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Identification of Protein Target and Selection of Suitable Drug Candidates Against Alzheimer's Disease by Docking Studies

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Abstract

Alzheimer's though a well-known condition since generations has no complete treatment till today and people suffer with the condition till date. There are several known medications available in the market and each in different combinations. However, the therapeutic effect of each drug may vary from person to person which is completely unpredictable. The current research work aims to enhance the efficacy of treatment by targeting a particular protein called APP rather than the general medication. Further the ligands used in the current study are all the derivatives of Tacrine which is known to possess anticholinesterase activity. Three variations of Tacrine compound namely 1,2,3,4-Tetrahydroacridin-9-amine chloride, Tacrine hydrochloride monohydrate and Tacrine hydrochloride were used. All the chemicals were screened for their drug likeliness using Lipinsky rule. Since all the three chemicals passed the screening, they were further subjected for docking with the selected receptor Amyloid Precursor Beta Protein. Docking was performed in two different software's Hex and Molegrow to obtain the accuracy in results. The docking results revealed that the compound 1,2,3,4Tetrahydroacridin-9-amine; chloride was proved to be a better ligand with a lower docking energy representing a great structural compatibility between the Ligand and receptor pair. The studies can be extended towards the clinical research to obtain a confirmatory result.

Keywords: Alzheimer's, APP, 1,2,3,4-Tetrahydroacridin-9-amine chloride, tacrine hydrochloride monohydrate, Tacrine hydrochloride, Molgrow, HEX, Rasmol

Introduction

Amyloids are starch like protein which gets deposited in the liver, kidneys, spleen, or other tissues in certain diseases, arising from 18 different proteins and polypeptides, and are associated with human diseases, known as amyloidosis, which play an important role in some neurodegenerative disorders (1). Amyloid is an extracellular, proteinaceous deposits exhibiting beta sheet structure and Figure 1, represents the structure of ribbon form of amyloid protein downloaded from the protein data bank (PDB).

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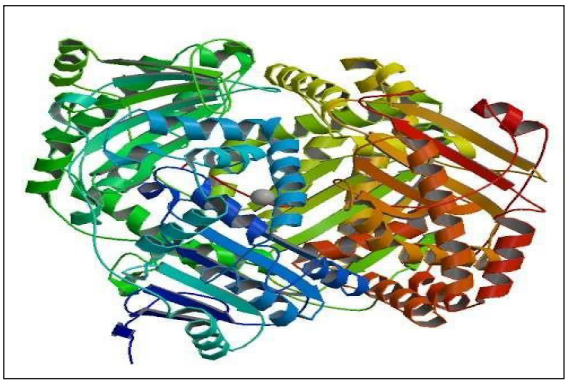

Fig 1. Structure of amyloid

Amyloid precursor protein (APP)

APP is a neuronal cell membrane protein called amyloid precursor protein (APP) is found abundantly at synapses of neurons in brain and is concerned as a regulator of synapse formation, neural plasticity and iron export. AAP (2) is proteolysed by enzymes like alpha-, beta- and gamma- secretase. Usually alpha and gamma secretase chop the APP and forms soluble proteins, while another enzyme called beta secretase along with gamma secretase chop the APP, resulting in formation of insoluble polypeptide with 37 to 49 amino acid residues called beta amyloid protein (Aβ), which is toxic to neurons and a principal component present in Alzheimer's disease patients. Another important protein called tau proteins a prion-like misfolded oligomers is also cited in the AD patients. Figure 2, decipites the structure of beta-APP.

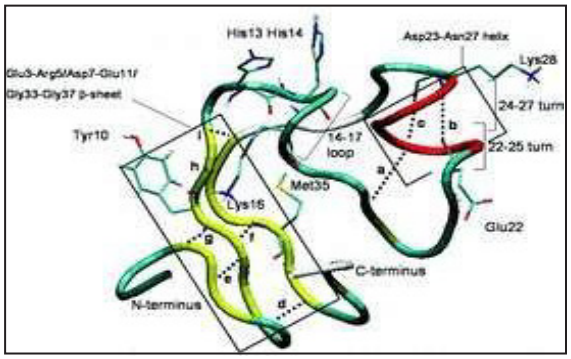

Fig 2. Represents the structure of Amyloid beta peptide (beta-APP)

Alzheimer disease

Alzheimer disease (AD) is a chronic long term neurodegenerative disease that typically develops slowly and gets worsen over time, leading to the characteristic features of dementia. The disease progress can be categorized into four phases, with a increasing pattern of memory loss, cognitive deficits and functional impairment like writing, reading and speaking (3). Short-term memory loss is the most typical initial sign. As the condition worsens, behavioral problems, linguistic difficulties, disorientation (including a tendency to get lost easily), mood swings, loss of desire, and behavioral disorders can be observed (4). Two major components involve in AD progression includes plaques and tangles in the brain and initial symptoms of which are mistaken for normal ageing (5). The characteristic feature is the atropy of the brain, where the gyri shrink and sulci present between the grooves becomes wider, with atropy the ventricles the fluid filled cavities in the brain gets wider as well. Figure 3 represents the characteristic differences between normal and AD brain. No medication can reverse its progress neither decrease the risk of AD, though some medications may temporarily improve symptoms. Exercises can potentially improve AD symptoms.

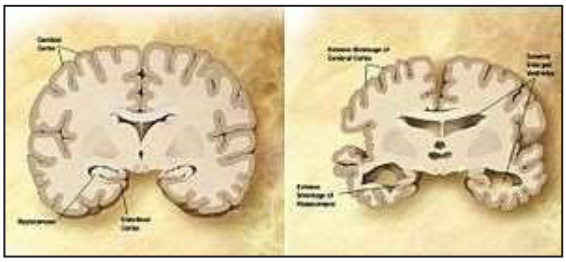

Fig 3. Represents the differences between healthy and Alzheimer's disease brain

Gamir-Morralla A et al. (6) performed a study with Kidins220, in patients with AD where it phosphorylated tau proteins indicating that Kidins220 can be a potential biomarker to identify the progress of AD. Perche F et al (7), used single chain variable fragment (scFv) for passive immunotherapy not only in AD but also for oth-

er neurological disorders, where the scFvs not only recognized the **amyloid** protein but also delayed amyloid β aggregation indicating that its a safer alternative compared to other anti-Aβ antibodies or adenovirus encoding antibodies. Yang SH et al. (8) conducted a study in AD models using necrostatin-1 (Nec-1) that targets amyloid **β**eta and tau proteins directly, and reduced the neuron cell death with simultaneous improvement of cognitive functioning indicating that Nec-1 can be a preventive approach for alzheimers disease. Xiong J Et al (9), performed an experiment in which they added myricetin to the peptide samples of amyloid **β**eta (25-40), which resulted in structural changes around amino acid residue 32 of Aβ (25-40) peptide, that resulted from hydrophobic interactions between the peptide or with the self-association of the peptide in this region. Enache TA and Oliveira-Brett AM (10), Performed and compared the oxidative behavior study with human amyloid beta and other similar peptides in a solution or using cyclic and differential pulse voltammetry. The electron transfer in Aβ peptides depended on peptides hydrophobicity and 3D structure, and observed that the highest oxidation peak were close to N-terminal of peptides. Gregori M et al (11) studied the effects of nanoliposomes with the retro-inverso peptide RI-OR2-TAT, in invitro on D3 cell monolayer, a model of BBB and concluded that low concentrations of these peptide was required to stop the formation of Aβ aggregates and can be potential therapy for the treatment of AD.

Material and Method:

Various online softwares like protparam, SOMPA, PHYRE, RASMOL, PDB, CHEBI, Pubmed, Lipinski applications were used inorder to identify and analyse the protein structure as well as to find the targetted chemical compounds. While docking was performed using softwares like Hex (Non-Targetted and Molgrow (Targetter) and compared their results to find the best suitable compound against the target 1MWP.

Major proteins identified in alzheimer's disease

An Extensive literature survey was conducted to select the protein that plays a vital role in the onset of the condition. Some of the important proteins selected were, Amyloid beta (A4) precursor protein, Presenilin 1, Presenilin 2 and Apolipoprotein E.

Amyloid beta precursor protein

APP are the proteins found in the neuron cell membranes and are involved in neuron cell growth, repair and iron transportation. The sequencial cleavage by beta and then gamma secretase results in Beta-amyloid 42 as well as Beta-amyloid 40, of which Beta amyloid 42 is stronger reductant than the Beta-amyloid 40. The peptides bind to lipoproteins and apolipoproteins where by arresting the metal-catalyzed oxidation of them. Further Beta-APP 42 activates the phagocytes in the brain and extort inflammatory responses. Mutations in APP gene located on chromosome 21, also results in familial AD.

Presenilin 1 & 2

Another protein called presenilin are also found in brain cells and are the subunits of gamma secretase that regulates the APP processing. There are two types of presenilin proteins called as PSEN-1 and PSEN-2, that are associate with early-onset familial AD. Presenilin 1 is located o chromosome 14, while presenilin 2 is located on chromosome 1. The patients with inherited Alzheimer's disease (AD) carry mutations in both presenilin proteins.

Apolipoprotein E

Apolipoprotein E (APOE), gene located on chromosome 19 is another major genetic risk factor for development of sporadic AD. Generally APOE helps breakdown of beta Amyloid and any mutations in APOE, results in anormous gathering of beta amyloid outside the neurons thus inhibiting the signal transmission between the neurons progressing the AD.

Primary structure analysis, physicochemical characterization of protein by protparam

The basic sequence level annotation was performed in Protparam (12) and the rest obtained are furnished below. Figure 4 and 5 represents the amino acid sequence of protein.

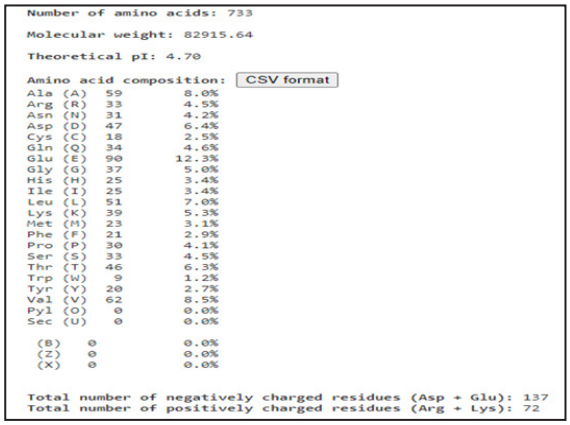

Fig 4. Represents the acidic nature of protein due to presence of negatively charged residues

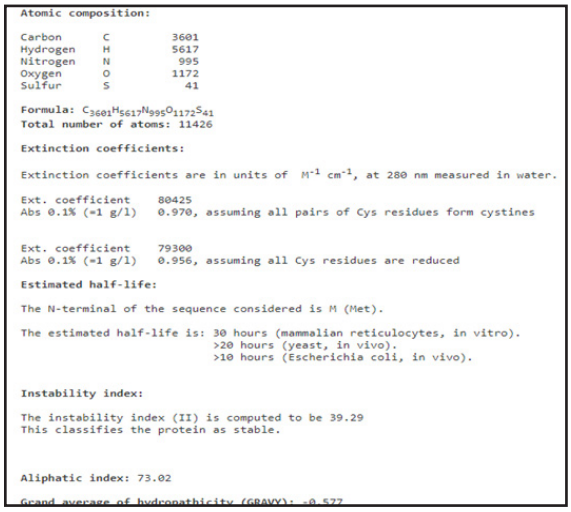

Fig 5. Represents the protein's instability index value 39.29, hydropathicity value -0.577 and Aliphatic index value 73.02 indicating that the protein is stable.

Secondary structure analysis using SOPMA

SOPMA (13) was used to identify the secondary structural confirmation within the proteins. Secondary structure analysis reveals the four major types of structural confirmations along with their contribution to the final protein structure. The details of the structural contributions by each type of secondary confirmation are shown below : Alpha helix (Hh) : 336 amino acids and constitutes 45.84%, Extended strand (Ee) : 79 amino acids and constitutes 10.78 %, Random coil (Cc) : 276 amino acids and constitutes 37.65 % and Beta Turn formed by 42 amino acids and forms 5.73%. and is shown in figure 6.

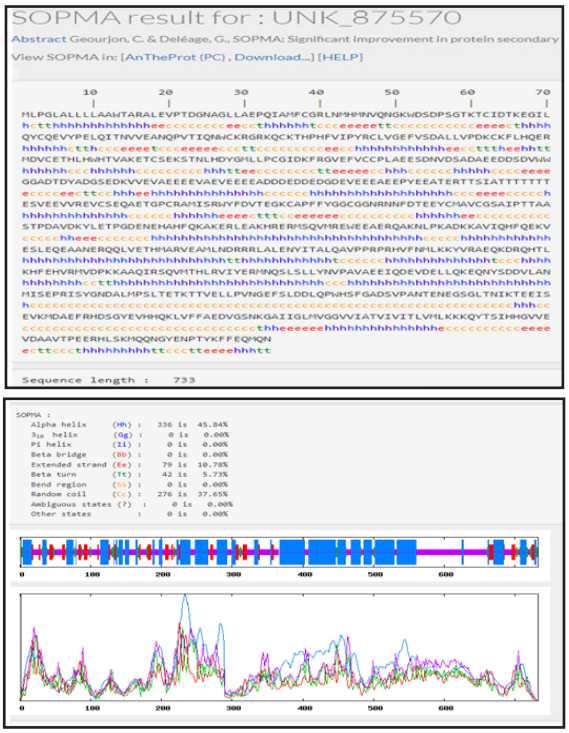

Fig 6. Represents the secondary structural analysis of protein using SOPMA

Tertiary structure analysis by PHYRE

PHYRE (14) server is online tool used for the identification of structure representative present in PDB Data bank that shows greater identity to the query sequence. Phyre, after performing an extensive sequence similarity search of the query with all the sequences of the proteins available a best hit result is displayed.

Based on the above results it can be observed that the PDB ID **1MWP** is showing a greater degree of identity with the protein thus can be selected as the structure representative of the protein as shown in Figure 7. And the retrieval of protein structure and annotations were done from rcsb pdb, Figure 8 (15).

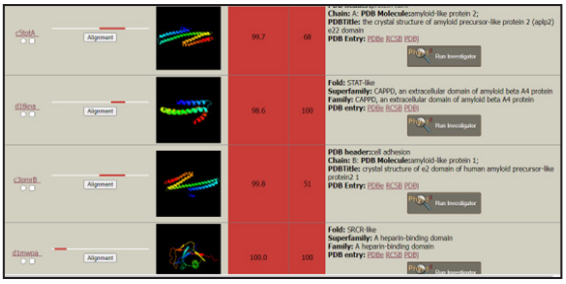

Fig 7. The Phyre output showing the positive hits with highest sequence similarity

Figure 8: The home page of 1MWP protein as obtained from RCSB PDB.

Visualization of protein by rasmol software

The 3D structure of the protein corresponding to 1MWP as obtained from PDB can be studied in RASMOL visualization software (16) with various commands as required by the user as shown in Figure 9.

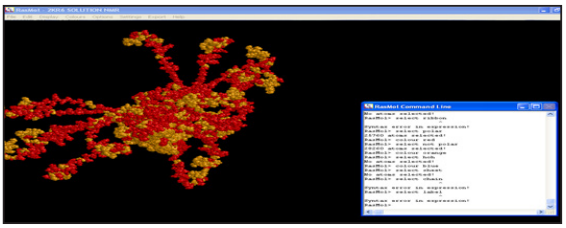

Figure 9: The Structure of 1MWP as revealed by Rasmol visualization software

Identification of suitable chemicals and generation of chemical library:

Based on the required function chemicals were screened from various data bases like Pubchem, CHEBI, which provides the basic information about the chemicals along with the information about the structural properties and function (17, 18). The three derivatives of Tacrine compound were used in the study as they are known to possess anticholinesterase activity which becomes an important property to treat a AD. All the three tacrine derivatives were underwent with primary screening for drug likeness (19) to find the effective drug that shows a good structural compatibility with the selected receptor APP.

Docking:

Docking of the selected compounds was performed in two softwares HEX (20) and MOLGROW (21), in order to get the accurate results. HEX is a non targeted method of docking, in which the active sites on the protein were not generated while in the MOLGROW sofware a grid was generated around the active sites of the protein where the ligands bind to it. Figure 10, 11 and 12 represents the results of docking performed in Hex software while the Figures from 13 to 21 represents the docking performed in MOLGROW software.

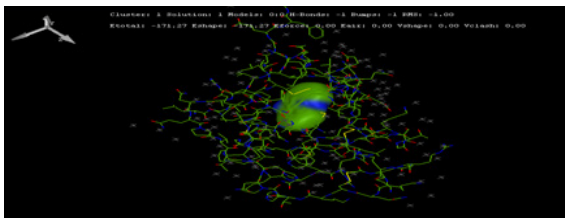

Fig 10. Docking of 1MWP with 1,2,3,4-Tetrahydroacridin-9-amine;chloride

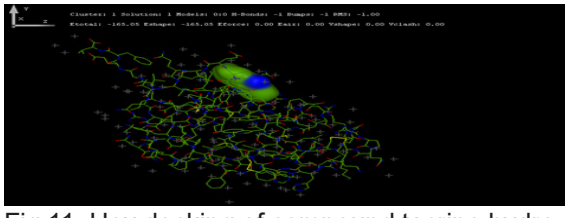

Fig 11. Hex docking of compound tacrine hydrochloride

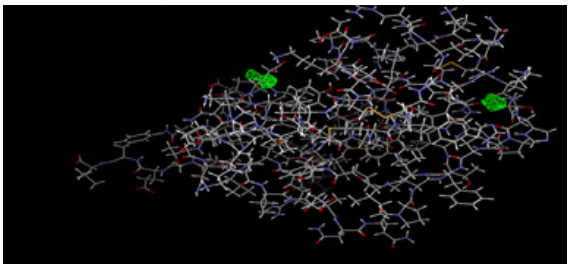

Fig 12. The figure shows the Wireframe mode of the protein structure with the 2 cavities selected for targeting in docking

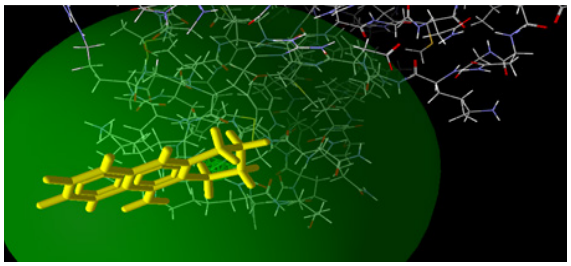

Fig 13. The Ligand and the binding site being shown in the grid of docking

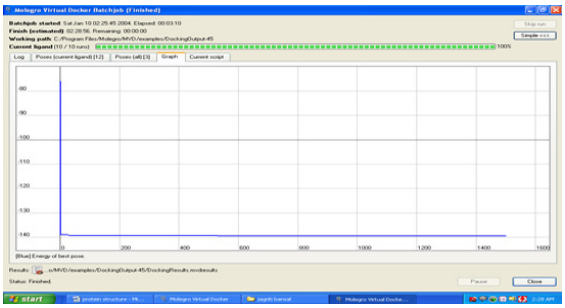

Fig 14. The energy calculations for various poses during the process of docking

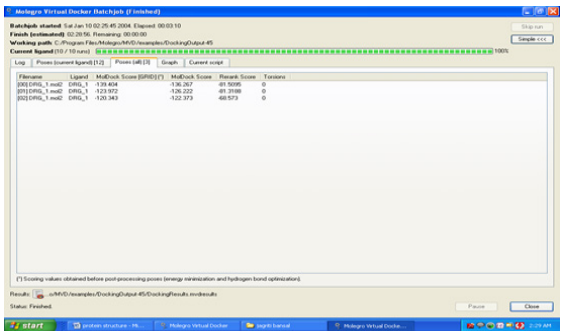

Fig 15. Final docking result showing the best docking poses and their energies calculated

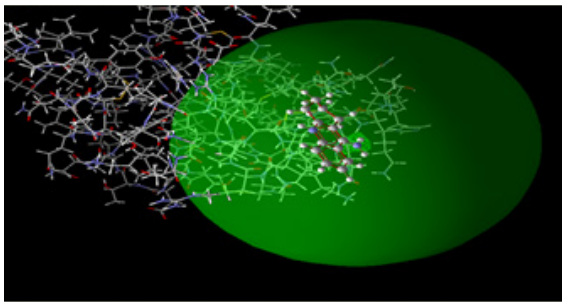

Fig 16. Docking of Compound 2 with 1MWP Showing the grid view and the energy calculations during docking process

Fig 17. The energy calculations for various poses during the process of docking

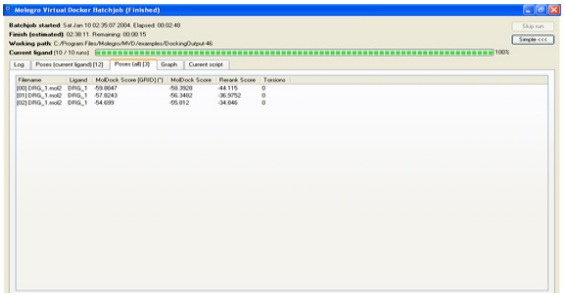

Fig 18. Final docking result showing the best docking poses and their energies calculated

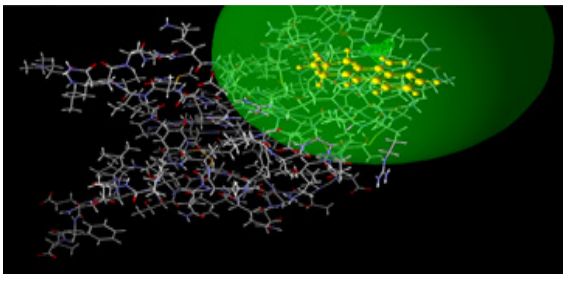

Fig 19. Docking of Compound 3 with 1MWP Showing the grid view

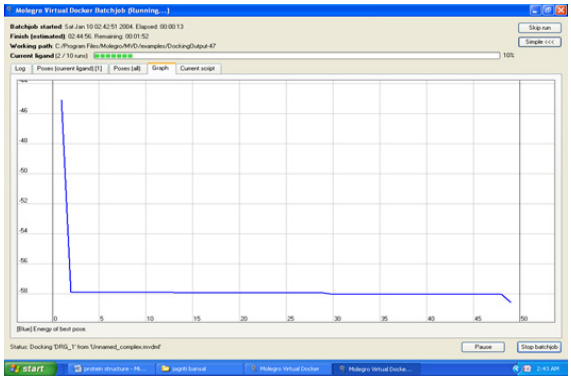

Fig 20. The energy calculations for various poses during the process of docking

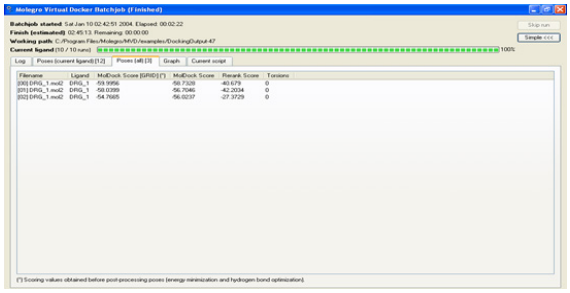

Fig 21. Final docking result showing the best docking poses and their energies calculated.

Results and discussion

By using the application called ProtParam, an online computation tool we identified the various physical and chemical parameters of the protein 1MWP The parameters identified includes molecular weight, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity. The secondary structural analysis performed using SOMPA application reveals that the major part of the protein is composed of alpha helix. This assigns the protein its polar and globular in nature. By using the PHYRE software, we found that the protein 1MWP showed the highest similarity with beta amyloid protein and can be chosen to perform the docking studies with the selected chemical compounds. Once protein structure was identified, from the Figure 8, the home page of the 1MWP data it can be identified that the structure corresponds to the protein N terminal domain of amyloid precursor protein, whose structure was obtained by X-ray diffraction studies. The resolution obtained for the structure was found to be 1.80 with an R value free of 0.242. This protein is classified under the group of sugar binding proteins. Further the protein was visualized using rasmol application. Based on the primary screening it was observed that all the three tacrine derivatives have passed the screening criteria of drug likeliness and were subjected to docking studies. Table 1, represents the druglikeness of the selected compounds. In the above study the derivatives of Tacrine were selected for docking in view of its function. The docking energies obtained were summarized in Table 2. As per the above data the best ligand selected among the three was CID 24188122 corresponding to 1,2,3,4-Tetrahydroacridin-9-amine, chloride. For this the HEX docking energy and MOLGROW docking energies obtained were -171.27 and –139.40 respectively.

Table 1: Respresents the chemicals obeying Lipinski rule of five

S.NO.	CID	Name of comp	Mol. wt	HBD	HBA	LogP	Canonical smiles
1.	24188122	1,2,3,4-Tetrahydroacridin-9-amine;chloride	233.71g/mol	**1**	**3**	**3.05**	C1CCC2=NC3=C-C=CC=C3C(=C2C1)N.(Cl-)
2.	6420002	Tacrine hydrochloride monohydrate	252.73 g/mol	**3**	**3**	**2.29**	C1CCC2=NC3=C-C=CC=C3C(=C2C1)N.O.Cl
3.	2723754	Tacrine hydrochloride	234.72 g/mol	**2**	**2**	**3.11**	C1CCC2=NC3=CC=C-C=C3C(=C2C1)N.Cl

Table 2: Represents the Docking energies of the chemicals in both Hex and Molgrow softwares

S. No.	Compounds	Hex docking energy	Molgrow docking energy
1.	1,2,3,4-Tetrahydroacridin-9-amine chloride	-171.27	-139.40
2.	Tacrine hydrochloride monohydrate	-165.05	-59.88
3.	Tacrine hydrochloride	-173.13	-59.99

Conclusion:

The current research was undertaken to study one of the common and most life influencing disorder Alzheimer's. This disorder being one of the common problems that influence the health along with the life style of an individual needs at-most attention for improving the treatment protocols. The study involved the application of insiico technology and softwares for the screening of chemical therapeutics available in the market to treat the condition. Literature mining was performed to study the disease in detail and identify the major protein involved in the onset of the condition. This screening enables the user to select a receptor target that can be used as a target in docking studies. The receptor selected in the study was Amyloid beta precursor protein. The sequence was retrieved from NCBI data base and subjected for structural and functional analysis. The structure selected from RCSB PDB for APP protein was 1MWP which was downloaded and subjected for further analysis. Chemical substances were screened from Pubchem and CHEBI data base and 3 compound were selected for docking studies. Both Molgrow and Hex were used for docking and the results were compared. Final results revealed that the compound **1,2,3,4-Tetrahydroacridin-9-amine; chloride** proved to be more efficient based on the structural comparability and docking results. Among all the three ligands used in docking this compound has yielded a lower docking energy in both the softwares which accounts for its greater structural compatibility.

Conflicts of interest: No

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