INSILCO STUDIES ON ASTAXANTHIN DERIVATIVES AGAINST TAU PROTEIN- A NOVEL APPROACH TO DESIGN ANTI-ALZHEIMERS DRUG TARGETS

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ABSTRACT: Alzheimer's disease, an irreversible, progressive brain disorder eventually causes neuronal death and thus finally culminating in loss of intellectual and cognitive abilities. Neurodegenerative diseases (NDDs) are traditionally defined as disorders with selective loss of neurons and distinct involvement of functional systems defining clinical presentation. However, the pathophysiology of these diseases have not been fully elucidated and effective treatments are still lacking. The tau proteins, like CDK5 and GSK3β kinases have a significant role in the abnormal hyperphosphorylation of MAPT (Membrane Associated Protein Tau) which causes Alzheimer's disease. In healthy condition, the Tau protein has the ability to bind and neutralize the internal ladder-like microtubule skeleton structure. In diseased condition the ladder-like microtubules are collapsed due to irregular hyperphosphorylation of tau and formation of Neuro Fibrillary Tangle deposits inside the brain, which subsequently results malfunctions in communication between nerve cells and finally neuronal death. In this revision we employed computational method to study the molecular interaction of Tau protein with algal derivatives of Astaxanthin and its analogues. The active pocket binding site Amino acids residues were predicted through CAST-p server and docking analysis was performed by using Auto Dock Vina tool. The 1300 Astaxanthin drug derivatives were downloaded from ZINC database as targets. Among all these, the compound ZINC 05281539 showed high binding energy interaction value i.e.-11.0Kcal/mol and showed tightly coiled protein ligand interaction on protein surface area. These results may be useful to develop the best anti-Alzheimers drug candidate. However, further investigations on the above compound are necessary to develop potential chemical entities for prevention and treatment of Alzheimer's disease.

Key words: Alzheimers Disease, Tau protein, Auto Dock Vina, Astaxanthin Derivatives etc.

INTRODUCTION:

Alzheimer's disease (AD) is characterized by progressive loss of cognitive functions, linked to marked neuronal death. The signs of AD occur due to the damage of nerve cells in certain regions of the brain such as cerebral cortex, formation of neurofibrillary tangles and senile plaques. Worsening of these nerve cells leads to damage of millions of the signalling connctions between nerve cells on the region of synapses. The neurons participate in the transmission of electric charges, resulting in the release of messages. In diseased condition, there will be break down of this signalling system and formation of abnormal senile amyloid-β plaques [1, 2]. Tau protein interacts with different components of the plaque- producing Amyloid system involved in phosphorylating the microtubule binding protein, tau that contributes to the formation of neurofibrillary tangles, Amyloid Plaques and further can influence presenilin and other AD-associated proteins [3-6].

Astaxanthin is an organically occurring carotenoid pigment responsible for such natural wonders as the pink colour of flamingo feathers and the rosy hue, famed endurance of aquatic animals such as salmon, prawn, and crab [7-10]. Astaxanthin has several essential biological functions including protection against oxidation of essential polyunsaturated fatty acids, protection against UV light effects, immune response, pigmentation, neuronal damage, reproductive behaviour, Anti-aging and improved reproduction[11]. Disturbance of the equilibrium status of pro-oxidant and anti-oxidant reactions in cells can lead to oxidative stress, which generate Reactive Oxygen Species (ROS) and free radicals [12]. It is worth mentioning that astaxanthin can act as a safeguard against oxidative damage through various mechanisms, by quenching of singlet oxygen, scavenging of radicals, inhibiting lipid peroxidation and regulating gene expression related to oxidative stress [13-16].

Oxidative stress is a key facilitator in the pathology of neurological diseases [17-19]. Recent research findings proved that astaxanthin disrupts the formation of dangerous compounds linked to neurodegenerative diseases, including Alzheimers disease. These dangerous compounds viz. *phospholipid hydroperoxides* (PLOOH) accumulate in the red blood cell of individuals with dementia. The astaxanthin reduces PLOOH levels,

protecting cells by preventing the damaging effects of free radicals, Reactive oxygen species and significantly decrease the risk of dementia.

An interesting feature about astaxanthin is, it can cross the blood-brain barrier which makes it available to the eye, brain and central nervous system to alleviate oxidative stress that contributes to ocular disease, condition called Age-related Macular Degeneration (AMD) and neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, stroke, high cholesterol etc. It is strongly suggested that treatment with astaxanthin may be effective for oxidative stress-induced neurodegeneration and a potential candidate for natural brain food.

Present day's insilico molecular docking approaches are routinely used in modern drug design to understand drug–receptor interaction. The most recent phase in the new drug discovery process is utilizing the knowledge of the three dimensional structures of target macromolecules or of related proteins. The same strategy is used in the present study also to know the binding of the marine bioactive compounds to selected enzyme sites since it was already theoretically predicted the way the inhibitors bind to the molecular targets and how specific interactions are important in the molecular recognition [20, 21].

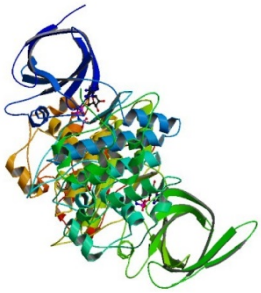
MATERIAL AND METHODS:

Software tools employed for this study:

In this study, we employed various tools and softwares to analyse the 3D structure of protein PDB ID: 1J1B, retrieved from Protein Data Bank (www.pdb.org/pdb/) [22]. The ligand molecules were downloaded from PubChem database [23] Analysis of protein and ligand-hydrogen bonding interaction and visualization studies were carried out by using PyMol tool [24] and protein energy minimization were evaluated by using Argus Lab tool [25]. Then molecular docking studies were done by using Auto Dock Vina and Pyrex [26] protein pocket binding site were predicted by using (CASTp) program (http://cast.engr.uic.edu) [27] and finally ADME properties were determined by using Molinspiration and PASS prediction etc.

Preparation of 3D Structure:

The target protein PDB Code: **1J1B** was retrieved from protein Data Bank and crystallographic water molecules were removed from the protein. All the hetero atoms including water molecules, bound ligands and any co-crystallized solvents were removed from the PDB file of target receptor. The polar hydrogen was added and partial charges were assigned using an Argus Lab force field.



Collection and preparation of ligands:

About 1300 Selected analogues for Astaxanthin were collected from Zinc Database by using key word search. Then these analogues were added with hydrogens, energy minimized with UFF force field using conjugate-gradient algorithm by Pyrex Virtual Screening tool, subjected to against 1J1B protein. Among these 1300 ligands, only 6 compounds viz. CID 05281539, CID14613036, CID06980194, CID14613037, CID0698751 and CID 89033358 showed high binding affinity and best docking score with 1J1B Protein. Hence, these Astaxanthin Derivatives were further used to study the binding interactions with Tau Protein and to perform bioactivity tests.

Molecular Docking:

To predict the best dock score of selected lead compounds dock against Tau protein through Auto Dock Vina tool. The software requires the receptor and ligand coordinates in either Mol2 or PDB format. Non polar hydrogen atoms were removed from the receptor file and their partial charges were added to the corresponding carbon atoms. The receptor PDB file was transformed in to the PDBQS format file containing the receptor atom coordinates, partial charges and solvation parameters. The ligand file was transformed into a PDBQ file, merged nonpolar hydrogen atoms and torsions were defined. The grid calculations were set up and maps were calculated with the program Auto Grid. The grid maps were centered on the ligand binding site and were of dimension 40x40x40 points. The grid spacing was $0.375A^0$ and the default Auto Dock parameter settings were used for docking. All docking runs were performed thrice using the Lamarckian genetic algorithm and the obtained dock

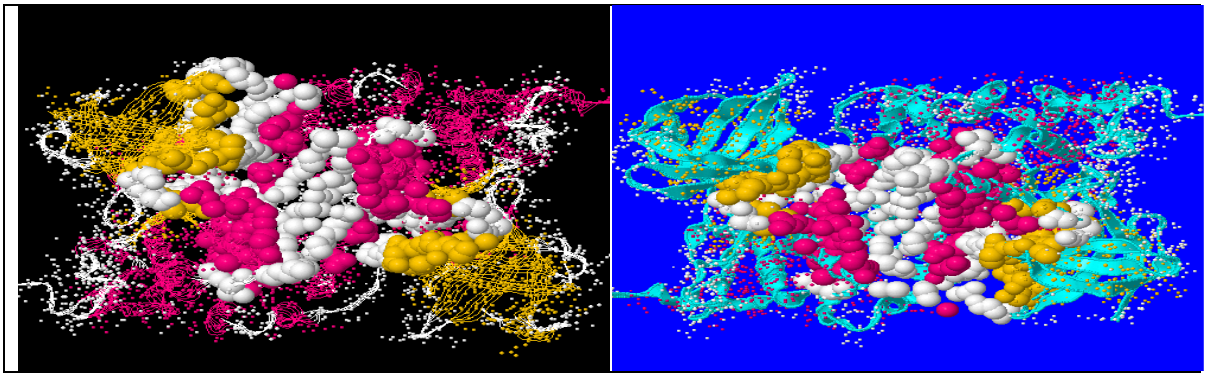
scores were reported in Kcal/mol [28]. The docking protocol utilized in the study consisted of 10 independent runs per ligand using an initial population of 50 randomly placed individuals, a maximum number 2,50,000 energy calculations, a mutation rate of 0.02, a crossover rate of 0.80, and an elitism value of 1. The probability of performing a local search on an individual in the population was 0.06 using a maximum of 300 iterations.

RESULTS:

Active site prediction through CASTp Server:

The CASTp server was used to locate and measure pockets and void regions and to annotate the functional information of specific residues on selected protein 3D structure. The Pymol interaction of selected drug molecules with the Tau protein active sites were predicted by using (CASTp) program as shown in Table.1. The following residues viz. **ASN-689,ASN-686,LYS-585P-700,ASP-700,LYS-585,ILE-562,ARG-96,ARG-180,GLU-97,ARG-96,LYS-585,GLN-89,GLN-795,ASP-700, LYS-585 etc**. were present in the active sites of selected Tau protein.

Picture1: Showing the CAST-p Pocket Binding Sites for TAU Protein 1J1B:



Based on above pocket binding sites of protein, it was evident that these drug molecules were able to tightly coil and bind to any one of the sub sites of selected target protein and thus inhibit tau protein activity.

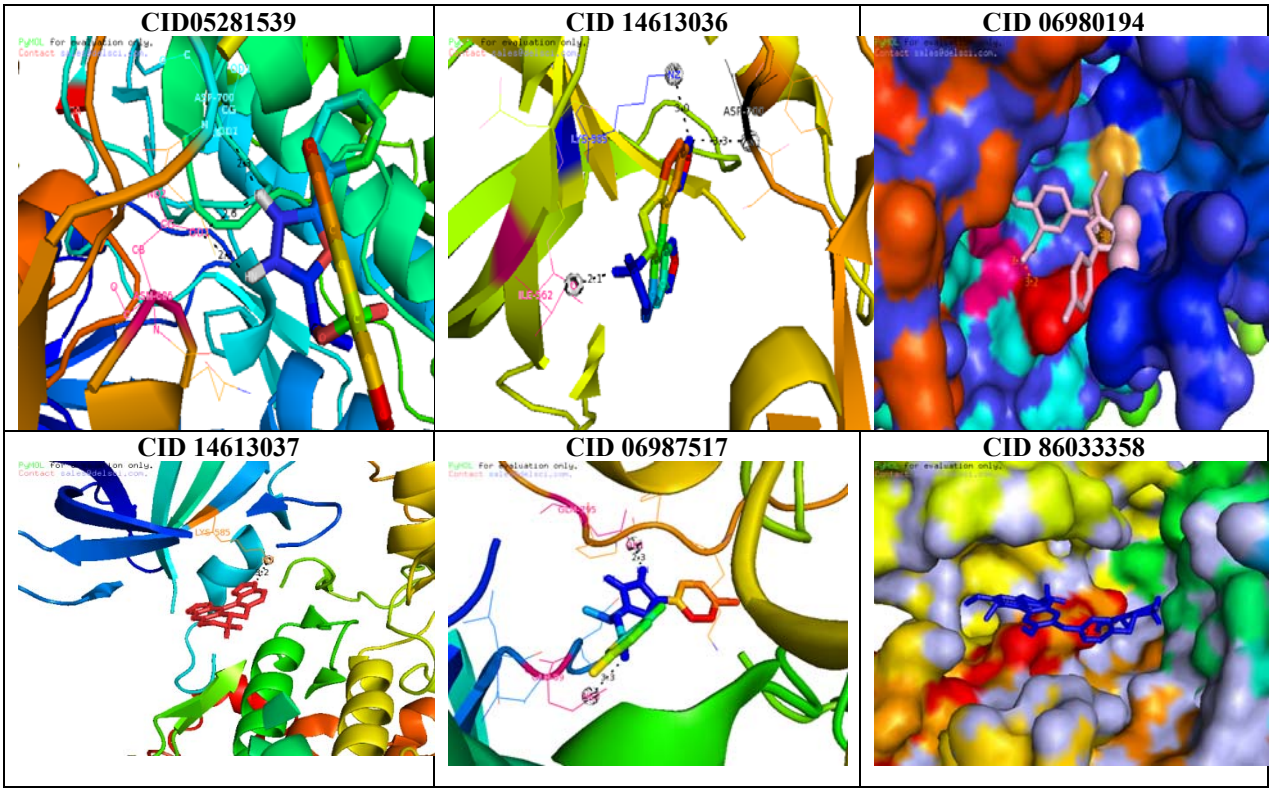
Studies on Hydrogen bond interaction through Pymol:

The hydrogen bond interaction, which contribute as one of the major parameter to understand the possible involvement of hydrogen bond formation with amino acid residues on receptor protein surface was shown below in Table 1&2.

Table 1: Interaction Studies Employed by 1J1B Protein through Pymol

Compound ID	Protein and ligand Interactions	Presented amino acid residues	Bond angle	Docking score
Compound 1 **CID05281539**	OD1----------NH OD1----------NH OD2----------NH	ASN-689 ASN-686 ASP-700	2.3 2.6 2.3	-11.0
Compound 2 **CID 14613036**	N----------O N2----------O O----------H	ASP-700 LYS-585 ILE-562	3.3 3.0 2.1	-10.2
Compound 3 **CID 06980194**	NH1----------O NH2----------O N----------O N----------O	ARG-96 ARG-180 GLU-97 ARG-96	3.2 3.3 3.2 3.1	-10.2
Compound 4 **CID 14613037**	N2----------O	LYS-585	3.2	-10.2
Compound 5 **CID 06987517**	NE2----------O OE1----------NH	GLN-89 GLN-795	3.3 2.3	-10.1
Compound 6 **CID 86033358**	OD2----------H	ASP-700	2.3	-10.1

Table 2: Picture showing the interaction between 1J1B and selected ligand Molecules:



Prediction of ADME properties:

Several physically relevant properties of bioactive compounds from Astaxanthin Derivatives, like Molecular weight, H-bond donors, H-bond acceptors and Log P value according to Lipinski rules performed by Molinspiration tool (Table 3&4). Lipinski rules of five is a thumb to evaluate drug-likeness or determine whether a chemical compound with certain pharmacological or biological activity has the properties that would make it a orally active and important for a drug pharmacokinetics in the human body. In this study all analogs showed values for the properties analysed and exhibited drug-like characteristics based on Lipinski rule of five.

Molinspiration:

Table 3: Data showing Biological Activity Properties for Selected Ligands of 1J1B

Compound ID	Log P Value	Molecular Weight	No. of atoms	Hydrogen Bond Donors	Hydrogen Bond Acceptors	TPSA Value	Volume
CID 05281539	3.45	394.43	30	5	6	65.23	349.46
CID 14613036	4.21	324.36	24	5	1	41.37	281.32
CID 06980194	4.15	396.40	29	1	9	123.08	347.13
CID 14613037	3.85	324.36	24	5	1	41.37	281.32
CID 06987517	4.42	336.39	25	5	0	65.23	311.16
CID 86033358	2.31	610.75	45	8	4	86.26	566.78

Table 4: Data showing Biological Activity Properties for Selected Ligands of 1J1B

Compound ID	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
CID 05281539	-0.28	-0.37	-0.19	-0.31	-0.24	-0.23
CID 14613036	0.52	0.22	-0.03	-0.18	-0.00	0.21
CID 06980194	-0.40	-0.47	-0.34	-0.52	-0.42	-0.35
CID 14613037	0.52	0.22	-0.03	-0.18	-0.00	0.21
CID 06987517	-0.35	-0.51	-0.28	-0.37	-0.36	-0.35
CID 86033358	0.08	-0.52	-0.40	-0.38	-0.06	-0.26

PASS Prediction:

S. No	Pa	Pi	Biological activity
1	0,242	0,239	**Dementia Treatment**
2	0,244	0,191	GABA aminotransferase inhibitor
3	0,525	0,056	Platelet aggregation stimulant
4	0,582	0,098	Membrane permeability inhibitor
5	0,359	0,065	Acetylcholinesterase inhibitor

By employing suitable with computer program it is possible to predict the Activity Spectra along with the biological activity for a compound on the basis of its structural formula. PASS predicts 3678 pharmacological effects, mechanisms of action, Mutagenicity, Carcinogenicity, Teratogenicity and Embryo toxicity. In the present study, all the best inhibitor compounds were analysed for their activity spectra using PASS. Following this, the ADME properties viz. Molecular weight, H-bond donors, H-bond acceptors, TPSA and Log P value for these six drug compounds were analysed according to Lipinski rules five (Table 3&4) through Molinspiration tool.

DISCUSSION:

Microtubule Associated Protein Tau (MAPT) was coined to be an abnormal hyper phosphorylated protein associated with Alzheimers Disease. Previous research data reported that CKD5 and GSK3β are the two important regulation kinases involved in the hyper phosphorylation of MAPT [29]. In this study, we have investigated various insilico strategies to find the role of GSK-3β kinases involved in hyper phosphorylation of Membrane associated Protein, Tau in Alzheimers Disease.

In this docking study, the Tau protein homosapiens PDB ID (1J1B) was taken as receptor for the binding site analysis with the marine bioactive compound from Astaxanthin derivatives. CASTp server predicts the number of active pocket binding sites and their residues presented in the receptor surface. Results on docking studies performed on Astaxanthin and its analogues, collected from ZINC database, it was noticed that out of 1300 analogues, only six were selected possessed for further studied based on their highest docking scores which were in between -11.0 to -10.1 Kcal/mol (Table 1). The hydrogen bond interaction plays a key role to predict the amino acid residues presented in between selected target protein region and ligand molecules. The hydrogen bond interaction region through Pymol software showed a group of polar residues such as ASN-689, ASN-686, ASP-700, LYS-585, ILE-562, ARG-96, ARG-180, GLU-97, GLN-89, GLN-795 etc. located on the binding cavity of GSK-3β (1J1B). Our docking results with all selected ligand molecules showed that almost all drug molecules were involved in hydrogen bonding interaction with the above mentioned amino acids in the binding region. In hydrogen bond interaction of the astaxanthin derivatives all exhibited the best binding affinity with tau protein void region. Among all these derivatives, CID 05281539 showed highest docking score (-11.0 Kcal/mol) and tightly bound on the protein surface area. The H-bond interactions between atoms OD1----NH, OD1----NH, OD2----NH with their amino acids are ASN-689, ASN-686, ASP-700 and their respective bond angles are $2.3A^0$, $2.6A^0$, & $2.3A^0$. Based on these results, it was inferred that the drug molecule will interact with the active sites of the Tau protein and inhibit the activity of tau protein segregation and tangle formation involved in Alzheimers Disease. Further, ADME properties such as Molecular weight, H-bond donors, H-bond acceptors, TPSA and Log P value of these six drug compounds analysed according to Lipinski rules five (Table 3&4) through Molinspiration tool. The PASS prediction results on the target drug molecule established their therapeutic biological activity like treatment of Dementia, inhibitory nature of Acetylcholinesterase, GABA, aminotransferase, membrane permeability, stimulator of platelet aggregation etc., (Table 5). On the whole, the overall results summarized from various insilico analyses suggested that the compound CID 05281539 is strongly involved in inhibition of Tau hyper phosphorylation with reference to Alzheimers Disease.

CONCLUSION:

The present insilico Molecular docking studies on the phytoconstituent of Marine Bioactive Astaxanthin derivative, CID 05281539 clearly demonstrates that it potentially inhibited the Tau protein hyper phosphorylation activity. Hence, it may serve as a useful lead molecule in developing clinically useful for Tau protein inhibitor. However, further investigations are necessary to confirm the biological activity through in-depth studies develop potential compounds for prevention and treatment of Alzheimers Disease.

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CONFLICT OF INTEREST: Nil

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