Huperzine A with Idebenone: Using Molecular Docking to analyze a potential Combination Therapy for Alzheimer's Disease by Inhibition of the Acetylcholinesterase Enzyme

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Abstract:

Those suffering from Alzheimer's disease (AD) have low concentrations of Acetylcholine (ACh), a neurotransmitter involved in learning, memory, and muscle contraction.[1] Acetylcholinesterase (AChE), a cholinergic type enzyme, plays a crucial role in concluding neurotransmission and degrading ACh.[2] The accumulation of the AChE protein in AD patients results in decreased levels of ACh, and contributes to the buildup of amyloid-beta (Aβ) plaques that are crucial in the onset of AD.[3] This study aims to examine how Idebenone could work as a potential inhibitor in addition to Huperzine A for AChE inhibition as a possible combination therapy for Alzheimer's Disease. Structure files of the inhibitors and AChE (PDB ID: 1VOT) were manipulated using PyMOL, GNINA, and Google Colab for molecular visualization and docking to find bond energies and possible ligand-protein interactions. The results confirmed that Idebenone has a significant binding affinity, though relatively lower than Huperzine A and in a different location, to be a useful component in this combination therapy due to its variety of interactions within AChE's binding cavity including hydrogen bonds, hydrophobic and aromatic interactions. Huperzine A, with its stronger binding affinity, also exhibited positive interactions with amino acids surrounding the binding cavity of AChE, including hydrophobic and aromatic interactions. Most notably, the placement of each of these molecules did not significantly impact one another. Therefore, we can conclude that when used in conjunction, the two inhibitors target distinct locations in the wide binding pocket of AChE, and complement one another in the areas each can not cover alone. Their neuroprotective qualities also aid in the treatment of the disease.[4,5,6] Future research could explore longer inhibitors to optimize binding within AChE's binding cavity and test the efficacy of this combination therapy on other neurodegenerative diseases involving cholinergic dysfunction.

Keywords: Alzheimer's Disease, Combination Therapy, Huperzine A, Idebenone, Molecular Docking, Acetylcholinesterase Inhibition, Cholinesterase Inhibitors.

Introduction:

Alzheimer's disease (AD) is a complex neurodegenerative disorder characterized by clinical dementia and prominent memory impairments.[7, 8] It is the most common form of dementia, accounting for approximately 50-70% of all dementia cases either by itself or in combination with other disorders.[8] Those suffering from AD have low levels of Acetylcholine (ACh), a neurotransmitter released by the brain that regulates memory, focus, and learning.[1, 9] When released by neurons in the extracellular space, acetylcholine binds to receptors on other neurons and activates sodium-ion pumps to generate electrical impulses.[10] Acetylcholine is the primary neurotransmitter in the parasympathetic nervous system while it is found in trace amounts in the central nervous system where it helps in preserving memories and moving memories from short to long-term storage.[10] As a treatment for Alzheimer's Disease, injecting calculated doses of acetylcholine directly into the central nervous system is therapeutically difficult.[11] Therefore, most of the drug therapies for AD use medications to inhibit the activity of Acetylcholinesterase to maintain ACh levels.[11]

Alzheimer's disease involves several pathological processes including amyloid-beta accumulation, oxidative stress, and neuroinflammation.[12] Monotherapies only target one of these pathological processes, failing to address the complicated interactions between them, and do not often cause the desired effect in the disease due to its multifactorial origin.[12] Given the polygenic and multi-faceted nature of AD, combination therapies have been increasingly recognized for

their potential to provide a comprehensive approach to the disease's treatment.[13, 14] Cholinesterase inhibitors, the primary inhibitors that help regulate ACh levels in AD patients, when combined with other drugs, can improve cognitive function and slow the rate of disease progression more effectively as compared to single-drug therapies.[15] Our study aims to analyze a potential combination therapy for AD with Huperzine A and Idebenone as inhibitors for the Acetylcholinesterase enzyme using molecular docking and visualization.

Acetylcholinesterase (AChE) is a glycosylphosphatidylinositol-linked (GPI-linked) glycoprotein that exists as a dimer in the erythrocytic membrane, an enzyme of the cholinergic type.[2, 16, 17] AChE's primary function is to break down ACh into choline and acetic acid, and carefully regulate its concentration at synapses in the parasympathetic nervous system.[2, 16]

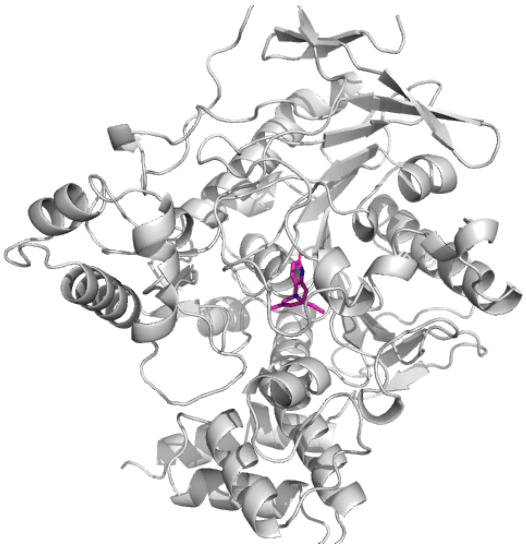


Figure (1): Acetylcholinesterase (gray) is shown in complex with inhibitor Huperzine A (magenta)

In patients with AD, overactivity of AChE leads to a decrease in ACh concentration, which in turn causes degeneration of the cholinergic system.[18] Moreover, the abundance of AChE also

contributes to the aggregation of amyloid-beta (Aβ) peptides and amyloid plaque formation.[19] As Aβ accumulation is one of the pathological processes of AD, in brains impacted by AD, there are large vascular deposits of insoluble fibrillar Aβ linked with death of cellular elements constituting brain cells and impairment of the blood-brain barrier.[20] The dual role of AChE in cholinergic dysfunction and amyloid-beta pathology makes it a significant target in drug therapies for AD.

Huperzine A, a naturally occurring sesquiterpene alkaloid compound found in the club moss and Chinese herb *Huperzia serrata*, is a known inhibitor for AChE that can penetrate the blood-brain barrier.[5] In addition to inhibiting AChE and reducing the risk of harmful interactions with oxygen, the inhibitor's healing and neuroprotective qualities help reduce and reverse the symptoms of AD.[4,5] A double-blind study conducted to assess the effectiveness of Huperzine A in AD treatment proved that the drug therapy considerably enhanced cognitive and task-switching abilities, relative to pre-medication performance of the patients.[21]

Huperzine A shares these qualities with another potential inhibitor for AChE called Idebenone, which is a synthetic quinone structurally similar to the naturally occurring antioxidant CoQ10, with an additional hydroxyl group.[22] It is used in the treatment of several other neurological conditions like Friedreich ataxia (FRDA), senile dementia, and Huntington's disease.[22] A clinical evaluation of Idebenone treatment for AD patients, 45mg given orally daily for 4 months, demonstrated that the drug was well-tolerated and significantly improved memory, behavior, and attention.[6]

Though as with many other treatments of AD, early targeting is crucial for positive, long-term outcomes. Notably, outcomes are significantly improved when Idebenone is administered orally in nanorod formations that maximize available compliance and improve bioavailability while reducing necessary dose sizes due to increased efficiency of drug delivery.[23] Another benefit of Idebenone is its nanorod's distinct ability to penetrate the blood-brain barrier (which is often disturbed in AD patients).[23]

Huperzine A and Idebenone share a similar carbon skeleton, obtaining structural length from their aromatic rings. The arrangement of carbon rings and polar atoms like oxygen and nitrogen in their molecular structures hints at possible hydrophobic interactions and hydrogen bonds with amino acids in AChE's binding cavity. Idebenone features an elongated structure compared to Huperzine A, which can relatively take up more space within the binding cavity of AChE. Thus, it can be hypothesized that in the inhibition of AChE, Idebenone will perform similarly, if not better than Huperzine A due to its similar structure with additional carbon rings to create hydrophobic interactions as well as similar oxygen and hydrogen groups and a shared protective quality and ability to penetrate the blood-brain barrier as a result of more effective distribution via nanorods.

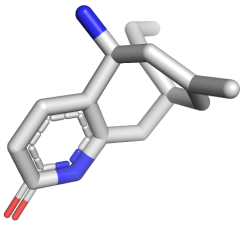


Figure 2(a): molecular structure of Huperzine A

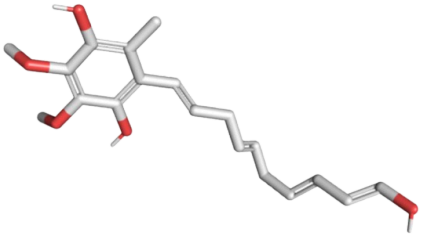


Figure 2(b): molecular structure of Idebenone

Methods:

Protein-ligand docking predicts a ligand's binding pocket with a protein by visualizing it in a "box". The box, generated based on the binding pocket's size or through trial and error, allows the program to check every residue for compatibility. Our team used GNINA and Google Colab to generate boxes for molecular docking, aiming for the best RMSD (Root Mean Square Deviation - the measure of the average distance between the atoms of superimposed molecules) values.[24]

The PDB ID (1VOT) of AChE was used as the structure for molecular docking and was first cleaned in PyMOL by removing the excess water molecules.[25] AChE's ligand in its crystal structure, Huperzine A, was extracted and saved as a separate PDB file from the protein receptor before being uploaded into GNINA for redocking to test the accuracy of our results and verify that the same program would be effective in examining the binding of Idebenone, a ligand without an existing crystal structure within AChE. A box size of 50x50x50Å was used with a box center of 5, 66, and 63 (X, Y, Z) for unbiased docking that encompasses the entire area immediately surrounding the inhibitor. Idebenone's structure, originally unavailable in the PDB database, was converted into a PDB file. It was then used for biased docking on GNINA with a box size of 40X40X40 angstroms and a box center of 0, 67, and 68 (X, Y, Z) for biased docking that encompasses a specific, targeted area surrounding the inhibitor.

Results were then visualized via PyMOL (Figures 3 and 4) and the binding affinities for the top nine poses were produced for Idebenone and Huperzine A respectively (Table 1).

Results:

Huperzine A, when re-docked to AChE, produced positive results in interactions with amino acids surrounding the binding cavity of AChE. Some of the notable interactions as shown in Figure 3 were hydrophobic (4.6Å and 3.9Å apart from Phenylalanine-331 and Glycine-119 respectively) and aromatic (4.2Å apart from Tryptophan-84).

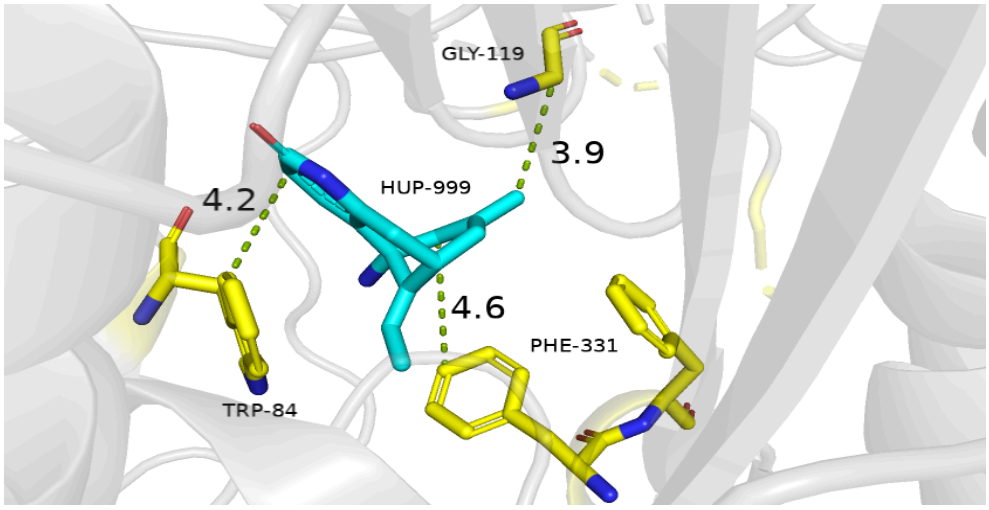


Figure (3); Huperzine A (teal) interactions with Acetylcholinesterase residues (yellow)

Idebenone, on the other hand, produced a variety of interactions due to its elongated structure with additional hydrogen and oxygen atoms. Figure 4 shows two hydrogen bonds (3.2Å and 2.4Å apart with Asparagine-280 and Serine-286 respectively), two hydrophobic interactions (3.5Å and 3.7Å apart with Phenylalanine-330 and Phenylalanine-331 respectively), and an aromatic interaction (3.9Å apart with Tryptophan-279).

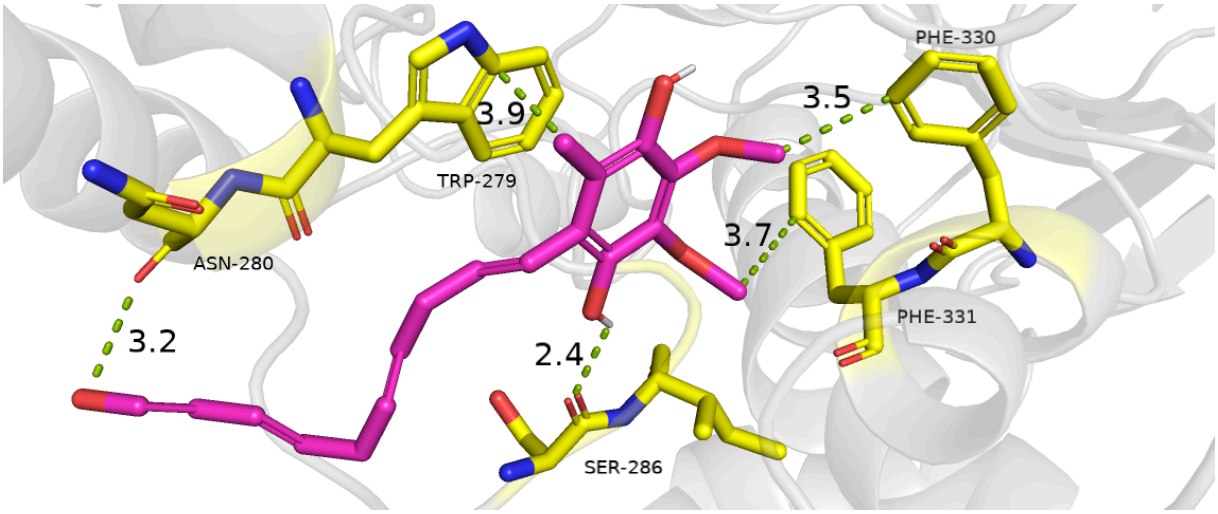


Figure (4); Idebenone (magenta) interactions with Acetylcholinesterase residues (yellow)

Table (1) shows the binding energies of the two inhibitors calculated using GNINA.

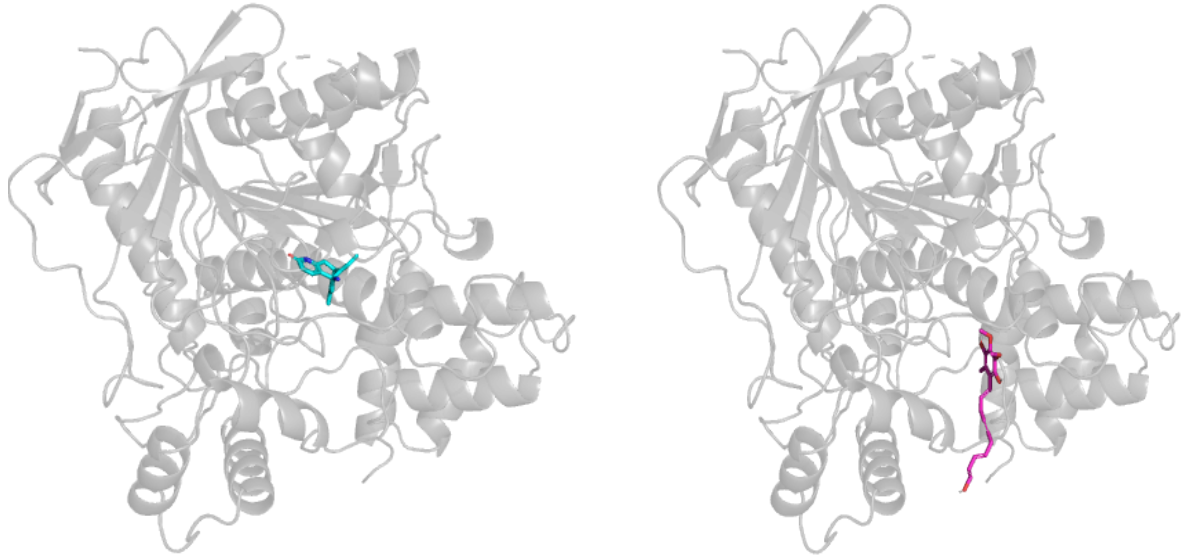
Idebenone	
Pose	Affinity (kcal/mol)
1	- 6.48
2	-4.55
3	-4.30
4	-4.24
5	-6.57
6	-5.75
7	-4.45
8	-5.27
9	-5.93

Huperzine A	
Pose	Affinity (kcal/mol)
1	-11.01
2	-10.23
3	-10.34
4	-4.60
5	-9.78
6	-6.58
7	-4.79
8	-6.42
9	-6.99

Table (1): Binding energies (kcal/mol) of Idebenone and Huperzine A produced from GNINA.

Energies are listed in kcal/mol and a more negative value indicates a more favorable binding of the inhibitor to the enzyme. In pose 1, Idebenone exhibits a relatively good binding energy of -6.48 kcal/mol, though not as strong as Huperzine A's -11.01 kcal/mol. Idebenone's binding affinity is still significant and suggests that it can complement Huperzine A in combination therapy for AD due to its positive interactions with AChE and other important qualities.

Figures 5(a) and (b) indicate the different locations Huperzine A and Idebenone take in the binding cavity of AChE. Huperzine A interacts with amino acids deep down in the binding cavity and more centrally relative to the protein itself while Idebenone interacts with residues at the mouth of the binding cavity near the exterior edge of the protein.

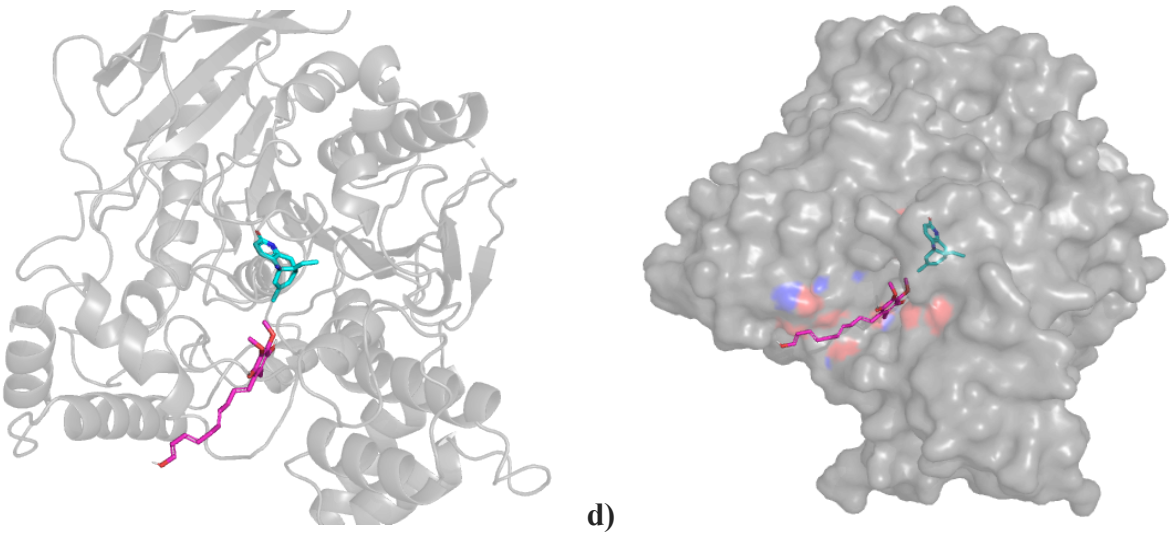


a) **b)**

Figure 5(a): independent docking of Huperzine A (teal) with AChE (gray), 5(b): independent docking of Idebenone (magenta) with AChE (gray)

When Huperzine A and Idebenone are co-docked into AChE as shown in Figure 5(c) and (d), it is apparent that even though the binding cavity is not entirely filled, both of the inhibitors

complement each other by covering different areas collectively than any one of them can do individually.



c) d)

Figure 5(c): co-docking of Huperzine A (teal) and Idebenone (magenta) with AChE (gray), 5(d): surface view of AChE (gray) binding cavity when Huperzine A (teal) and Idebenone (magenta) are co-docked

Discussion:

Combination therapies for AD have garnered considerable support over time as they tend to produce better outcomes in terms of cognition, daily life activities, global outcomes, and behavior in patients.[26] For example, the combination therapy of donepezil, a cholinesterase inhibitor, with memantine (an N-methyl-D-aspartate receptor antagonist of glutamate - the primary excitatory neurotransmitter in the human brain) demonstrated a significant improvement in the cognitive function of patients with mild to severe AD.[26] *The International Journal of Alzheimer's Disease & Associated Disorders* reports in different articles the study of donepezil and memantine combination therapy which involved 400 patients subjected to tests in 24 weeks

of treatment, showed signs of improvement in Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory, and clinicians reported improvement based on the individualized interviews with their patients.[27, 28]

Moreover, another study demonstrated that after four years of combined drug therapy with cholinesterase inhibitors and memantine, participants showed signs of slow cognitive and functional decline in AD as compared to no treatment and monotherapies involving cholinesterase inhibitors only.[29] The benefits were observed to increase with treatment duration.[28]

In light of the aforementioned studies, the combination therapy of Huperzine A and Idebenone can also offer a potentially powerful therapeutic approach for AD considering their complementary mechanism of action as suggested by the results. Comparative analysis of the binding affinities and ligand-protein interactions confirms the initial hypothesis of Idebenone being a potential inhibitor in addition to Huperzine A as a combination therapy for AD by inhibition of the AChE enzyme.

Huperzine A showed a strong binding affinity and positive interactions with amino acids surrounding the binding cavity of AChE, including hydrophobic and aromatic interactions. Idebenone, with its elongated structure, showed a relatively good binding affinity although not as strong as Huperzine A, and exhibited various hydrogen bonds, and hydrophobic and aromatic interactions in AChE's binding cavity.

Idebenone's binding affinity with AChE, though relatively lower than Huperzine A, is still significant enough for it to be a useful component of the combination therapy as it has also been regularly used as a treatment for AD with nanorod delivery methods. It will contribute positively

by interacting more diversely with residues of AChE, and complementing the stronger binding affinity of Huperzine A with its additional qualities such as antioxidant activity and mitochondrial function improvement.

When used in conjunction, the two inhibitors do not entirely occupy the wide AChE binding cavity. However, they cover significant areas in different locations and more area than either single molecule could cover alone, suggesting potential synergistic effects.

The two inhibitors are also known for their unique qualities that can help alleviate symptoms of AD. Huperzine A increases cholinergic transmission and improves memory and cognitive function in AD patients with no severe side effects.[30] Furthermore, its neuroprotective qualities provide a line of defense against neuronal damage by protecting neurons from hydrogen peroxide, β-amyloid protein, glutamate, ischemia, and staurosporine-induced cytotoxicity and apoptosis.[31]

On the contrary, Idebenone, known for its potent antioxidant properties, helps neutralize oxidative stress, which is one of the pathological processes of AD and a critical factor in the disease's progression.[32, 33] Moreover, it also improves mitochondrial function, and thus, enhances cellular energy metabolism which is often debilitated in patients with AD.[32, 33] When used in conjunction with early detection, both of these drugs have the potential to minimize the impacts of AD. This combination therapy could address both the symptomatic and pathological aspects of AD more effectively than either drug can do individually.

Despite the promising synergistic effects this combination therapy implies, there might be challenges in its practical applications in real life. Idebenone is usually delivered via nanorods while Huperzine A is used in the forms of tablets and capsules.[23,34] Combining

nanotechnology-based formulations with conventional tablets or capsules might complicate the drug formulation and delivery processes, though both of these drugs can feasibly be contained within a single capsule to simplify drug delivery. Given the similar carbon skeletons and functional groups, there can also be negative drug-drug interactions between Huperzine A and Idebenone. This can decrease the efficiency of the inhibition process, thereby, slowing the rate of drug action. Therefore, the safety and efficacy of this combination therapy need to be addressed through further preclinical and clinical studies before practical use.

Conclusion:

Our study supports the potential of Idebenone as a complementary inhibitor to Huperzine A for the inhibition of AChE as a combination therapy for AD. Using molecular docking, we demonstrated that Idebenone effectively interacts with amino acids within AChE's binding cavity, potentially inhibiting AChE activity, thereby, increasing ACh levels. When used in conjunction, the two inhibitors cover distinct locations in the binding pocket and complement each other's mode of action in the inhibition process. Their healing and neuroprotective qualities also aid in the treatment of AD.

The binding cavity of AChE offers a wide space, and future pharmaceuticals may benefit from a longer molecule that fills the entire binding cavity. Accurate spatial dimensions for long inhibitors and their binding energies and ligand-protein interactions will have to be calculated using relevant tools (PyMOL, GNINA, and AutoDock Vina, etc.) to maximize efficient inhibition activities within AChE's binding pocket. Future research may also explore the

possibility of using this combination therapy for other neurodegenerative diseases involving cholinergic dysfunction such as dementia with Lewy bodies (DLB) and Parkinson's disease.

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