In-Silico Drug Identification In The Treatment of Neurodegenerative Disease; Alzheimer

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Abstract:

Alzheimer's disease is a fatal neurodegenerative disorder where neuronal cell die at an accelerated rate. This disease has characteristic pathological findings of senile plaques and neurofibillary tangles along with less production of neurotransmitter acetylcholine. Currently available medication aims at affecting the acetylcholine cycle thereby preventing the breakdown of acetycholine. This offer relatively small symptomatic benefit for some patients but do not slow disease progression. A new approach utilizing the genomic and chemi-informatics tools such as Modelling software tool Swiss Model, docking programs – AUTODOCK, ARGUSLAB and Discovery Studio 2.5 are used to identify a better lead compound which can possibly slowdown the progression of neuronal death by targeting plaque proteins. Beta –amyloid peptides (forming the senile plaques) are formed by action of beta-secretase and gamma secretase enzyme on amyloid precursor protein (APP). The lead compound designed in such a way that it specifically binds to the active site of APP thereby preventing the cleavage of APP to beta-amyloid peptide, eventually preventing the formation of plaques. The lead molecule determined by this approach is intended to overcome the shortcomings of existing drugs against AD progress. 3D structures of the protein was generated using Homology Modelling. Active compounds of medicinal herbs were selected as these herbs have properties of memory enhancement. Chemical structures of the active component of these herbs were accessed using Zinc Database & converted to *.pdb. The protein was successfully docked with active component.

Keywords*:* Alzheimer Disease, Amyloid β protein, Modelling, Lead Optimization, Lipinski Rule, Docking, AUTODOCK 4.2.

Introduction

Alzheimer's disease (AD) is the most common cause of dementia and the fifth leading cause of death among Americans aging 65 and more with an afflicting rate of 70 s [1]. In the absence of effective therapeutic approaches, more than 25 million people would be expected to be affected by AD in the near future [2]. One of the histological hallmarks of AD is the presence of brain senile plaques. The major constituent of these fibrillar plaques is a self-aggregating peptide named β-amyloid peptide (Aβ) [3]. The Aβ is generated by the successive action of two proteases, β- and γ-secretases, on the amyloid precursor protein (APP) [4]. The most abundant variants of Aβ found in AD, are $A\beta_{1–40}$ and $A\beta_{1–42}$ containing 40 and 42 amino acids, respectively. $A\beta_{1–42}$ is the dominant form in senile plaques and forms amyloid fibrils more rapidly than $A\beta_{1–40}$ [5]. According to amyloid cascade hypothesis, the fibrillar Aβ aggregates induce a cascade of events that lead to neuronal cell death [6]. The A peptide is rich in hydrophobic regions of β -sheet structures which forms strong Aβ–Aβ intermolecular β-sheet interactions, leading to fibril formation [7].

Material and Methods

A. Prediction of 3D structure of β-Amyloid

The following software programs and database were used for homology modeling

1. PDB (http://www.rcsb.org/pdb/)

2. NCBI (www.ncbi.nlm.nih.gov/)

3. Swiss Model

4. SAVS server

5. PyMol (Visualization Software)

The basic aim of homology modelling is to build a three-dimensional (3D) model for a protein of unknown structure (the target) based on one or more related proteins of known structure (the templates). The necessary conditions for getting a useful model are that the similarity between the target sequence and the template structures is detectable and that the correct alignment between them can be constructed. This approach to structure prediction is possible because a small change in the protein sequence usually results in a small change in its 3D structure. For generating an initial model of the target protein we used the homology modeling web server tool Swiss Model.

For the prediction of 3D structure of β-Amyloid protein (Homo sapiens), firstly, sequences in fasta format from NCBI site (www.ncbi.nih.nlm.gov/) are taken.The protein sequence is of 770 aa length. The 3D structure for this sequence is modelled by using the Swiss Model.

Steps undertaken during homology modeling:-

1. Taken target sequence of amino acid in
FASTA format.

2. PBLAST search for finding homology to sequences of known structure.

3. 10 BLAST hits were found in which 2 hits were good. Identity for both sequences was found 99% respectively.

4. Downloaded both pdb files.

5. Taken both templates in FASTA format.

6. By taking target and template files in FASTA format, multiple sequence alignment was done by Clustal W.

7. After modelling the 3-D structure of Amyloid β protein validate the structure in SAVES online server and find the quality factor score. After that I am doing docking with this build protein with suitable molecule. The SAVES result shows the modelled protein is of Good quality it shows 92.8% residues in favoured region.

Selection of Compound:

A Research article which is published in the " International Journal of Biomedical and Pharmaceutical Sciences", Based on phytochemical and pharmacological studies carried out there are several phyto-constituents which can be potential drug targets for AD treatment. These substances such as alkaloids, bi-phenolics lignans, curcuminoids, caffeic acid derivatives, diterpenes, Asiatic acid, zeatin, crocin, magnolol, vitamin C and sinapic acid. However there are some phytochemical substances which have already been launched or in the clinical trial phase. It should be also mentioned that these substances, examples of which galantamine and hyperzine A are only being used in the management of AD patients. The compound is extracted from this Research article and in-silico analysis is carried out. Next the similar compounds are extracted from the Zinc database and from the PubChem.

Filtering out Drug like Molecules:

The compounds I took filtered according to anti-amyloid drugs like properties (Molecular weight and Log P values) and selected those molecules which fulfilled the anti-amyloid drug like properties. In order to find the basic range of anti-amyloid drugs like properties, we evaluated their molecular weights and log P values.

After finding the molecular weight and log P values, The compounds applied to the Lipinski rule of five for checking the drug likeliness property.

B. Lipinski's Rule of Five

- Not more than 5 hydrogen bond donors
- (OH and NH groups)
- Not more than 10 hydrogen bond acceptors (notably N and O)
- A molecular weight under 500 g/mol
- A partition coefficient log P less than 5
- Rotatable bonds less than 10

The compound which satisfies the Lipinski's rule of five is taken as drug molecules and docking procedure is carried out.

C. Dock working principle:s

In general docking process can be divided in to two phases. One is the searching algorithm, which finds possible binding geometries of the protein and its ligand. The other is the scoring function, which ranks the searching results and selects out the best binding geometry based on the energies of the complexes. Autodock 4.2 is used for docking.

Working with AutoDock4 includes 3 steps:

1. Preparation of receptor & ligand files.
2. Calculation of affinity maps by using a 3D grid around the receptor & ligand.
3. Defining the docking parameters and running the docking simulation.

The preparation step starts with pdb files of receptor (R.pdb) and ligand (L.pdb), which are added hydrogens and then saved as RH.pdb & LH.pdb. The calculation of affinity maps in the "Grid" section requires the above pdb files to be assigned charges & atom types, and also that the nonpolar hydrogens are merged. This is done automatically by ADT, and the resulting files need to be saved as RH.pdbqt & LH.pdbqt, which is the only format AutoGrid & AutoDock can work with. Calculation of affinity maps is done by AutoGrid, and then docking can be done by AutoDock. The newest docking algorithm is LGA (Lamarckian Genetic Algorithm).

RESULTS AND DISCUSSION

3-D structure of β-Amyloid protein:

The modelled protein of Aβ(1-42) is shown in figure. The modelled protein is visualized by using the PyMol viwer.

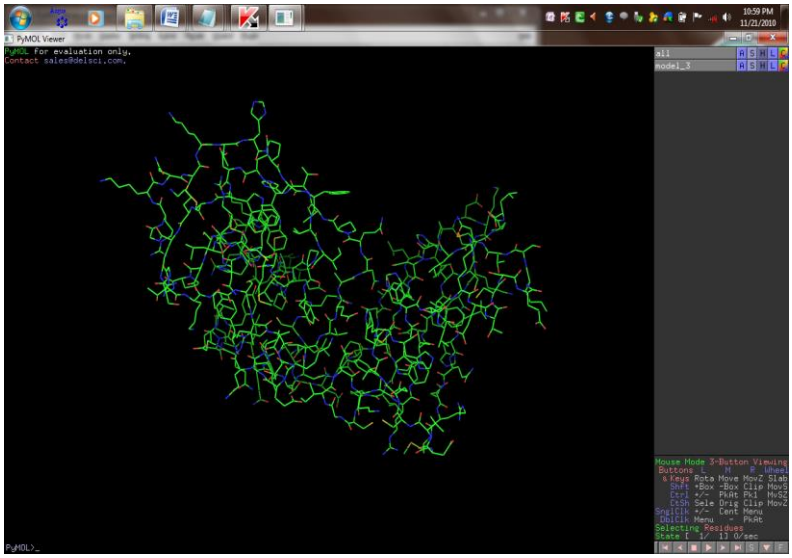


Fig: 1 3D-structure of Aβ (1-42) protein

A. VALIDATION:

Structure validation of above protein was done by the SAVES server. Validation was done for Procheck, What check, and Errat plots. Overall Quality Factor of 3D- Structure of Aβ(1-42) is found 79.7. That means this structure is of good quality.

Ramachandran Plot: The Ramachandran plot shows the phi-psi torsion angles for all residues in the structure (except those at the chain termini).

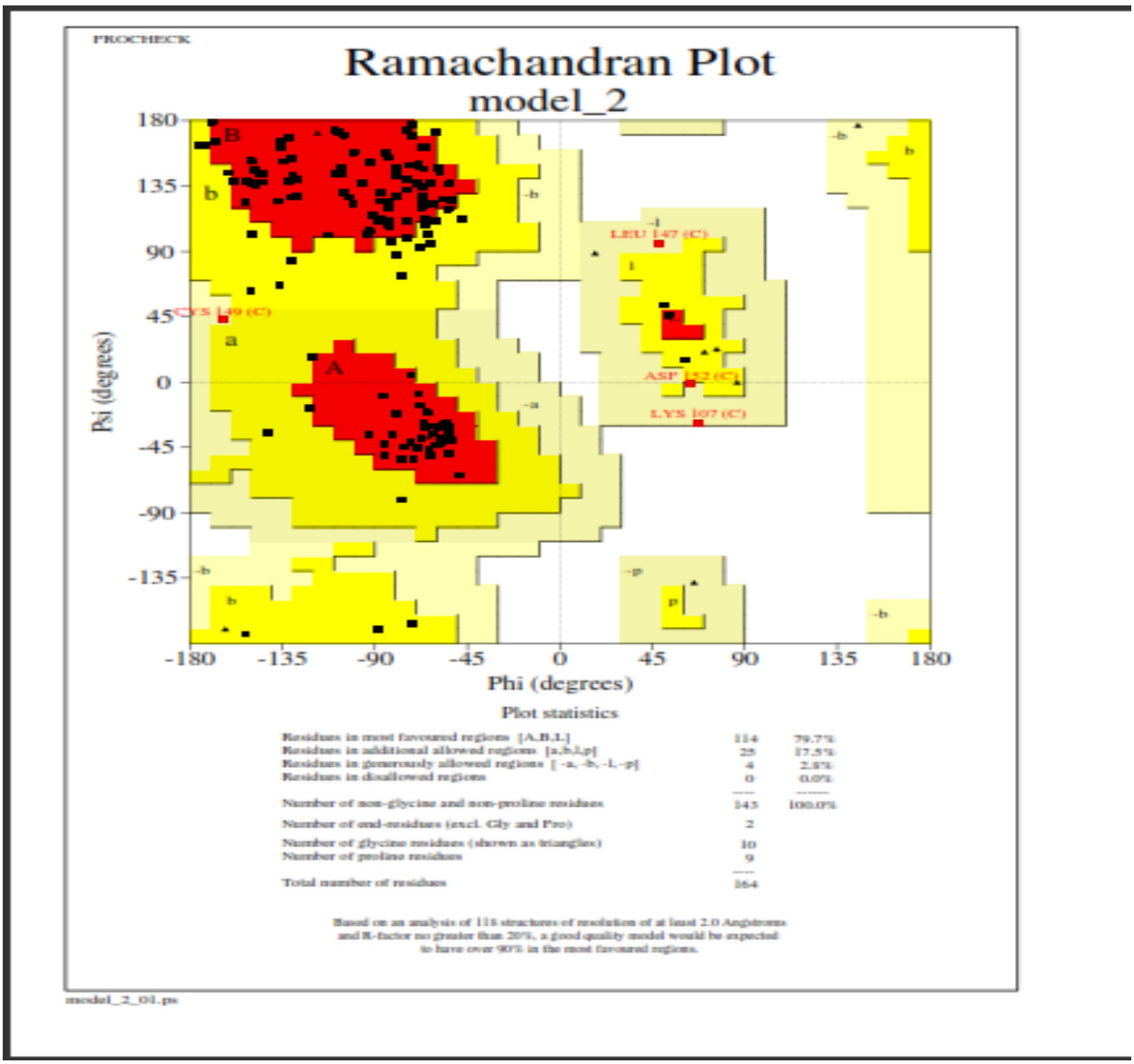


Fig.2:Ramachandran plot for Aβ(1-42) generated by PROCHECK.

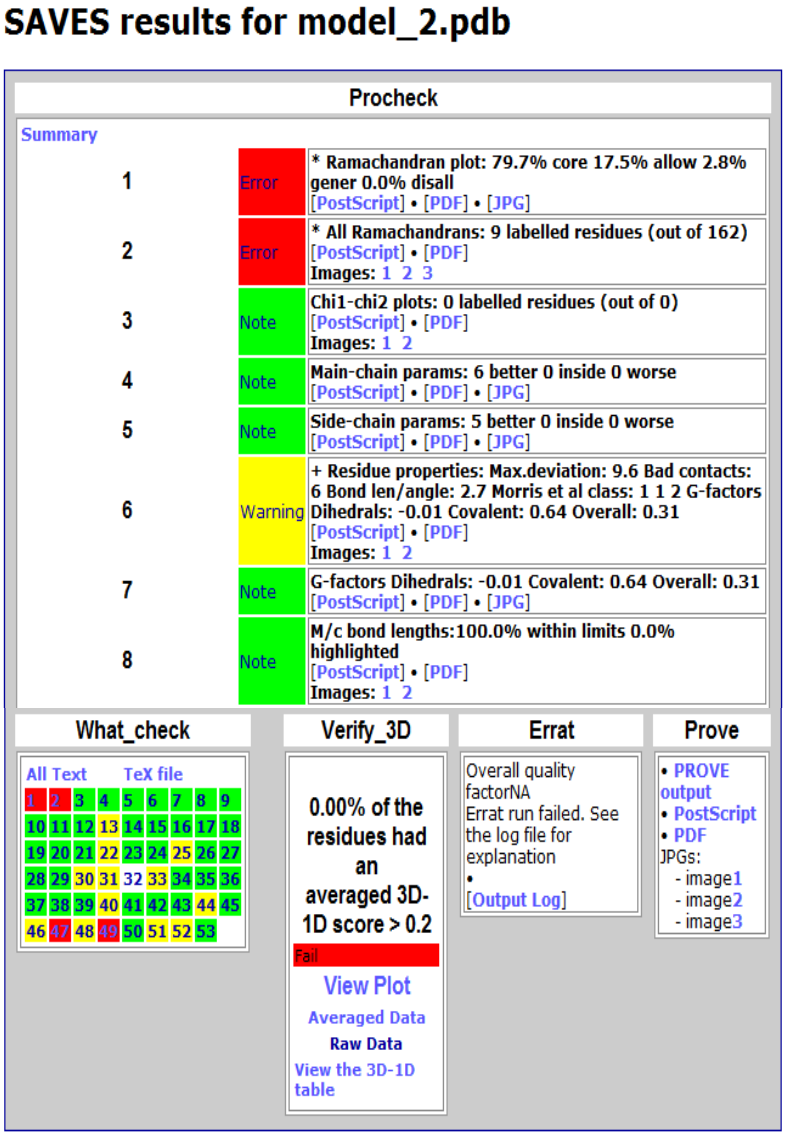


Fig.3 SAVES server result for modelled protein.

The active site of the protein is taken from the CASTp server. After that I am doing docking with this build protein with suitable molecule.

The in-silico analysis is carried out. Next the similar compounds are extracted from the PubChem.

The drug likeliness check for the Pubchem compounds .I make the table of drug likeliness compounds and their molecular weight and LogP value is shown below.

B. ADME analysis result:

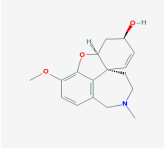
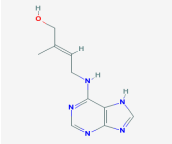
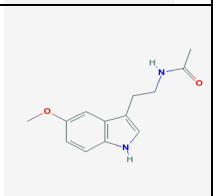
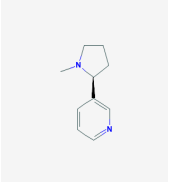
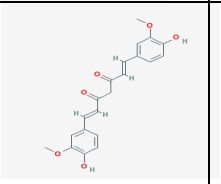
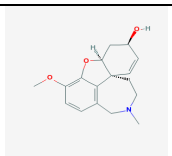
S.No.	Structure	ADME analysis result					
		Compound ID	Mol. formula	Mol. weight [g/mol]	LogP	H-Donor	H-Acceptor
1.		**9651**	$C_{17}H_{21}NO_3$	287.35354	1.8	1	4
2.		**449093**	$C_{10}H_{13}N_5O$	219.24312	0.7	3	5
3.		**896**	$C_{13}H_{16}N_2O_2$	232.27834	0.8	2	2
4.		89594	$C_{10}H_{14}N_2$	162.23156	1.2	0	2
5.		**969516**	$C_{21}H_{20}O_6$	368.3799	3.2	2	6

Table: 1 ADME analysis result.

C. Docking Result

The docking energy for the following compounds is given below. β-Amyloid (1-42) is docked with following compounds and the lowest binding energy is calculated.

S. No.	ADME analysis result		
	Structure	Compound ID	Predicted binding Energy (kcal/mol)
1		**9651**	-6.1951 Kcal/mol

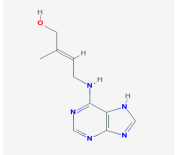
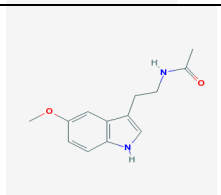
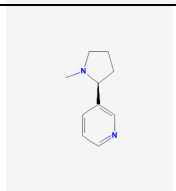
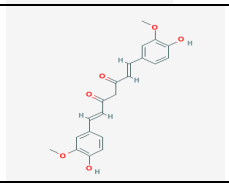
2		**449093**	-7.0828 Kcal/mol
3		**896**	-6.0252 Kcal/mol
4		89594	-3.7302 Kcal/mol
5		**969516**	-8.3432 Kcal/mol

Table: 2 Docking Result

From the above Lowest Binding energy analysis, The two compounds has the best Minimum Energy Those are-

Compound ID	Binding Energy (kcal/mol)
969516	-8.3432
449093	-7.0828

Table: 3 Best compounds lowest binding energy

Their Docking Screenshot is given below-

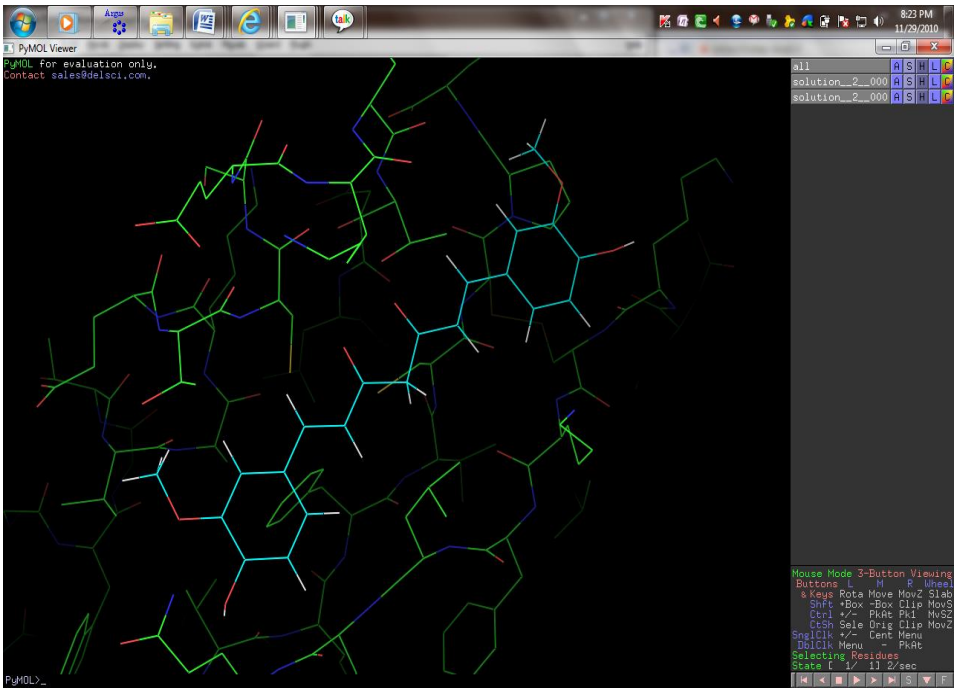


Figure:4 Best out of Two compounds Docking result

DISCUSSION

This in-silico-herbal work makes use of ayurvedic herbs in Computer Aided Drug Designing. The principle outlined in Homology Modelling is used to model the 3D structure of the Amyoid β protein. Since suitable template was not found by searching across pdb database and BLAST search, so the Swiss Model search engine has been used to determine appropriate templates and used them to model the proteins. The mention of herbs Galanthamine, Zeatin, Melatonin, Nicotine and curcumin is found in the works as memory enhancer as they are found to have brain & CNS regenerative property. Hence,forth the combination of the active components of the herbs for docking with the proteins of Alzheimer's disease has been utilized. Again, since the work is done in in-silico platform, the combination needs to go to clinical testing to establish its efficacy.

CONCLUSIONS

The successful docking of Amyloid β protein with phytochemicals proves that the combination can be effective in the treatment of Alzheimer's disease. Again, docking scores as per patch dock server are shown in Table.2.

FUTURE WORK

The future work includes, performing the wet lab experiments, involving chemical synthesis and testing the designed molecules in vivo using specific cell lines would be necessary to arrive at definite conclusions.

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