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Molecular Docking Studies on Evolvulus Alsinoides Compounds Against TAU Protein in Alzheimer's Disease

Bioinformatics

KEYWORDS : Alzheimer's, *Evolvulus alsinoides*, Tau protein, Docking programes.

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ABSTRACT

Tau proteins, involved in Alzheimer's disease are microtubule-associated proteins that are abundant in neurons of the central nervous system. In the present study, the active site of the tau protein was found by using docking programs and software such as Argus lab, AutodockVina, Pymol etc.The ligands against tau protein were taken from the medicinal plant, Evolvulus alsinoides for evaluation of its potential against Alzheimer's disease. The exact confirmation and configuration of the ligand was calculated to find the best molecule with minimum binding energy in order to develop potential drug molecules against the disease. The results showed that among 11 ligands selected, five compounds viz. CID222284, CID5280863, CID439514, CID2497066 and Caffeoyl quinic acid exhibited highest binding affinity values like -8.5, -8.4,-7.9,-7.5 and -8.3 respectively. However, these lead molecules have to be evaluated further before they are suggested as the best potential lead molecules.

Introduction:

Alzheimer's disease (AD) is the most common form of dementia characterized by progressive impairment in cognitive functions such as memory, reasoning, thinking and behavior (Mattson, 2000), which become severe as the age progresses. Most often, AD is diagnosed in people over 65 years of age. By 2050, the number of AD patients is expected to reach 106 million globally (Brookmeyer et al., 2007). The pathological features of AD include neuritic plaques and neurofibrillary tangles (NFT) of hyperphosphorylated tau protein, and neurotransmitter deficit (Nordberg, 2004). Plaques are dense, mostly insoluble deposits of beta amyloid peptide and cellular material around neurons while, the tangles (neurofibrillary tangles) are aggregates of the microtubule-associated protein tau which has become hyperphosphorylated and accumulate inside the cells themselves. Over a period of time, neurons lose their ability to function and communicate with each other, and eventually die. A research report in 2004 showed that deposition of amyloid plaques does not correlate with neuron loss (Schmitz et al., 2004).

AD is also considered as tauopathy due to abnormal aggregation of the tau protein which forms the connector pieces of the microtubule tracks. In neurons of AD patients, tau is changed chemically and can no longer hold the pieces together (Gail et al., 2004). A changed form of a Protein Kinase hyperphosphorylates tau and causes cytoskeleton to collapse which results in collapse of the transport system between nerve cells and finally to neuron death (Singh et al., 2007).

The major constituent of Alzheimer's disease Paired Helical Filaments (PHF) core is Intrinsically Disordered Protein (IDP) tau. In spite of a considerable effort, insoluble characters of PHF together with inherent physical properties of IDP tau have precluded so far reconstruction of PHF 3D structure by X-ray crystallography or NMR spectroscopy. Using monoclonal antibody, MN423 specific to the tertiary structure of the PHF core, the in vivo PHF structure was imprinted into recombinant core PHF tau. Crystallization of the complex led to determination of the structure of the core PHF tau protein fragment 386TDHGAE391 at 1.65 A° resolution. Structural analysis suggests important role of the core PHF C-terminus for PHF assembly. It is reasonable to expect that this approach will help to reveal the structural principles underlying the tau protein assembly into PHF and possibly will facilitate rational drug design for inhibition of Alzheimer neurofibrillary changes (Jozefsevcika et al., 2007).

Since a long time, many herbs have been used as pain relievers and even today we depend on the curative properties of herbal remedies. Brain is a much complex organ of our body. Only a few drugs from plant sources are available for alzheimer's disease. One such plant which shows anti-alzheimer's effect is Evolvulus alsinoides. This plant extract also has exhibits antioxidant and immunomodulatory activities (Gupta et al., 2009).This plant belongs to the family Convulvulaceae. It is commonly known as shankhpushpi (Deepaiyer et al., 2011). It also contains alkaloids viz.betaine, sankhapushpine and evolvine, scopoletin, scopolin, umbelliferone, 6-methory-7-0-ß-glucophyranoside coumarin queretine-3-o-ß-glucophyrenoside (Singh et al., 2008). Two new flavonol-4'-glycoside, kaempferol 4'-O--d glucopyranosyl-(1.2)-.-d-glucopyranoside and kaempferol 4'-O-. -l-rhamnopyranosyl-(1.6)-d-glucopyranoside from E. alsinoides were also reported (Aulakh et al., 1988;Baveja et al., 1969). The structures of the compounds hexatriacontane, octyl octadec-9-enoate, nonyl octadec-9-enoate, dodecanyl octadec-9,12-dienoate, tetracosanyl hexadec-9-enoate, heptacosan-14-b-ol, stigmast-5, 22-dien-3b-ol and stigmata-5-en-3b-ol compounds have been isolated from E. alsinoides aerial parts for the first time, on the basis of spectral data analysis and chemical reactions **(**Sohail et al., 2009**)**. The plant is used in Ayurveda as brain tonic, in treatment of neurodegenerative diseases, asthma and amnesia (Goyal et al., 2005). *Evolvulus alsinoides*L. potential memory enhancing agent used in treating dementia (Satyavati et al., 1996). Many reports shows tau protein is also responsible for AD where it forms the neurofibrillary tangles.

MATERIAL AND METHODS:

The present work was focused on the molecular docking studies on *Evolvulus alsinoides* compound against tau protein, 2V17. pdb. responsible for Alzheimer's disease. The protein sequence was retrieved from NCBI by using URL www.ncbi.nlm.nih.gov and the 3D Structure of the protein from PDB by using URL http://www.rcsb.org/PDB. Ligands against this protein were selected from the plant *(Eolvulus alsinoides*) (Singh et al., 2008; Aulakh et al., 1988;Baveja et al., 1969; Sohail et al., 2009) and they were retrieved from PubChem database. Argus Lab, Cast-P server, Autodockvina (Pyrx), Pymol, Osiris, Pass Prediction were the software's and Bioinformatics tools employed in the present work.

Structural details of the protein, 2V17:

The protein sequence for 2V17, retrieved from PDB is known as Monoclonal Antibody Fab Fragment (MN423). It contains a fragment of Tau protein. The crystal structure of the tau protein (JozefSevcika et.al 2007) contains a complex of 3 biomacromolecules A, H&L contains 6, 222& 214 residues respectively. The protein structural weight is 47905.40. This protein contains two domains and disulphide bonds between l 214- h137 and l 214-h137.

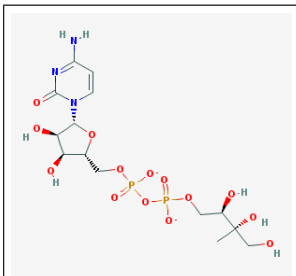
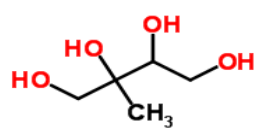
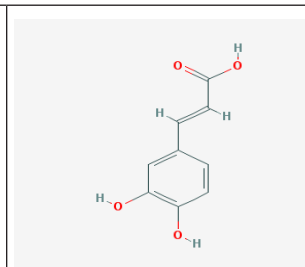
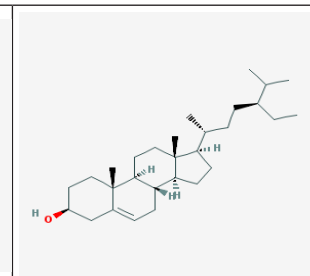
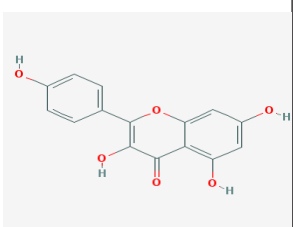
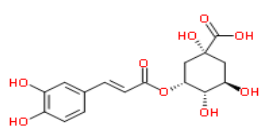
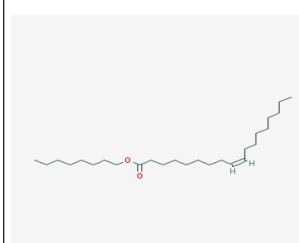
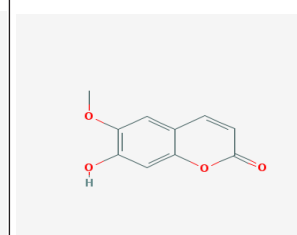
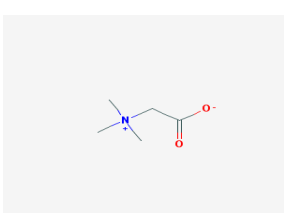
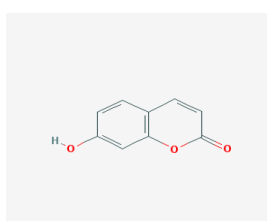
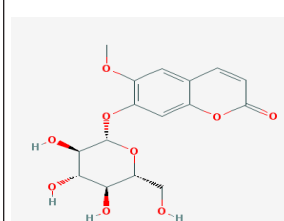
3D Structure of protein 2V17:



Ligands:

Based on the literature, the ligands were taken from the medicinal plant, Evolvulus alsinoides which contains Alkaloids, flavanoids, saponins, tannins and glycosides etc (Om ogbai et al., 2010). These selected ligands were retrieved from PubChem database in 3D.sdf format and Docking studies were carried out on these 11 selected ligands against tau protein as shown below.

Ligands selected for Docking studies:

 2-c-methyl erythritol	 2-Methyl 1, 2, 3, 4-butanetetrol	 Beta-Sitosterol	 Caffeic acid
 Kaempferol	 Caffeoylquinic acid	 Octyl octadec-9-eonate	 Scopoletin
 Betaine	 Umbelliferone	 Scopolin	

Results:

Protein-Ligand interaction plays a significant role in structure based drug Designing. In the present work, we have docked the ligands (β-Sitosterol and kaempferol etc.) with Tau protein (2V17) which is used as one of the target for Alzheimers disease. The Docking studies were carried out through Auto Dock Vina (Pyrx) and based on the highest binding affinity, we Predicted the active site of the protein by using CAST-P server.

Active site Prediction:

CAST-P results showed that 2V17 was having the following Amino acid residues at the protein binding pocket which participate in the protein ligand interactions.

SER-9 TYR-10 GLU-38GLN-39LYS-39PRO-40 GLY-41 PRO-41LYS-42 GLY-42 THR-43 ALA-44ASN-44 PRO-80 GLU-81PHE-83 ALA-84 MET-85 TYR-87 SER-93 ALA-94 ILE-95 TYR-97 ALA-100 GLY-101 THR-102 LYS-103 LUE-104 GLU-106 TRP-112 GLY-113 GLN-114 GLY-115 THR-116 LEU-117 VAL-118 THR-119 VAL-120 SER-121 LYS-142TYR-154 PHE-155 GLU-157 SER-158 VAL-159 THR-160 VAL-161 SER-162 TRP-163 THR-164 ASP-165 GLN-166 ASP-167 SER-168 SER-177 TYR-173 THR-174 PHE-176 ALA-177 LEU-178 LEU-179 TYR-184 MET-186

Docking Studies:

The docking studies will aid in proper understanding of the protein active site, binding mode and interaction of tau protein (2v17) target with ligand molecules and an advanced molecular docking program viz. AutoDock Vina was used in this study. The rotational bonds of the ligands were treated as flexible while those of the protein were kept rigid. Grid boxes were fixed around an active site of the protein. Genetic Algorithm (GA) was

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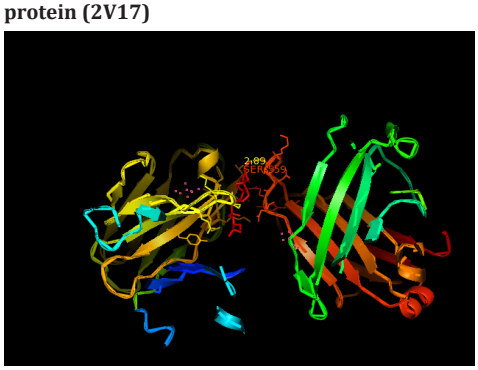
used for searching; the scoring functions for the interactions were calculated and docking evaluation was carried.

Table No: 1 Showing binding affinity between protein (2V17) and the ligands:

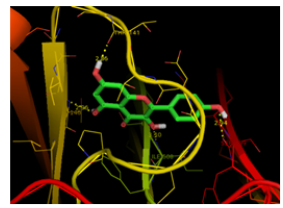
LIGAND	HYDROGEN BONDING			DISTANCE	BINDING AFFINITY
	RESIDUE	ATOM	LIGAND		
1. β – SITOSTEROL	SER-559	O	O	2.89	-8.5
2. KAEMPFEROL	THR-599	O	O	2.94	-8.4
3.	ILE-508	N	O	3.10	
4.	THR-146	N	O	2.96	
5.	THR-141	O	H	2.86	
6. CAFFEOYL QUINIC ACID	THR-141	N	O	3.01	-8.3
7.	THR-141	O	O	2.82	
8.	LYS-598	N	O	2.89	
9.	THR-146	O	O	2.82	
10.	ILE-508	N	O	2.81	
11.	LYS-598	N	O	2.89	
12.	SER-507	O	O	3.21	
13.	THR-146	N	O	3.04	
14. SCOPOLIN	ASP-139	N	O	3.04	-7.9
15.	SER-599	O	O	3.33	
16.	THR-141	O	O	3.11	
17.	THR-141	O	O	3.03	
18.	LYS-598	N	O	3.02	
19.	SER-507	O	O	3.07	
20.	SER-144	O	H	2.94	
21.	THR-146	N	O	3.15	
22.	THR-146	O	O	2.86	
23. 2-c-METHYL ERYTHRITOL	SER-559	O	O	3.03	-7.5
24.	SER-559	O	O	3.11	
25.	SER-559	O	O	2.85	
26.	SER-559	O	O	3.14	
27.	SER-559	N	O	3.07	
28.	GLN-557	N	O	2.94	
29.	ASP-558	O	H	3.23	
30.	ASP-556	O	N	3.32	
31.	ASP-556	N	O	3.20	
32.	GLN-146	O	O	2.85	

Protein ligand Interaction and Visualization by Pymol:
From the Protein-Ligand interaction studies, it was observed that the selected lead molecules showed the best interaction with the following amino acids, SER-559, THR-599, ILE-508, THR-146, THR-141.

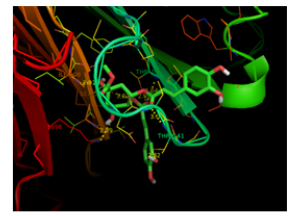
Pictures showing interaction between seleced ligands and protein (2V17)



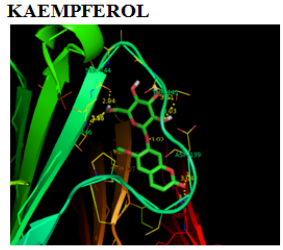
B-sitosterol



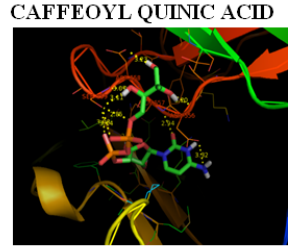
KAEMPFEROL



CAFFEOYL QUINIC ACID



SCOPOLIN



2-C METHYL ERYTHRITOL

Bioactivity analysis for ligands: (Table : 2)
Results obtained from the Osiris output, revealed that β-Sitosterol and Kaempferol showed the best drug related properties such as Solubility, Drug Likeness, and Drug Score etc. Further, we found that they did not show any mutagenic properties when compare to other lead molecules. The results are shown below.

Table No: 2 Data generated on biological activity properties for selected analogues by Osiris

S.NO	Ligand	C log p	Solubility	Molecular weight	Drug likeness	Drug score
1.	B-Sitosterol	8.24	-6.84	426.0	-4.31	0.1
2.	Kaempferol	2.1	-2.79	286.0	0.9	0.22
3.	CaffeoylQuinicacid	-0.44	-1.5	354.0	0.17	0.7
4.	Scopolin	-0.56	-1.90	354.0	-5.11	0.45
5.	2 C-Methyl Erythritol	-5.18	-1.34	462.0	-29.88	0.24

Table No:3 Data generated for selected analogue (β-sitosterol) for the prediction of biological Activity (Pass prediction).

S.NO.	Pa	Pi	Activity
1.	0,957	0,001	Cholesterol antagonist
2.	0,427	0,015	Antiparkinsonian
3.	0,523	0,116	Nootropic
4.	0,395	0,055	Dementia treatment
5.	0,314	0,021	Vascular dementia treatment
6.	0,218	0,051	Neurotrophic factor enhancer
7.	0,177	0,072	Antioxidant
8.	0,377	0,038	Auntituberculosic
9.	0,480	0,259	Antitoxic
10.	0,143	0,020	Beta amyloid protein antagonist

Table No: 4 Data generated for selected analogue(Kaempferol)for the prediction of biological activity (Pass prediction).

S.NO.	Pa	Pi	Activity
1.	0,102	0,057	Beta amyloid protein antagonist
2.	0,959	0,001	Kinase inhibitor
3.	0,864	0,003	Antioxidant

4.	0,261	0,025	Neurotrophic factor enhancer
5.	0,220	0,020	Huntington's disease treatment
6.	0,271	0,072	Antitoxic
7.	0,182	0,019	Amyloid beta aggregation inhibitor
8.	0,217	0,087	Dopamine release stimulant
9.	0,348	0,273	Nootropic
10.	0,040	0,030	Cholesterol absorption inhibitor

Discussion:

In the present study, 11 ligands were selected to dock against Tau protein (2v17). Among these compounds, only five showed the best binding affinity results. From these studies, it is inferred that the β-**Sitosterol** was having the highest binding affinity when compared to other ligands. **Kaemperol** also has significant interaction with Tau protein next to the β-Sitosterol. The docking studies are helpful for understanding the binding mode and interaction of β-Sitosterol and Kaempferol. From the Docking result, β-sitosterol O atom type is bonded with protein atom type SER 559(O) with bond distance of 2.89 and Kaemperol O, N, N, O atom types are bonded with protein residual atoms THR599(O), ILE508(O), THR146(O), THR141(H) with bond distances of 2.94, 3.10, 2.96 and 2.86 respectively.

The lead hit compounds (β-Sitosterol and Kaemperol) satisfied the Lipinski's rule of five with zero violations and also the octanol/water partition coefficient (miLogp), a useful parameter for predicting the drug transport properties like absorption, bioavailability, permeability and penetration. As well as topological molecular polar surface area (TPSA), number of atoms, their molecular weight (MW), number of hydrogen donors and number of hydrogen acceptors. All the five lead molecule followed the 'rule-of-5' and it is found that all the molecules have rotatable bonds in the range of 3-5 except CID5280863 (Kaempferol). CID222284 (β-sitosterol) has satisfied all the parameters with clogP 8.24, solubility-6.84, molecular weight 426.0, Drug likeness -4.31 and Drug score 0.1. The other four compounds also satisfied the above parameters. Our investigation revealed that the selected compounds have exhibited significant binding affinity with the of tau protein (2v17). This insilico approach can be further investigated to generate more effective and potential drug through ligand based drug designing approaches. The above results demonstrated that both β-Sitosterol and kaempferol can be suggested as potential drugs for Alzheimer's.

ACKNOWLEDGMENT:

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