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Identification of Novel Lead Moieties as Anti-Cholinesterase Agents: A Computational Approach

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative condition with a complicated pathophysiology. Cholinesterase inhibitors work to slow down the acetylcholine's deterioration. Cholinesterase inhibitor drugs slow down the process of ACh decomposition, inhibiting AChE activity and keeping ACh levels stable. The current research involved in identifying novel cholinesterase (ChE) inhibitors with the application of computational tools. The known cholinesterase inhibitors were used to develop pharmacophore hypothesis using Pharmagist webserver. Through the ZINC Pharmer web server, 1000 most comparable pharmacophoric ligands that are feasible were identified. Data warrior tool was used to filter the compounds and molecular docking was carried out for the selected compounds. Depending on the binding affinity and amino acid interaction top five ligands are subsequently evaluated for ADMET properties using PKCSM webserver. ZINC03242470 compound with the binding score and showed good interaction with key amino acids of cholinesterase has the potential for further development as an Anti-cholinesterase agent.

Key words: *Alzheimer's disease, cholinesterase inhibitors, Molecular docking, Pharmacophore modeling*

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INTRODUCTION

Cholinesterase inhibitors work to slow down the acetylcholine's deterioration. By inhibiting its normal degradation into acetate and choline, they are a family of drugs that increase the levels and efficacy of acetylcholine in the peripheral as well as the central nervous systems. Acetylcholinesterase inhibitors have a variety of uses. The neurogenerative diseases Parkinson's disease, dementia and Alzheimer's disease are the ones they are most usually used to treat. ACh-producing cells are destroyed by various physiological processes in several degenerative illnesses, which also impair cholinergic transmission in various parts of the brain[1,2].

The four possible factors of AD is viruses- Herpes Simplex type 1 Virus (HSV1), Defective DNA pair, Aluminium as possible factor and Head injury.

- The areas of the brain that are most impacted by AD and HSV encephalitis are identical. It is uncertain how the virus may enter the brain, however when the virus is repeatedly reactivated, it may spread from the TGG to the mesial temporal lobe, then to other limbic regions, and finally to neocortical regions. This could explain the severe hippocampus damage and memory loss seen in AD patients. Since the immune system weakens with age, entry into the brain would be more likely in the elderly. The creation and maintenance of latency appear to involve the immune system.
- It has been discovered that several neurological diseases have a defect in DNA repair. In nondividing cells, like neurons, the effects of such a defect are likely to be especially severe. The known repair defect discovered in the cells of Down's syndrome patients is more directly related to AD.
- Additionally, since AD patients could be seen as cases of accelerated ageing in some ways and since DNA damage seems to increase with age (although it is unknown whether repair decreases concurrently), they may be more vulnerable to DNA-damaging agents than age-matched normals. A

higher susceptibility would, of course, imply that the damage is a contributing factor to the disease or perhaps a side effect of it rather than a cause.

- There is no agreement on the sensitivity, or even correctness, of the several methods that have been used to look at levels of aluminium in the brain, or more precisely, levels in plaques and neurofibrillary tangles; another point of disagreement is the potential for aluminium contamination. Despite not being fully understood at the molecular level, aluminium is well recognised to be neurotoxic. Aluminium has been linked to AD pathogenesis due to its neurotoxicity and the fact that rabbits given intracerebral injections of aluminium salts showed signs of neurofibrillary degeneration, poor memory, and decreased learning ability. Studies on the levels of aluminium in the human brain soon after suggested that AD had higher levels than the normall brain.
- Boxers have been reported to acquire clinical dementia after suffering repetitive head trauma. Additionally, it has been shown that their brains contain a lot of NFT and amyloid plaques. There seems to be a link between head trauma and AD in several studies. The blood-brain barrier (BBB) may get damaged because of head trauma, which might then allow toxins to enter the brain and/or cause the immune system to become less effective. In fact, a BBB malfunction in AD patients has been identified by two investigations [3].

The foundation of Alzheimer's disease therapy is pharmaceutical and nonpharmacological management and care planning based on physician psychotherapy, shared self-improvement, and judgement established by a strong connection between the doctor, the patient, and the caretaker. Even though they are often used as symptomatic treatments, the N-methyl-d-aspartate (NMDA) antagonist memantine and cholinesterase inhibitors (ChEIs) licenced by the Food and Drug Administration (FDA) for AD can have moderate "disease course-modifying" benefits through improving cognition and minimising independence loss. The AD drugs permitted by the FDA are the AChEIs- donepezil, rivastigmine, galantamine and the NMDA antagonist memantine[4]. The side effects of cholinesterase inhibitors are Abdominal cramps, headache, dizziness, anorexia, nausea, diarrhoea, muscle cramps, indigestion, and weight loss [5].*In-silico* drug design, also known as computer-aided drug design (CADD), is a process that begins with the identification of a chemical compound that exhibits biological activity and concludes with the optimization of the biological profile and the chemical synthesis of a new chemical compound [6-8].

Hence the present study aims to identify new leads as cholinesterase inhibitors for the better treatment of AD.

MATERIAL AND METHODS

Identification of drug molecules: Based on the literature research of the disease, the current identified medications were Donepezil, Galantamine, Rivastigmine, Phenserine, Tacrine, Huperazine-A. The compounds structures were obtained in SDF format from NCBI Pubchem [9-11].

Pharmacophore modelling: A three-dimensional framework known as a pharmacophore is produced by mapping the physiological **p**owerful chemicals necessary for the ligand to attach to the appropriate target protein and interact with it. Molecule are subjected for pharmacophore modeling by uploading to free online tool called pharmagist in Sybyl Mol 2 form with mail ID. The provided molecules are combined in a variety of methods by the server to create the pharmacophore in Jmol file [12].

Collecting data from zinc database: It produces the number of aligned molecules like 7 aligned molecule one up on another gives a cluster in the JMol format, where the molecule with maximum aligned 7 or 6 molecules with high scoring are selected and downloaded, further these molecules were uploaded in free online tool for pharmacophore modelling i.e., ZINC pharma. Which will screen the zinc database and gives the molecules having same pharmacophoric features by submitting to query? After queries over zinc database give around more than a lakh hits of molecule, further obtained hits are filtered and get total of 950 small molecules. Those above molecules are uploaded to the data warrior, where data warrior is free ware tool for doing pharmacophore modelling, these molecules are screened based on the standard drug physicochemical properties like molecular weight, log P, H donor, H acceptor, polar surface area, rotatable bond and steric centre. The SDF files for each of these compounds were generated and utilised for the autodock studies [13-16].

Molecular Docking: After being generated from data warrior, molecular docking studies is carried out to those molecules, the goal of docking studies is to determine the best fit and binding affinity of the drug towards the targeted protein. First thing is preparation of protein for docking studies, the protein is selected and downloaded in the form of pdb file, form the Protein Data Bank with PDB ID 7BO3[13]. The X-ray diffraction of targeted protein Human Butyrylcholinesterase in complex withN-(2-(1H-Indol-3-yl) ethyl)-2-cycloheptyl-N-methylethan-1-amine, with resolution of 2.20 angstrom. Using the "Swiss pdb viewer," hetero compounds, molecules of water, and undesirable ligands were removed from the protein during protein production. AutoDock Vina was used for docking, and all of the ligands were created,

reduced, and optimised. PyMol version 2.4, a molecular viewing programme from Discovery Studio, was used to further validate the docking method. The grid generation process is crucial to the docking process because it establishes the location where the ligands will bind with the protein. The grid is defined by the target protein from the database of proteins using the co-crystallized ligand that is present with the protein. The top 10 structures with the best docking scores were selected from a screening of the docked result from each of the ligands [17].

ADME-T studies: The ADMET characteristics play a serve a significant purpose. In the body. When a molecule is administered orally, a good absorption is a desired property. The distribution and absorption of a molecule throughout the system will be governed by the balance of hydrophilic and lipophilic groups in the structure. From a therapeutic standpoint, effectiveness and toxicity are important. The ADME-T studies are carried out using PKSCM online software [17].

RESULT AND DISCUSSION

Ligand-based pharmacophore modelling: Using the PharmaGist website, the pharmacophore hypothesis was derived using preclinical cholinesterase inhibitors**.** The compound generated from PharmaGist has one aromatic ring and two hydrogen bond acceptors separated apart. The result obtained from the PharmaGist is uploaded in the ZINC Pharmer, it will screen and recover the compounds having same pharmacophoric features from the ZINC database. The derived pharmacophore model aids in determining the structural need for regulating cholinesterase. Screened molecules were uploaded to the DataWarrior, further the molecules are filtered based on the physicochemical properties, I.e., the log p value, molecular weight, polar surface area, hydrogen donor and acceptor of the standard Cholinesterase inhibitors. The SDF files for each of these compounds were downloaded and utilised for the molecular docking research.

Molecular docking: Cholinesterase inhibitors were studied via molecular docking**.** The objective was to identify the primary binding contacts in the target protein's active site that may be bioactive conformations using PDB ID: 7BO3. This action was crucial in determining the connections between compounds found in the ZINC database. Utilizing AutoDock Vina, molecular docking investigations were carried out. Galantamine, Huperazine-A, Phenserine, Rivastigmine, Tacrine are currently used as Cholinesterase inhibitors. Table 1 displays the docked result and binding activity. Phenserine was identified among the inhibitors with a binding energy of -9.4 kcal/mol and as a carbon hydrogen bonding with HIS438, ASP70 Pi-Sigma interaction with PHE329, Pi-Pi T shaped interaction with TRP321, Pi-alkyl interaction with TRP430, TYR332, ALA328.These molecules attach to the protein by hydrogen bonding, the Pi-Pi T shaped interaction, Pi-alkyl interaction and the Pi-Sigma interaction. The residue tacking part in interaction was HIS438, ASP70, PHE329, TRP321, TRP430, TYR332, ALA328. As a result, the above interaction might be crucial and explain why molecules behave as Cholinesterase inhibitors.

Table 1 shows the docking result along with the kind of interaction of standard drugs.

Table 1: Docking score and type of interaction of standard drugs

Compound	Docking Score	Interacting residues	Type of interaction
GALANTAMINE	-8.7	THR120 ASN83 SER198, HIS438 TRP82	Conventional Hydrogen Bond Carbon Hydrogen Bond Unfavourable Donor-Donor Pi-Pi Stacked
HUPERAZINE-A	**-**8.4	TYR128 GLY121 TRP82 PHE329, HIS438	Conventional Hydrogen Bond Carbon Hydrogen Bond Pi-Sigma Pi-Alkyl
PHENSERINE	**-**9.4	HIS438, ASP70 PRO285 PHE329 TRP231 TRP430, TYR332, ALA328	Carbon Hydrogen Bond Unfavourable Acceptor-Acceptor Pi-Sigma Pi-Pi T-Shaped Pi-Alkyl
RIVASTIGMINE	-7.1	GLY116, GLY117 PRO285 PHE329, TRP231 VAL288 LEU286	Conventional Hydrogen Bond Carbon Hydrogen Bond Pi-Pi T-Shaped Alkyl Pi-Alkyl
TACRINE	-8.4	HIS438 TRP82 ALA328	Conventional Hydrogen Bond Pi-Pi Stacked Pi-Alkyl, Alkyl

The compound was docked after extracting the molecule from the ZINC database using ZINCPharma based on the pharmacophore. In this case, 5 molecules were chosen for docking studies with the target PDB ID: 7BO3. The docking was analysed to find compounds with the best docking scores (most negative) to correlate with the high affinity of standard medications. This comparison helps to recognize the hit

molecule as a cholinesterase inhibitor. The molecules are identified based on their binding interaction. The docking score ranges from -6.9 to -9.5 kcal/mol. Molecular docking has identified a compound ZINC03242470 with best docking score of -9.5 kcal/mol and exhibit Conventional hydrogen bond interaction with GLY116, GLY117, SER198, Pi-Anion interaction with ASP70, Pi-Sigma interaction with TYR332, and Pi-alkyl interaction with LEU286, TRP231. Likewise, another compound ZINC55580150 with docking score of -10.1 kcal/mol showed conventional hydrogen bonding interaction with TYR407, TYR444, carbon hydrogen bonding interaction with GLU216, halogen interaction with GLY443, Pi-Pi sacking interaction with PHE208, alkyl interaction with LEU337, MET350 and Pi-alkyl interaction with PHE352, LEU337, ILE335. Similarly, another molecule ZINC66113202 with docking value of -9.1 kcal/mol showed conventional hydrogen bonding interaction with TYR332, TRP430, Pi-Pi T-Shaped interaction with TRP231, PHE329, TRP8, alkyl interaction with HIS438 and Pi-alkyl interaction with LEU286, ALA328. AutoDock tools and the Biovia Discovery Studio Visualizer were used to identify binding interactions between docked compounds and proteins. The molecular docking analysis demonstrated that the chemicals discovered bind similarly to the molecules found in typical medications. As a result, these 5 substances can inhibit Cholinesterase. Table2 shows the chemical structures of five docked molecules.

Table 2: Docking score of molecules obtained from ZINC database

Compound	Docking Score	Interacting Residues	Type of Interaction
ZINC03242470	-9.5	GLY116, GLY117, SER198 ASP70 TYR332 PHE329, HIS438 TRP231, LEU286	Conventional Hydrogen Bond Pi-Anion Pi-Sigma Pi-Sulphur Pi-Alkyl
ZINC35320714	-8.5	GLY117 HIS438 TRP82 LEU286 TRP231, PHE329 GLY116	Van der waals Pi-Cation Pi-Sigma Pi-Alkyl Pi-Pi T-Shaped Amide- Pi stacked
ZINC66113202	-9.1	TYR332, TRP430 TRP231, PHE329, TRP82 LEU286, ALA328 HIS438	Conventional Hydrogen Bond Pi-Pi T-Shaped Pi-Alkyl Alkyl
ZINC74520445	-8.5	HIS438 TRP82, TYR332 PHE329 ALA328	Carbon Hydrogen Bond Pi-Pi stacked Alkyl Pi-Alkyl
ZINC93954902	-6.9	SER198, GLY116, GLY117 LEU286 HIS438 TRP82 ALA328 TRP430, MET437	Conventional Hydrogen Bond Carbon Hydrogen Bond Pi-Cation Pi-Pi T-Shaped Pi-Alkyl Alkyl

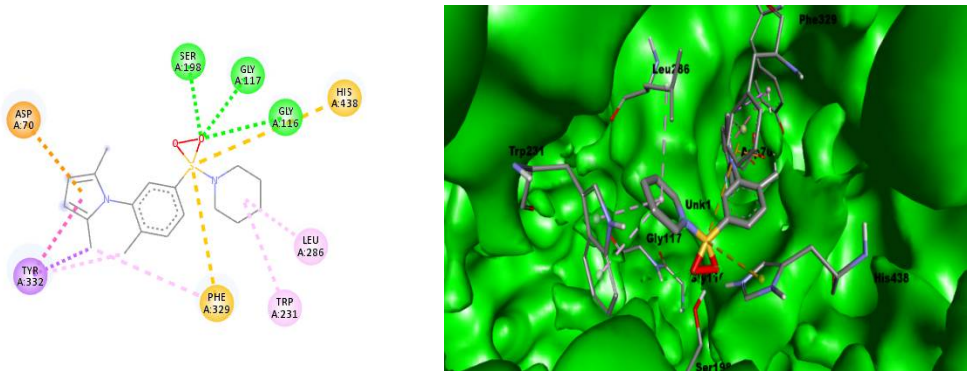

Fig.1: 2D and 3D interaction of ZINC03242470 with 7BO3

ADMET studies: The Lipinski rule of five is used to determine drug similarity, which states that the drug must have a molecular weight of 500, an A Log P of 5, a number of hydrogen donors of 5, and a number of hydrogen acceptors of 10, then by these five rules have better activity like permeability, bioavailability, and absorption.The idea of drug-likeness serves as a helpful guide during the early stages of drug research.The number of hydrogen bond donors (HBDs) is five, the octanol/water partition coefficient (A log P) is five, and the number of hydrogen bond acceptors is five (HBAs) The first and most well-known rule-based filters of drug-likeness were 10 and were introduced by Lipinski in 1997.The Rule of Five

states that a molecule would not be orally active if it violated two or more of the four requirements. Later, the "Rule of Five" and additional drug-likeness guidelines/filters were put forward. For example, the research discovered that more than 80% of the compounds met the following specifications: 2.9 (A log P) 3.8, 280 (MW) 332, 105 Surface areas 138, and the rotatable bonds below 10, based on 4 molecules in the zinc database from pharmacophore modelling. Drug-likeness rules based on physicochemical characteristics expedite drug development, although there is research that demonstrates that these rules/filters have their limitations.

CONCLUSION

AChE has an important role in the concept of cholinergic damage, and targeting AChE is probably the key to developing new anti-AD medicines. Based on the energy value of the docking and the prediction value of the activity, our study suggests that compound ZINC03242470 could serve as a promising inhibitor for the treatment of Alzheimer's disease**.**

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CONFLICT OF INTEREST

The authors declare that no conflict of interest.

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