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Molecular docking studies of various acetylcholinesterase inhibitors targeting acetylcholinesterase in *Myasthenia gravis*

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Abstract
This study aims to investigate the potency of AChE (Acetylcholinesterase) inhibitors targeting the AChE (Acetylcholinesterase enzyme). The various inhibitors (ligands) were docked against acetylcholinesterase enzyme (PDB ID: 7D9O), and the binding affinities of different inhibitors (retrieved from PubChem) were compared. Binding energy in molecular docking refers to the strength of the interaction between a protein and a ligand. It is a measure of how tightly the ligand binds to the protein and is an important factor in determining the efficiency of the docking process. The binding energy is typically calculated in terms of kcal/mol and can be used to compare the binding performance of different proteins and ligands. It is influenced by various factors such as hydrogen bonding patterns and hydrophobic interactions. The goal of molecular docking is to predict the binding orientation and stability of the ligand-protein complex, and the binding energy provides valuable information in this regard. Of all the binding energies, binding energy of Donepezil was found to be highest i.e -8.75 Kcal/mol. The present study proved that Donepezil is effective anti-virulent AChE Inhibitor of acetylcholinesterase against Myasthenia Gravis with binding energy (-8.75 Kcal/mol).

Keywords: Acetylocholinesterase, Alzheimer's disease, AChE inhibitors, molecular docking, simulation techniques, peripheral anionic site, catalytic active site

Introduction
Acetylcholine (ACh) is a key neurotransmitter in the central and peripheral nervous systems, regulating memory, learning, attention, and motor control. At the neuromuscular junction (NMJ), it mediates signal transmission from motor neurons to muscle fibers, triggering contraction. The rapid termination of this signal is essential to prevent continuous stimulation and is accomplished by acetylcholinesterase (AChE), a serine hydrolase that hydrolyzes ACh into acetate and choline within microseconds (Berg *et al.,* 2011) [1]. This recycling ensures precise synaptic transmission and highlights AChE as one of the most efficient enzymes in human physiology. In mammals, AChE is encoded by a single gene, generating diverse isoforms through alternative splicing and post-translational modifications. A related paralog, butyrylcholinesterase (BCHE), shares ~50% amino acid identity but differs in function. Beyond its classical role in neurotransmission, AChE participates in non-classical processes, including neurite growth, synaptic organization, and cell adhesion. Its dysregulation has been implicated in several neurological disorders, notably Alzheimer's disease, Parkinson's disease, and Myasthenia gravis (MG) (Peitzika *et al.,* 2023) [2].

MG is a chronic autoimmune neuromuscular disorder caused by autoantibodies targeting acetylcholine receptors (AChRs) at the NMJ, leading to impaired neuromuscular transmission and fluctuating muscle weakness. Symptoms include ptosis, diplopia, dysarthria, dysphagia, limb weakness, and respiratory difficulty. Disease subtypes are distinguished by age and thymic pathology: early-onset MG (EOMG), frequently observed in women under 50, is strongly associated with thymic follicular hyperplasia, while late-onset MG (LOMG), more common in men over 50, presents distinct immunological features. The thymus in EOMG often harbors autoreactive B cells and plasma cells capable of producing AChR-specific auto antibodies, directly contributing to disease pathogenesis.

Therapeutically, AChE inhibitors such as pyridostigmine, neostigmine, rivastigmine, and physostigmine remain first-line treatments. By inhibiting ACh hydrolysis, these agents increase synaptic ACh availability and enhance neuromuscular transmission (Munoz-Muriedas *et al.,* 2004) [3].

However, the molecular interactions between AChE and its inhibitors remain incompletely understood. Furthermore, evidence that AChE accelerates amyloid-β aggregation suggests broader roles in neurodegeneration, reinforcing its relevance as a therapeutic target. This study aims to revisit the role of Acetylcholinesterase in Myasthenia gravis, with particular emphasis on its immunopathological involvement, therapeutic targeting, and non-classical functions, thereby providing new insights into the enzyme's potential as both a biomarker and a therapeutic target in neuromuscular and neurodegenerative diseases.

Material and Methods

To study the role of AChE in Myasthenia Gravis and to study the effect of various AChE inhibitors for AChE, molecular docking was performed using different tools. Also the binding affinities of various AChE inhibitors were compared to find most effective inhibitor. Various tools, databases used are as follows:

AUTODOCK: https://autodock.scripps.edu/. [4] Auto Dock is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure.

MGL Tools: https://ccsb.scripps.edu/mgltools/downloads/.[5] MGL Tools is a software for 3D visualization and analysis of molecular structures. It uses Python and OpenGL to deliver a cross platform interactive molecular viewer suitable for producing publication-quality images.

Python: https://www.python.org/ [6]

Discovery Studio Visualizer: BIOVIA Discovery Studio Visualizer is a free, feature-rich molecular modeling application for viewing, sharing and analyzing protein and small molecule data. Experts and their colleagues can seamlessly and efficiently exchange results, without loss of either time or scientific information. https://www.3ds.com/products/biovia/discovery-studio/visualization [7]

PDB (Protein Data Bank): https://www.rcsb.org/. [8] RCSB Protein Data Bank (RCSB PDB) enables breakthroughs in science and education by providing access and tools for exploration, visualization, and analysis of: Experimentally determined 3D structures from the Protein Data Bank (PDB) archive Computed Structure Models (CSM) from Alpha Fold DB and Model Archive These data can be explored in context of external annotations providing a structural view of biology. PubChem https://pubchem.ncbi.nlm.nih.gov/. [9] PubChem is a database of chemical molecules and their activities against biological assays. The system is maintained by the National Centre for Biotechnology Information (NCBI), a component of the National Library of Medicine, which is part of the United States National Institutes of Health.

Optimization of the target protein

The crystal structure of the AChE was downloaded from the Protein Data Bank (PDB). After the removal of water, AD4 atoms were assigned along with non-polar hydrogens to the protein structure using AutoDock Tools and the optimized structure was saved as PDBQT file in the folder created for docking.

Optimization of the inhibitors

Retrieval of 3D structure of inhibitors was done using PubChem and was downloaded in SDF format. To convert it into PDB format Discovery Studio Visualizer was used, the file was saved in PDBQT format after optimization in AutoDock Tools.

Running the docking stimulation

This involves running the docking stimulation which will search for the best binding conformation of the inhibitors within the defined search space i.e where the grid box is generated. The result for docking stimulation is analyzed by the binding affinities between AChE and inhibitors.The 3D structure of AChE inhibitors was retrieved from PubChem in the form of SDF format and then converted into PDB format using Discovery Studio. Inhibitors along with compound id is mentioned in table below:

Table 1: Inhibitors along with compound ID and their activity

Names	Pubchem ID	Activity
Donepezil	3152	Increase neurotransmitter levels Improve mental ability
Galantamine	9651	Helps prevent breakdown of acetylcholine
Huperzine a	854026	Improve cognitive function, memory, and overall mental performance
Pyridostigmine bromide (mestinon)	7550	Improve muscle strength
Neostigmine	4456	Inhibits AChE, increases levels of acetylcholine in the body
Physostigmine	5983	Improves muscle function. Also used for certain conditions such as glaucoma, and anticholinergic toxicity
Rivastigmine	77991	Used to treat mild to moderate dementia (memory loss and
Tacrine	1935	Improves thinking ability in patients with Alzheimer's disease

Result and Discussion

The modelling study performed showed great interactions between various AChE inhibitors and human acetylcholinesterase. Molecular dynamics simulations (MDS) can provide valuable information in deciphering functional mechanisms of proteins/peptides and other biomolecules, overcoming the rigid sampling limitations in docking analysis (Salsbury *et al.,* 2010] [10].

The tables deduced from AUTODOCK results given below demonstrate the binding energies of the inhibitors used for docking and are mentioned in the table below:

Binding Energy	Binding Energy	Binding Energy	Binding Energy
-8.75	-7.22	-7.42	-4.44
-7.89	-7.02	-7.17	-4.17
-6.87	-6.69	-7.11	-4.38
-5.95	-6.40	-7.13	-4.14
-5.60	-6.40	-6.51	-3.90
-5.29	-6.02	-5.59	-3.89
-4.84	-5.83	-5.37	-3.83
-4.72	-5.17	-5.35	-3.76
-4.55	-4.88	-5.34	-3.15
-4.50	-4.75	-5.22	-3.11
(a)	(b)	(c)	(d)

Binding Energy	Binding Energy	Binding Energy	Binding Energy
-5.25	-6.50	-5.62	-5.91
-4.60	-6.41	-5.54	-5.86
-5.24	-6.28	-5.47	-5.78
-5.11	-6.17	-5.46	-5.76
-4.77	-6.10	-4.07	-5.65
-4.60	-5.76	-3.85	-5.64
-3.76	-5.38	-3.66	-5.63
-3.73	-4.90	-3.60	-5.54
-3.72	-4.92	-3.42	-5.31
-3.56	-4.59	-3.28	-5.02
(e)	(f)	(g)	(h)

Ligands representation: (a) Donepezil (b) Galantamine (c) Huperzine A (d) Pyridostigmine Bromide (Mestinon) (e) Neostigmine (f) Physostigmine (g) Rivastigmine (h) Tacrine

Among all the inhibitors docked with "7D9O", Donepezil gave the best suited binding affinity. The .dlg file generated this compound gave the RMSD table. Docking RMSD can be most naïvely calculated with the assumption of direct atomic correspondence, or in other words, the assumption that the atomic labels between ligand structures in the given structure files are ordered and should remain static in the docking process (Bell & Zhang, 2019) [11]. This RMSD table gives us the binding affinities on the basis of the docking performed by AutoDock4 tools.

Table 2: Binding affinities on the basis of docking performed

Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	9	-8.75	0.00	51.54	RANKING
2	1	5	-7.89	0.00	50.73	RANKING
3	1	6	-6.87	0.00	45.09	RANKING
4	1	3	-5.95	0.00	55.82	RANKING
5	1	7	-5.60	0.00	48.83	RANKING
6	1	4	-5.29	0.00	55.45	RANKING
7	1	2	-4.84	0.00	53.00	RANKING
8	1	8	-4.72	0.00	54.85	RANKING
9	1	10	-4.55	0.00	57.81	RANKING
10	1	1	-4.50	0.00	44.68	RANKING

Donepezil 3D structure retrieved from PubChem with the compound ID "3152" is shown in Fig

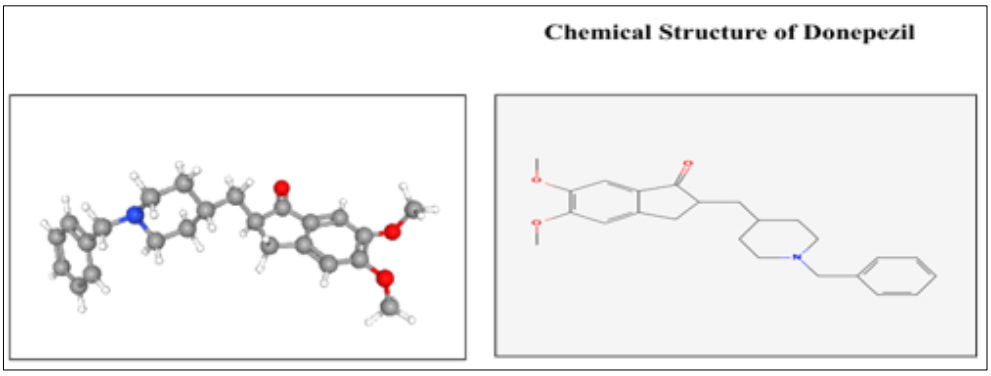

Fig 1: Chemical structure and Ball and stick model of Donepezil

The studies were in corroboration Ferreira *et al*., 2015 [12] who stated that Donepezil is a selective acetylcholinesterase inhibitor that is widely prescribed for Myasthenia Gravis (MG), Alzheimer's disease (AD). It has been shown to be of benefit in mild, moderate and severe stages of MG, AD, vascular dementia and dementia associated with Parkinson's disease. The main mechanism of action through which it influences cognition and function is presumed to be the inhibition of acetylcholinesterase enzyme in the brain. Donepezil not only acts at the neurotransmitter level, but also at the molecular and cellular level in almost all stages involved in the pathogenesis of MG, including the inhibition of various aspects of glutamate-induced excitotoxicity, the reduction of early expression of inflammatory cytokines, the induction of a neuroprotective isoform of AChE and the reduction of oxidative stress-induced effects.

Conclusion

Acetylcholinesterase (AChE) plays a pivotal role in terminating cholinergic signaling and has been strongly implicated in the pathophysiology of neuromuscular and neurodegenerative disorders, including Myasthenia gravis (MG). In this study, molecular docking was employed to assess the potency of selected AChE inhibitors against the AChE enzyme (PDB ID: 7D9O). Comparative binding affinity analysis revealed that Donepezil exhibited the strongest binding interaction with AChE, with a binding energy of –8.75 kcal/mol, surpassing other tested ligands. These findings highlight Donepezil as a promising AChE inhibitor with potential therapeutic value in MG, supporting its role in enhancing acetylcholine availability at the neuromuscular junction. The observed high binding affinity suggests that Donepezil could effectively stabilize the AChE–ligand complex, thereby prolonging neurotransmitter activity. While these in silico results provide important mechanistic insights, further validation through *in vitro* and *in vivo* studies is required to confirm its efficacy and safety in MG management. Overall, this study underscores the therapeutic relevance of AChE inhibitors, particularly Donepezil, and provides a molecular framework for the design and optimization of next-generation inhibitors targeting neuromuscular autoimmune disorders

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