregates are formed in the cortex and hippocampal region of the brain which causes neural degradation. (Hardy *et al.*, 1992; Khurana, 2018; Khatik, 2018; Ali, *et al.*, 2019). Plaques begin forming several years before the disease onset and can be diagnosed clinically only during an autopsy (Dorice *et al.*, 2018). The β -amyloid peptide is formed from a precursor protein known as β -amyloid precursor protein (APP) through a sequence of cleavage events. This cleavage is carried out by an enzyme known as β -secretase followed by γ -secretase activity. Alternatively, APP can also be cleaved by α -secretase. Genetic defects identified include mutations in the Presenilin 1 gene on chromosome 14, the Presenilin 2 gene on chromosome 1, and the amyloid precursor protein (APP) gene on chromosome 21. (Jankowsky *et al.* 2004)

The Role of Secretase Enzyme in Alzheimer's Disease

Secretases are a class of enzymes that cleave off lengthy protein fragments present in the cell membrane. There are three types of secretases; components for secretase, α , β and γ -secretase. (Venu gopal *et al.*, 2010) β and γ -secretases are responsible for cleaving the APP into pathogenic β -amyloid protein which then forms the degenerative plaques. More specifically, the APP is cleaved into three fragments by secretase activity. This is shown in figure 1. At first the β secretase carries out cleavage; then

that fragment is further acted upon by γ -secretase to form the malicious protein. If instead of β secretase, the α -secretase acts first on APP, then no harmful plaque is formed. This is because α -secretase identifies a sequence of the target protein which closer to the cell surface than the portion identified by β -secretase.

The enzyme combo of β and γ -secretase acts on APP to create peptides of varying lengths, namely $A\beta_{40}$ and $A\beta_{42}$. While the $A\beta_{40}$ is soluble in nature, the $A\beta_{42}$ is insoluble and forms plaques. The development of Alzheimer's disease is highly sensitive to ratio between $A\beta_{40}$ and $A\beta_{42}$. The, γ -secretase determines the solubility of the $A\beta$ fragments and hence has a huge potential for drug development. The cleaving activity of α -secretase takes place at the surface of the cell, whereas that of β -secretase occurs at the endoplasmic reticulum. If the cleaving activity happens in the endoplasmic reticulum, then γ -secretase produces $A\beta_{42}$. If it happens in the trans-golgi network, $A\beta_{40}$ is produced. It is shown in figure 2. These three secretases vary widely in structure. While the α -form is a metalloproteinase, the β -secretase (BACE) is a transmembrane protein γ -secretase is an integral membrane protein.

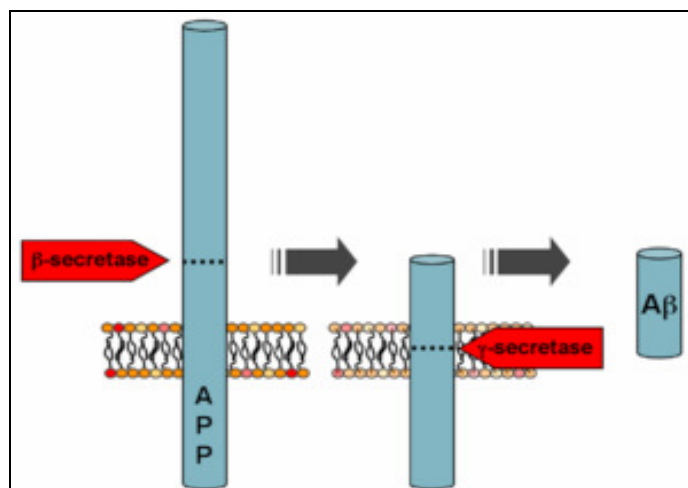


Fig. 1 : Amyloid- β -Amyloid being created from amyloid precursor protein (Venugopal *et al.*, 2010)

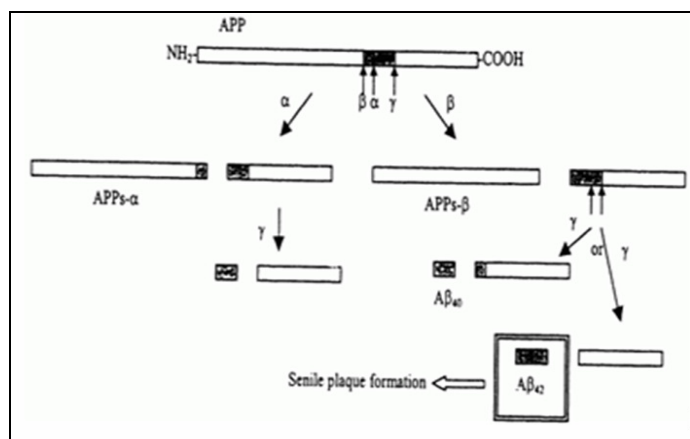


Fig. 2 : Mechanism of action of Secretases involved in the formation of senile plaque (Cole *et al.*, 2007)

β-secretase as a Therapeutic Target

As is clear from the above discussion, β-secretase is responsible for the amyloid β protein accumulation in brain leading to Alzheimer's disease. As a result, it is an attractive target for therapeutic purposes for the development of inhibitor drugs. In this work, it has been attempted to employ structure based drug design to develop a potent inhibitor of β-secretase enzyme from a list of candidate molecules serving as ligands.

Materials and Methods

As is clear from the above discussion, β-secretase is responsible for the amyloid β protein accumulation in brain leading to Alzheimer's disease. As a result, it is an attractive target for therapeutics. (Guner *et al.*, Igarashi *et al.*) Using a variety of bioinformatics tools and databases such as RCSB PDB, ZINC DATABASE, PyMOL, PMAP, PATCHDOCK, DRUG BANK, MARVIN SKETCH and MOLINSPIRATION, the following drug designing steps were taken for inhibitor development (Berman *et al.* 2008; Maier *et al.* 2011; Leach *et al.*, 2007; Madsen *et al.*, 2002).

1. The PDB file was downloaded of the target from the RCSB PDB link "Download Files" on the top right corner of the description page.
2. The protein (1FKN) was viewed and analyze in PYMOL. Here, it exists as a complex of the protein (dimer).
3. Only one chain of the protein complex was selected excluding the inhibitor.
4. It was saved by the name "TARGET.pdb".
5. The active site of target (PDB ID-1FKN) was found using PMAP.
6. All the known structures of small molecules which bind to the same active site of target was found through DRUG BANK. The pdb files of these molecules were downloaded.
7. The SMILES format of the small molecules was found from ZINC DATABASE.
8. Superimposing the various structures in PYMOL, a pharmacophore was designed on the basis of the common structure among the selected structures.

9. The structure of the pharmacophore was drawn in the online tool MARVINSKETCH and saved in the pdb and mol format, so as to obtain its 3D and 2D structure.
10. The SMILES format of the pharmacophore was obtained which can be generated by MARVINSKETCH.
11. In the MOLINSPIRATION window, The SMILES format was pasted and predicted the bioactivity of the molecule. This value is noted in the excel sheet.
12. The pharmacophore was docked with the target to check the binding of it with the target protein.
13. The pharmacophore was modified in MOLINSPIRATION for bioactivity. If it comes higher than the earlier one then the structure is kept or else some other change is done in it.
14. The structure with the higher value of bioactivity was drawn in MARVIN SKETCH so as to save it in the pdb format with the name "Ligand1 .pdb" and the value of the was updated bioactivity in the excel sheet.
15. The Ligand was docked with the target molecule and the results are saved in the concerned folder named as "TARGET WITH LIGAND 1".
16. Steps 13 to 15 were repeated, and draw as many structures which increase the bioactivity of the molecule higher than the earlier one with better docking results (Here, 7 such models were obtained with increasing bioactivity).
17. The Best result is chosen on the overall values of bioactivity (highest), ACE value minimum and can be reported as the potent inhibitor of the target protein.

Results

Step 1:

PDB file of the target molecule (β-secretase) has been downloaded from RCSB PDB link. In this it gives the information about structure of Beta-Secretase complexed with inhibitor. Chain A and B consists of molecule: Memapsin

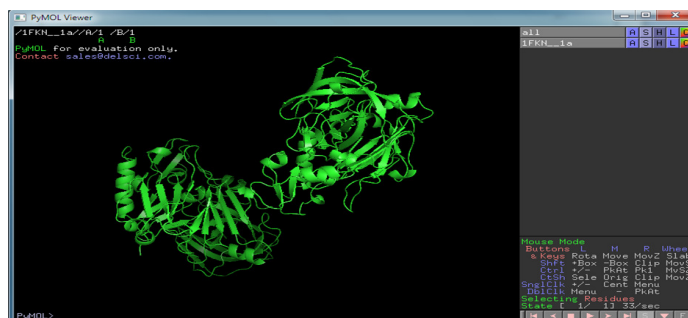
Chain C and D consists of molecule: Inhibitor

Step 2:

In PyMOL viewer the structure of β-secretase has been viewed and analyzed. The protein exists in complex form (Dimer).

Step 3:

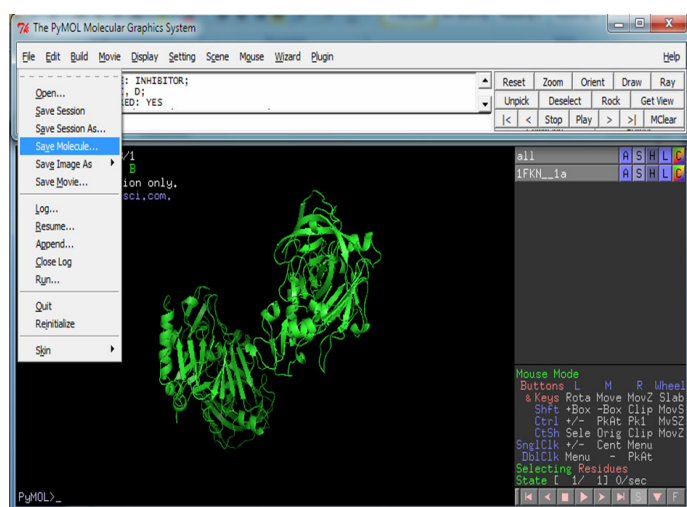
In this step the inhibitor has been excluded from the protein complex. Only one portion of protein is selected (Chain A and B).



Step 4:

HEADER	HYDROLASE/HYDROLASE INHIBITOR	09-AUG-00
1FKN		
TITLE	STRUCTURE OF BETA-SECRETASE COMPLEXED WITH INHIBITOR	
COMPND	MOL_ID: 1;	
COMPND	2 MOLECULE: MEMAP SIN 2;	
COMPND	3 CHAIN: A, B;	
COMPND	4 FRAGMENT: PROTEASE DOMAIN;	
COMPND	5 SYNONYM: BETA-SECRETASE;	
COMPND	6 EC: 3.4.23.46;	
COMPND	7 ENGINEERED: YES;	
COMPND	8 MOL_ID: 2;	
COMPND	9 MOLECULE: INHIBITOR;	
COMPND	10 CHAIN: C, D;	
COMPND	11 ENGINEERED: YES	
SOURCE	MOL_ID: 1;	
SOURCE	2 ORGANISM_SCIENTIFIC: HOMO SAPIENS;	
SOURCE	3 ORGANISM_COMMON: HUMAN;	
SOURCE	4 ORGANISM_TAXID: 9606;	
SOURCE	5 EXPRESSION_SYSTEM: ESCHERICHIA COLI;	
SOURCE	6 EXPRESSION_SYSTEM_TAXID: 562;	
SOURCE	7 EXPRESSION_SYSTEM_VECTOR_TYPE: PLASMID;	
SOURCE	8 EXPRESSION_SYSTEM_PLASMID: PET11A;	
SOURCE	9 MOL_ID: 2;	
SOURCE	10 SYNTHETIC: YES	
KEYWDS	ALZHEIMER'S DISEASE, BETA-SECRETASE, MEMAP SIN 2, BASE, ASPARTIC	
KEYWDS	2 PROTEASE, HYDROLASE-HYDROLASE INHIBITOR COMPLEX	
EXPDTA	X-RAY DIFFRACTION	
AUTHOR	L. HONG, G. KOELSCH, X. LIN, S. WU, S. TERZYAN, A. GHOSH, X. C. ZHANG, J. TANG	
REVDAT	6	13-JUL-11 1FKN 1 VERSN
REVDAT	5	05-MAY-09 1FKN 1 ATOM HETATM SEQRES
DBREF		

After excluding inhibitor the remaining portion of protein has been saved by selecting 'Save molecule' option in PyMOL menu bar as 'TARGET.PDB'

**Step 5:**

For finding active site of target the tool is used named as PMAP. It gives the information about active site of Beta-Secretase which has been found in 1FKN PDB file. It predicts category and active site residues present in protein. The category is Aspartic protease and active site residues are Asp 32, Asp 228. These residues form an active site on protein which allows to design a drug like molecule that bind inside the active site to inhibit unusual effects of β -secretase.

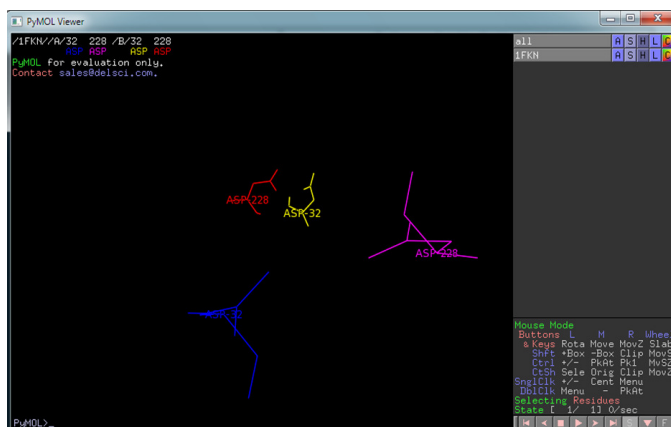
1FKN.pdb

Prediction: aspartic protease

Prediction: aspartic protease

zscore, score = 0.12016 0.89273 # zscore, score = 0.12208 0.89121

Active site residues: ASP32 ASP228 ASP32 ASP228

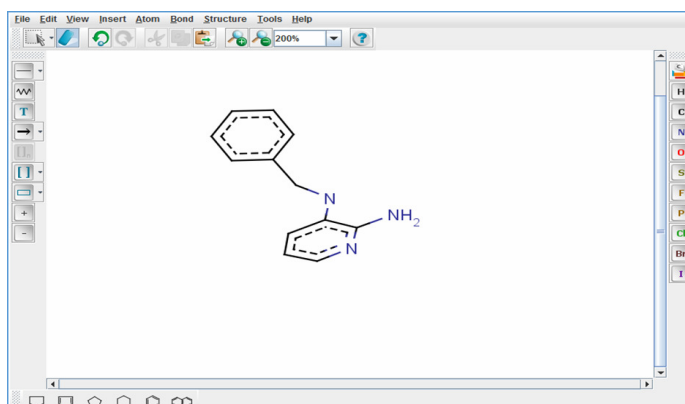
**Step 6-8:**

Drug Bank database is used to find the known structures of small molecules which have been bind on same active site of target. It gives the information about Drug Bank ID, Drug name, Drug type and Drug groups.

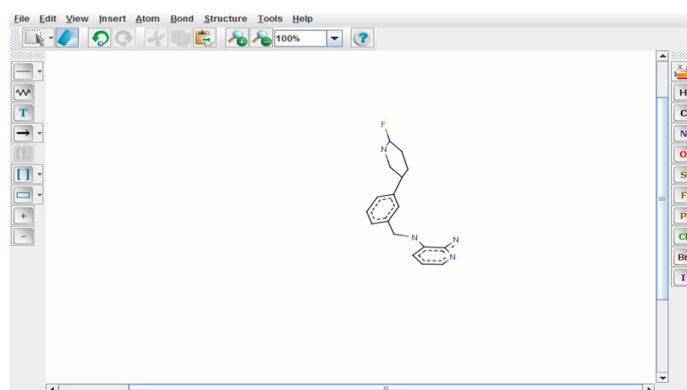
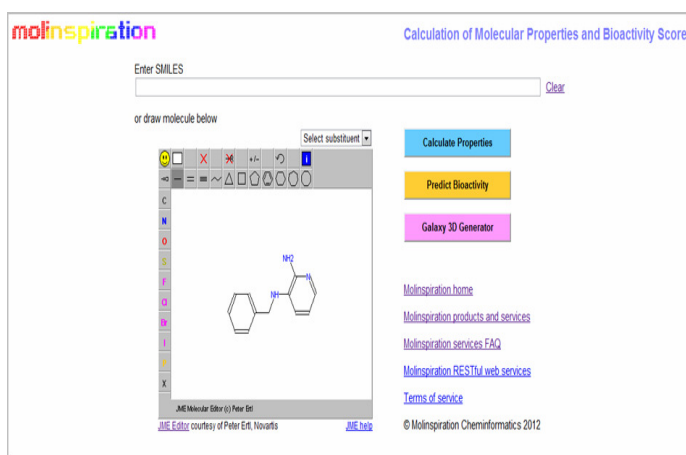
SMIES format for small molecules has been retrieved from ZINC database. After superimposing the various structures in PyMOL, a pharmacophore has been designed on the basis of common structure among the selected structures.

Step 9-10:

The structure of designed pharmacophore has been drawn in the online tool MARVINSKETCH. The structure of molecule has been saved in Pdb and mol format for the ease to obtain its 3D and 2D structure. Also got the SMILES format of the pharmacophore.

**Step 11-13:**

The generated pharmacophore structure has been pasted in the Molinspiration window which predicts the bioactivity of the molecule. It gives the bioactivity score of different receptors. In this the value of enzyme inhibitor is 0.17. Docking of pharmacophore with target gives the binding result in terms of atomic contact energy value. Modification of pharmacophore can be done to get the higher bioactivity.

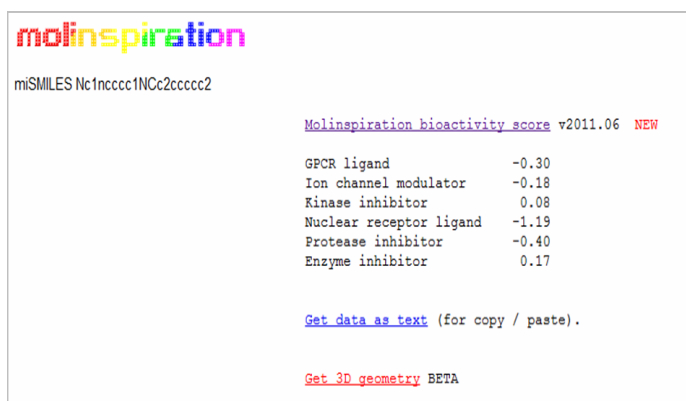
**Step - 15-16:**

Ligand molecule docked with target after modification which has higher bioactivity and minimum ACE (Atomic contact energy) values.

Step 17:-

The docking and bioactivity results are shown in this table. The best one has been chosen from this on the overall values of bioactivity and ACE minimum which can be reported as potent inhibitor of the target.

This table shows the result for selected reference molecules to generate a pharmacophore and for designed inhibitors.

**Step 14:**

Structure of higher bioactivity value has been drawn in MARVINSKETCH and saved in Pdb format with name "Ligand1.Pdb".

Table 1 : Bioactivity and docking

S. No.	Molecule name	Bioactivity	Smiles format	Binding on the same active site (yes/no)	Docking result (atomic contact energy-value)
1	PHARMACOPHORE	0.17	<chem>NC1=NC=CC=C1NCC1=CC=CC=C1</chem>	YES	-181.61
2	LIGAND-1	0.76	<chem>Nc1nc1cccc1NCc1cccc(c1)-c1ccc(F)nc1</chem>	YES	-208.85
3	LIGAND-2	0.63	<chem>Nc1nc1cccc1NCc1cccc(c1)C1=COc2cncnc12</chem>	YES	-238.17
4	LIGAND-3	0.62	<chem>Nc1nc1cccc1NCc1ccc(cc1S)-c1cncnc1</chem>	YES	-288.1
5	LIGAND-4	0.44	<chem>Nc1nc1cccc1NCc1cccc(CC2COC=N2)c1</chem>	YES	-219.33
6	LIGAND-5	0.54	<chem>Nc1nc1cccc1NCc1ccc(ccc1-c1cncnc1)C1=COc2cncnc12</chem>	YES	-224.26
7	LIGAND-6	0.55	<chem>Nc1nc1cccc1NCc1ccc(ccc1Br)-c1cnc2cc[nH]c2c1</chem>	YES	-236.77
8	LIGAND-7	0.67	<chem>Nc1nc1cccc1NCc1ccc2COCc2cc1-c1ccc2C=CNCc2c1</chem>	YES	-219.8

Table 2 : Bioactivity and docking of reference molecules

S. No.	Reference molecules (pdb ids)	Bioactivity	Smiles format	Binding on the same active site (yes/no)	Docking result (atomic contact energy-value)
1	2OHU	0.45	<chem>c1ccnc(c1)COc2ccc(cc2CNc3ccc[nH+]c3N)c4ccc5cc[nH]c5c4</chem>	YES	-298.19
2	2OHT	0.52	<chem>c1ccc(cc1)c2ccc3cc[nH]c3c2)CNc4ccc[nH+]c4N</chem>	YES	-241.31
3	2OHM	0.17	<chem>c1ccc(cc1)CNc2ccc[nH+]c2N</chem>	YES	-197.95
4	2OHS	0.46	<chem>COc1cc(cnc1)c2cccc(c2)CNc3ccc[nH+]c3N</chem>	YES	-198.17

Conclusion and Discussions

Alzheimer's disease is a neurodegenerative disease that, in its most common form, is found in people over age 65. Approximately 24 million people worldwide have dementia of which about 60% is due to Alzheimer's disease. Alzheimer noted two further abnormalities in the brain. The one being senile plaques, a structure previously found in the brain of elderly people (Tiwarly *et al.*, 2014).

Out of the three enzymes (alpha, beta, and gamma secretases) being found, β -secretase is chosen as target molecule on the basis of its function and location in the nerve cells.

A pharmacophore modelling strategy has been followed for designing efficient inhibitor to treat Alzheimer's disease. Initially, a drug like database of small molecules was made and selected the molecules which bind with target on its same active site. These molecules were docked with target using the molecular docking algorithm PATCHDOCK, and the compounds with better binding characteristics were selected for further study. Common feature pharmacophore model was developed on the basis of experimentally known inhibitors. The modifications were made to generate a highly potent inhibitor.

Among all the ligands generated using the pharmacophore for chosen target, LIGAND-3 gives the one of the highest bioactivity and lowest Atomic Contact Energy value. Hence, it can be considered as a potent inhibitor for the target molecule than the existing.

Further biological testing would be necessary to absolutely determine the success rate of this work and optimize the designed inhibitor.

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