RESEARCH ARTICLE

Insilico Binding Studies on Tau protein and PP2A as Alternative Targets in the Treatment of Alzheimer's Disease

Uma Anusha Nukala[1]*, P. Sahithi[2]

[1]M. Tech, Department of Biochemical Engineering & Biotechnology, University College of Technology, Osmania University, Hyderabad, India

[2]Former Assistant professor, University College of Technology, Osmania University, Hyderabad, India

[*]**Address for Correspondence:** Ms. Uma Anusha Nukala, M. Tech, Department of Biochemical Engineering & Biotechnology, University College of Technology, Osmania University, Hyderabad, India

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ABSTRACT- Neurodegenerative diseases (NDDs) are traditionally defined as disorders with selective loss of neurons and distinct involvement of functional systems defining clinical presentation. Alzheimer's disease, being one of the most detrimental neurodegenerative diseases, irreversible, progressive brain disorder with changes in nerve cells, resulting in their death and furthermore leading to the loss of intellectual and cognitive abilities. Despite the commercial availability of few drugs, Alzheimer's disease is the sixth leading causes of death globally and the major public health concern, owing to a constraining coercion for action against it. Since the same, many drug targets were identified of which one among the most potential one's is the Microtubule- associated Tau protein that contributes to the pathological lesions of Alzheimer's disease, the Neurofibrillary Tangles or Paired Helical Filaments. Believing that preventing the formation of these pathological lesions, the Neurofibrillary Tangles, is much more a prominent strategy, in the present study, two approaches one approach plying compounds belonging to the class of Cannabinoids and the other approach employing a set of small molecules were used, with the help of Bioinformatics tools, such as Discovery Studio in designing the therapeutic molecules that can obliquely and potentially combat against these Neurofibrillary Tangles or NFTs and actuate to reduce their content in neurons, which might result in an improved communication and thereby enhancing the condition of the diseased person.

Key-words- Alzheimer's disease, Cannabinoids, Cognitive abilities, Discovery Studio, Neurofibrillary Tangles, Tau protein

INTRODUCTION

Of all the devastating infirmities prevailing the world contemporaneously, brain related disorders have been proven to be an enormous disease burden in terms of human suffering and economic cost. Peculiarly brain disorders termed as "Neurodegenerative diseases/disorders (NDDs)" that involve the progressive loss of structure or function of neurons, including death of neurons, are the most fatal ones.[1] One among such deleterious NDDs is the Alzheimer's disease which is an irreversible, progressive brain disorder related to alterations in nerve cells that result in their death and furthermore leading to the loss of intellectual and cognitive abilities, such as thinking, remembering and reasoning, that is excruciating enough to interfere with daily functioning.

Alzheimer's disease being the sixth leading cause of death with as high a mortality rate as 1 in every 3 seniors dies, the prevalence and incidence projections denote that the number of people with Alzheimer disease will perpetuate to grow, categorically among the oldest old, and countries in demographic transition will experience the greatest magnification. The total number of people with AD ecumenical in 2010 is estimated at 35.6 million and is projected to proximately double every 20 years, to 65.7 million in 2030 and 115.4 million in 2050. Much of the incrementation will be in developing countries, with the most rapid magnification in the elderly population taking place in China, India, and their South Asian and western Pacific neighbours. In 2010, Europe had an estimated 10 million disease cases and predicated on Amalgamated Nation's demographic forecast this figure will elevate to 14 million in 2030. It is projected that by 2050, people aged 60

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and over will account for 22% of the world's population with four-fifths living in Asia, Latin America or Africa. [2]

Visually examining these data, it is ostensible that there is an imperative duress for action owing to the fact that the Alzheimer's disease has become a major public health concern as the world's population ages. For the same, many drug targets were identified of which one among the most potential one's is the Tau protein, which usually has a certain number of phosphate molecules attached to it, binds to microtubules and appears to stabilize them.

One of the major hallmark of Alzheimer's disease is the abnormal state of the Microtubule – associated protein tau in neurons. It is both highly phosphorylated and aggregated into Neuro fibrillary tangles or paired helical filaments, and it is commonly assumed that the hyperphosphorylation of tau causes its detachment from microtubules and promotes its assembly into NFTs which will further lead into Alzheimer's disease. Several studies consummated showed that this hyperphosphorylation is carried out by several kinases viz., CDK5, MARK, PKA, MAPK, GSK3 which have been targeted for inhibition in the interest of providing a therapeutic strategy to Alzheimer's disease.[3-5] However, this might affect other physiological processes as these kinases play a key role in several of them. Hence, as an alternative strategy, targeting this microtubule associated protein Tau and making it unavailable for these kinases might provide a better therapeutic approach to combat Alzheimer's disease. In this study, as for one task, 10 natural compounds belonging to the class of Cannabinoids found to be having a neuroprotective activity were taken and subjected to protein-ligand docking studies to obtain a best compound. [6-8]

As an additional novel approach to the Alzheimer's disease therapy, the focus of the therapeutic strategies of second task has been aimed at developing molecules to enhance the activity of the principal phospho-tau phosphatase, protein / phosphoprotein phosphatase 2A (PP2A) which is a major serine/threonine phosphatase, whose function relies on proper activation of PP2A catalytic subunit (PP2Ac) accounting for a plethora of cellular functions majorly including the de-phosphorylation of the hyper-phosphorylated tau protein. More than a dozen small molecules known to show a similarity to several PP2A activators in cellular assays have been identified, but it is unclear whether they act on PP2A directly or indirectly. [9] Among such cell-based molecules, some physiologically available molecules have been selected and tested for their activity to activate PP2A by subjecting them to protein ligand docking studies. [10]

Although, there are a few drugs commercially available so far, these drugs are not devoid of their side effects. Hence, this in-silico study is an attempt to detect more physiologically suitable and compatible therapeutic molecules with least possible side effects.

MATERIALS AND METHODS

Materials

Uniprot (http://www.uniprot.org/), Protein Data Bank (http://www.rcsb.org/), RAMPAGE Server (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php), and Pubchem (https://pubchem.ncbi.nlm.nih.gov), which are the online servers / databases and the Accelrys Discovery Studio 2.5, which is a suite of software for the drug design and discovery process, have been employed to carry out the tasks of the study to completion at Averin Biotech Pvt Ltd, Hyderabad, India during the period from April, 2015 to November, 2015.

Methodology for First task

Target Protein Structure Preparation

With the target protein being Tau (human) protein for the first task, the Uniprot is searched for all the available Tau (human) protein structures of which structures of the Tau (human) protein with Accession number entry as "P10636" have been submitted to RAMPAGE server for Ramachan-dran Plot Analysis that yielded the best protein structure bearing PDB ID: "4FL5", which was downloaded from PDB for protein preparation. Thus, the obtained Tau protein structure is loaded into the 3D-Window of *Discovery Studio* 2.5 and then submitted to protein modelling by enabling the parameters viz., Incomplete residue, Atom order, Add hydrogens, Valency, & Charge, which is then subjected to energy minimization to obtain a stable configuration by applying "Forcefield" and "Simulation" with the aid of "Steepest Descent" and "Conjugate Gradient" minimization algorithms which are repeated until the minimization criteria shows a "Gradient Tolerance Satisfied" message indicating that the protein has reached a stable configuration which is then saved in '.mol' format.

Identification and construction of Active site spheres

The final minimized Tau protein structure with PDB ID: "4FL5" was then loaded into a 3D window for the identification and construction of active site spheres by selecting the "Receptor- ligand interactions" tool and applying "Forcefield: CharmM", followed by selecting the "Binding Site" and then the "Define selected molecule as receptor" to "Define active site spheres". Several active sites were generated among which the active site that was close to the ligand of the selected protein structure was selected and site spheres were constructed around the selected active site (Fig. 1).

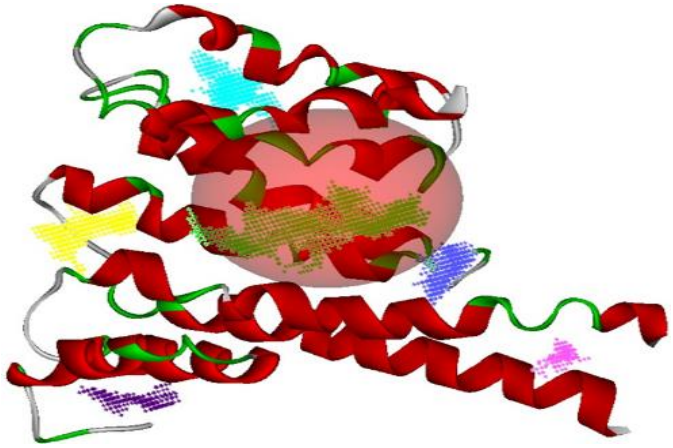

Fig. 1: Active sites of minimized tau protein identified using discovery studio

Ligand Preparation from Cannabinoids

The natural compounds, namely CANNABICHROMENE (30219); CANNABICYCLOL (Cannabipinol) 30607; Cannabidiol (644019); Cannabidivarin_11601669; Cannabinol (2543); Cannabitriol (11551959); Cannabivarin (622545); Delta9-Tetrahydrocannabinol (Dronabinol) 16078; TETRAHYDROCANNABIVARIN (93147); and Tetrahydrocannabivarol_34180 (28172-17-0), belonging to the class of Cannabinoids [6-8], that were found to possess a neuroprotective role, were culled and downloaded from Pubchem server and were then subjected to Ligand preparation procedure using the Discovery Studio, by employing the "Receptor-Ligand interactions" tool and selecting "Prepare Ligands" with "Lipinski filter" selection criteria as "True" which were then employed for further docking process to be conducted.

Docking by Libdock algorithm

Following the preparation of Ligands from the selected Cannabinoids, the Docking studies were then consummated in Discovery Studio, with the aid of Libdock algorithm, by taking the Minimized Tau protein structure with PDB ID: "4FL5" as the Input receptor, the prepared ligands from the selected Cannabinoids as the Input Ligands and the identified and defined active site spheres as the Input Site Spheres.

Methodology for Second task

Target Protein Structure Preparation

The same procedure that has been followed for first task was applied for the Selection of the protein structure, Protein preparation and Energy minimization of the protein for carrying out the second task with the target protein structure being human Protein Phosphatase 2A (PP2A) with the PDB ID: "4LAC" of the Accession entry: "P67775".

Identification and construction of Active site spheres

Considering the presence of ligands in the selected target protein structure i.e., the Protein Phosphatase 2A (PP2A) with the PDB ID: "4LAC" of the Accession entry: "P67775", the active site spheres were defined around the sites of ligand's presence viz., MES and AGS site spheres, by the same procedure that followed for the first task. These Active site spheres are depicted in the below Fig. 2.

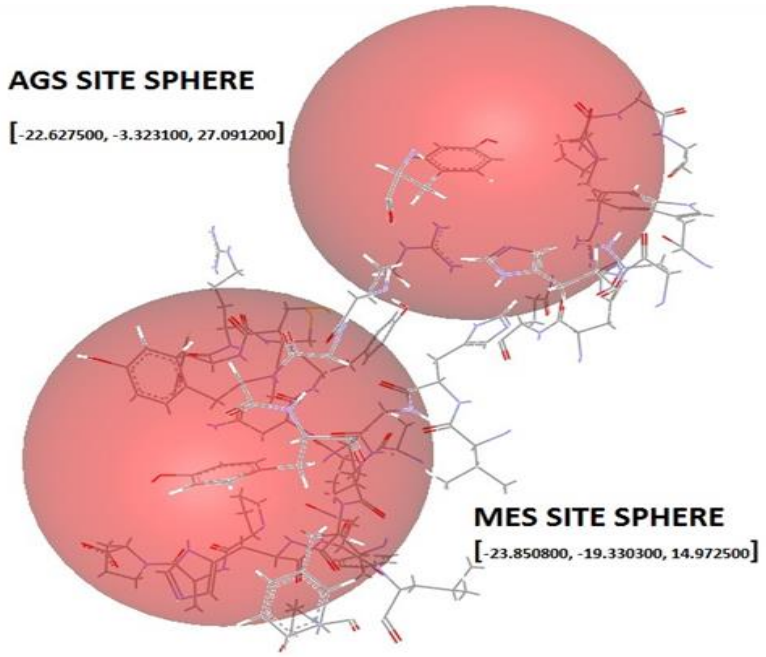

Fig. 2: Active sites of minimized tau protein identified using discovery studio

Ligand Preparation from Small Molecules

Some of the physiologically present compounds, namely 4-Hydroxynonenal; Dithiolethione; Ebelactone B; Epigallocatechine Gallate; Forskolin; Melatonin; Palmitic Acid; Progesterone; Taurolidine; Troglitazone, that were believed to possess an unclear activating effect on PP2A protein were downloaded from Pubchem for testing their activating effect on PP2A in this task.[10] Thus downloaded Pubchem compounds were then screened and employed in the preparation of ligands for further analysis by following the general ligand preparation procedure like that followed for the first task.

Docking by Libdock algorithm

Following the preparation of Ligands from the selected Cannabinoids, the Docking studies were consummated in Discovery Studio, with the aid of Libdock algorithm, by taking the Minimized PP2A protein structure (PDB ID: "4LAC") as the Input receptor, the prepared ligands as the Input Ligands and the MES and AGS site spheres as the Input Site Spheres.

RESULTS AND DISCUSSION

Libdock Docking Results of first task

The protein-ligand docking studies consummated with the aid of Libdock algorithm, with the "11" number of prepared ligands taken as the Input Ligands, the Minimized Tau protein structure with PDB ID: "4FL5" as the Input receptor and the defined active site spheres as the Input Site Spheres, yielded results stating that there were 904 poses of

ligands docked to protein with 1167 generated conformers. The complete docking scores of all the small molecules docked, along with the Interacting amino acids for the active site spheres were tabulated in the following Table 1 that yielded in the best Ligand molecule as Cannabitriol with the highest libdock score as "97.614" (Fig. 3).

Table 1: Libdock scores of the minimized tau protein and prepared ligands

Compound Name	Libdock Score	Interacting Aminoacids
Cannabichromene (30219)	83.536	Lys122, Asp126
Cannabicyclol (Cannabipinol) 30607	78.975	Lys122
Cannabidiol (644019)	83.731	Asn175, Ser45, Asp126
Cannabidivarin_11601669	76.929	Tyr130, Asn175
Cannabinol (2543)	84.563	Lys49, Lys122
Cannabitriol (11551959)	97.614	Lys49, Arg129, Asn175, Asp126
Cannabivarin (622545)	77.032	Tyr130
Delta 9-Tetrahydrocannabinol (Dronabinol) 16078	83.809	Asn175
Tetrahydrocannabivarin (93147)	78.709	Asn175
Tetrahydrocannabivarol_34180 (28172-17-0)	82.824	Lys49, Asp126, Ser45, Phe119, Lys122, Met123, Tyr127, Leu222

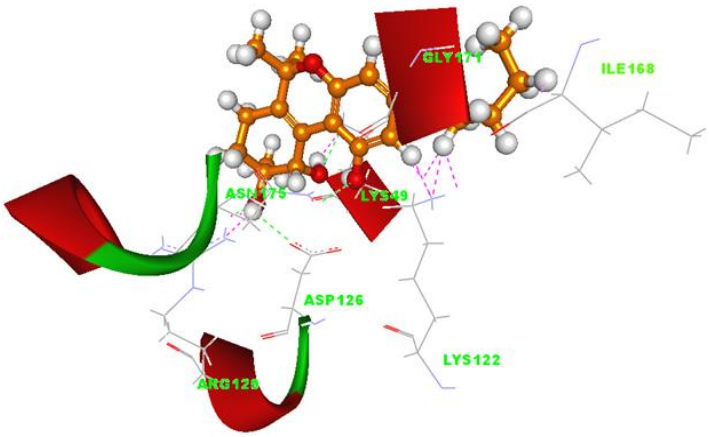

Fig. 3: Cannabitriol with highest libdock score

Libdock Docking Results of Second task

The protein-ligand docking studies consummated with the aid of Libdock algorithm, with the "18" number of prepared ligands taken as the Input Ligands, the Minimized PP2A protein structure (PDB ID: "4LAC") as the Input receptor and the active site spheres defined around the AGS and MES Site Spheres as the Input Site Spheres, yielded results stating that the AGS Site Sphere had 1455 docked poses while the MES Site Sphere had 1613 docked poses with 1547 generated conformers each. The complete docking scores of all the small molecules docked, along with the Interacting amino acids for the MES site sphere were tabulated in the following Table 2 that yielded in the best Ligand molecule as Epigallocatechin_gallate with the highest libdock score as "162.467" (Fig. 4).

Table 2: Libdock scores of the docking process of minimized pp2A protein and prepared ligands at MES SITE SPHERE- [-23.850800 -19.330300 14.972500]- COORDINATES

Compound Name	Libdock Score	Interacting Aminoacids
4-Hydroxynonenal_5283344	80.212	Tyr91, Arg89, Tyr267
Dithiolethione__68296	41.565	Glu67
Ebelactone_B_6436821	134.255	Asp64, Cys266, Glu67, Pro291, His63
Epigallocatechin_Gallate_65064	162.467	Phe62, Gly60, Gln61, His63, Met66, Glu67, Asn264, Tyr267, Ala292
Forskolin_47936	114.234	Tyr267, Gln61, Asp64, His63, Arg89
Melatonin_896	107.082	Asn264, Pro263, Cys266, Tyr91
Palmitic_Acid_985	112.16	Arg70, Asn264, Cys266, Pro291, Gln61, His63
Progesterone_5994	106.759	Asn264, Gln61, Tyr267, Pro291
Taurolidine_29566	94.054	Cys266, Arg89, His63, Tyr91, Gly60, Tyr267, Gln61, Tyr92
Troglitazone_5591	160.817	Tyr267, Asp64, Glu67, His63, Gln61

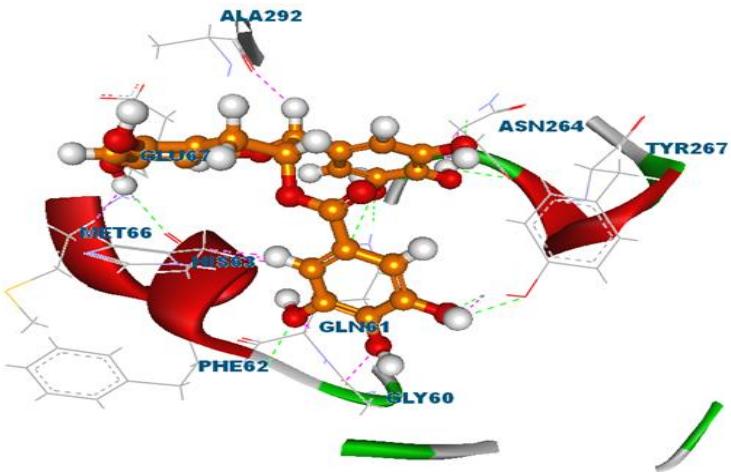

Fig. 4: Epigallocatechin_gallate with highest libdock score 162.467 at MES Site Sphere

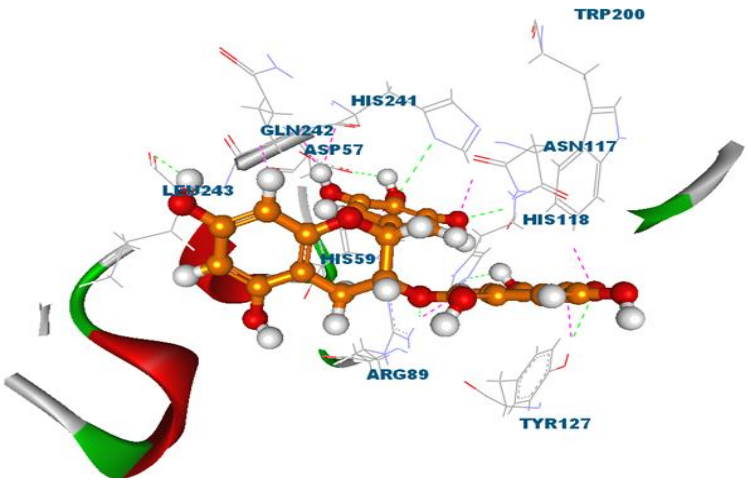

Fig. 5: Epigallocatechin_gallate with highest libdock score 138.415 at AGS Site Sphere

The complete docking scores of all the small molecules docked, along with the Interacting amino acids for the AGS site sphere were tabulated in the following Table 3 that yielded in the best Ligand molecule as Epigallocatechin_gallate with the highest libdock score as "138.415" (Fig. 5).

Table 3: Libdock scores of the docking process of minimized pp2A protein and prepared ligands at AGS Site Sphere– [-22.627500 -3.323100 27.091200]– Coordinates

Compound Name	Libdock Score	Interacting Aminoacids
4-Hydroxynonenal_5283344	79.311	His241, Asp57, His59, Tyr265, Leu243, Arg89
Dithiolethione__68296	42.335	His59
Ebelactone_B_6436821	116.564	Asp85, Asn117, Trp200, Pro213, His241, Ser212, Arg214, Arg89
Epigallocatechin_Gallate_65064	138.415	Asn117, Leu243, Asp57, His118, His59, Trp200, Tyr127, His241, Gln242, Arg89
Forskolin_47936	99.83	Leu243, His59, Arg89, Arg214, His241, Leu243, Gln242
Melatonin_896	100.545	Leu243, Asp85, Asp57, His59, His118, Tyr127, Trp200, Arg89
Palmitic_Acid_985	106.778	Arg89, Trp200, Arg214, Ala216, His241
Progesterone_5994	97.283	Gln242, Arg214, Ser212, Pro213
Taurolidine_29566	94.628	Arg89, Tyr127, His241, Trp200
Troglitazone_5591	136.71	His241, Asp202, Asp57, Pro213, Gln242, Arg214

CONCLUSIONS

Alzheimer's disease being a global and burning problem, providing a potential treatment for it has become the most quintessential need of the hour that has laid the pavement to this study. For the aforesaid intent, by taking the Tau and PP2A as the targets, therapeutic molecules were ascertained to tackle Alzheimer's disease and ameliorate the condition of the patient. With the recent advancements in the biological research, the customary in-vivo models have been replaced by the in-silico models, which were as well employed in this study.

Although in-silico studies represent a relatively new avenue of inquiry, it has begun to be used widely in studies, which predict how drugs interact with the body and with pathogens. The In-silico models used in this study exactly mimic the real models, hence reducing lot of money and time consumed in the process of drug discovery. But as it stands that it still is an artificial environment quite different from that of living models, it is imperative that these molecules viz., Cannabitriol and Epigallocatechin_gallate, be tested under wet lab conditions to ensure the competence and efficacy of the conclusive data obtained from the bioinformatics work.

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