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The discovery of new acetylcholinesterase inhibitors as potential therapeutics for Alzheimer's disease

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Abstract

Alzheimer's disease is an irreversible, progressive neurodegenerative disease that is the most common form of dementia. It has been shown that the neurotransmission controlled by acetylcholine plays a critical role in nervous system function, and the lack of this neurotransmitter contributes to Alzheimer's disease. There are numerous ways to design therapeutics for Alzheimer's disease, one of which includes the inhibition of the enzyme acetylcholinesterase. The aim of this work was to identify new inhibitors of acetylcholinesterase that would serve as starting points to designing more efficient inhibitors. Computational studies were employed to virtual screen potential inhibitors and analyze their interactions with acetylcholinesterase. As a result, potential inhibitors that bind more favorably to acetylcholinesterase compared to currently approved inhibitors and have appropriate drug-like properties for human use were discovered. This work pioneers future research efforts seeking to develop new therapeutics for Alzheimer's disease.

Keywords

Alzheimer's Disease, Acetylcholinesterase, Virtual Screening, Drug Discovery, Therapeutics

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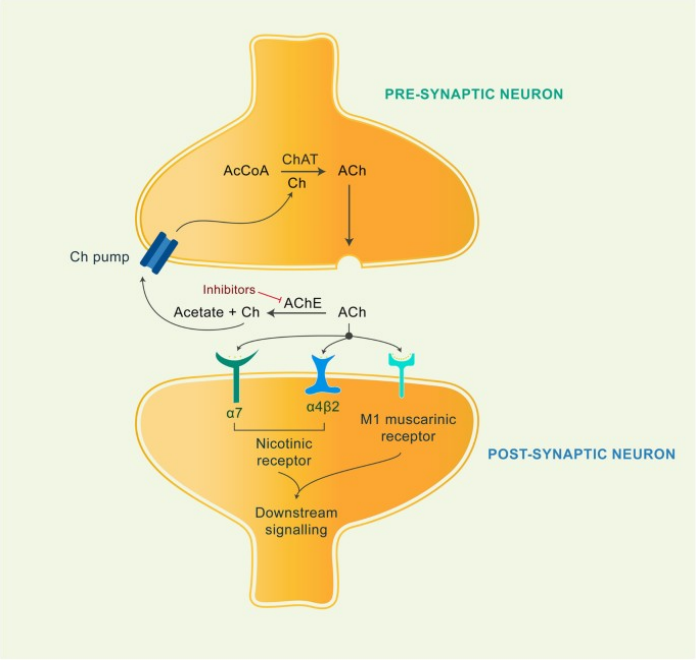
Introduction

Alzheimer's disease (AD) is an irreversible, progressive neurodegenerative disease that is the most common form of dementia (1-3). Dementia is the general term describing patients who experience physiological symptoms such as cognitive decline, delusions, hallucinations, and so on (1-3). It has been estimated that about 50 million people are currently living with AD worldwide, with the number of cases of dementia projected to double every five years and to reach 152 million by 2050 (1). Furthermore, between 2000 and 2019, the number of people dying from AD worldwide has increased by 145.2% (4, 31), and AD is the third major leading cause of death among elders (5). With the global population aging, it is crucial to locate the exact cause of AD and develop viable and versatile drugs to prevent or cure AD.

While researchers attempt to fully understand the cause of AD, there are three main hypotheses: Cholinergic Hypothesis, Tau Hypothesis, and Amyloid Hypothesis. The Cholinergic Hypothesis suggests that the decrease in the concentration of the neurotransmitter acetylcholine (ACh) in the brain contributes majorly to cognitive decline, which is one of the hallmark symptoms of AD (6). It has been shown that enzymes such as acetylcholinesterase (AChE) decrease the presence of ACh by hydrolyzing it into acetate and choline (10). Furthermore, according to numerous antemortem and post-mortem studies in those afflicted with AD, a large number of aberrations regarding cholinergic pathways such as changes in the movement of choline, the release of ACh, and nicotinic receptor expression present themselves as convincing evidence for the Cholinergic Hypothesis (6). Figure 1 shows the mechanism of ACh becoming hydrolyzed by AChE into acetate and choline.

Figure 1: The hydrolysis of ACh to acetate and choline facilitated by AChE (1).



The Tau Hypothesis argues that the hyperphosphorylation of the tau protein, which is involved in maintaining the stability of microtubules in the axons of neurons (7), is the major cause for the development of AD (5, 8). This alternate form of the tau protein aggregates together to form neurofibrillary tangles (NFT), leading to neurodegeneration (7). In post-mortem studies of patients with AD, researchers found that NFT occurred in places where brain degeneration was the most significant and in regions that explained the symptoms of AD, such as those associated with language and memory. However, several therapies that prevented the buildup of tau proteins failed to pass the clinical trials. For example, Tideglusib, an inhibitor of the tau hyperphosphorylation contributor glycogen synthase kinase 3, did not demonstrate significant changes in patients with AD (16).

Finally, according to Amyloid Hypothesis, the buildup of amyloid-β (Aβ) protein plaques from aggregates of misfolded Aβ found in between neurons is the primary cause of AD (8). These misfolded Aβ are produced by mutated versions of enzymes such as β-secretase and γ-secretase, which cut the amyloid precursor protein (APP) that Aβ derives from. Some caveats with regards to this hypothesis include studies that found that there are normal patients who have a significant amount of Aβ plaques and patients with AD who have little Aβ plaques (14). Meanwhile, some researchers suspect that all three hypotheses are contributors to AD. For instance, both Aβ and tau proteins can be found in cholinergic neurons located in the basal forebrain system in early AD. Likewise, abnormal cholinergic neurons may contribute to alterations in APP metabolism and tau hyperphosphorylation, leading to neuroinflammation and neuronal death (34).

Currently, there are no medications that cure AD; however, numerous drugs have been developed to decrease the rate of clinical decline, while others aid in lessening the cognitive symptoms of AD related to memory, thinking, language (9). The majority of the drugs approved has been targeting AChE from the Cholinergic Hypothesis. Donepezil (Aricept®) is an AChE inhibitor that treats mild to severe stages of AD. By competitively binding to the peripheral anionic site of AChE, Donepezil has been shown to improve the cognitive function of patients with severe AD (9). The inhibitory potency (IC_{50}) towards AChE is 55.0 nM (32). Moreover, the drug has 100% bioavailability as it crosses the blood-brain barrier easily and has slow excretion rates (10). The side effects of Donepezil include nausea, muscle cramps, diarrhea, anorexia, and abdominal pain (9, 10). Rivastigmine (Exelon®) is a slow-reversible inhibitor that decreases the activity of AChE by binding at the esteratic part of the active site (10). It generally treats mild to moderate cases of AD and is able to slow the decline of cognitive function, as well as improve activities of daily living. If ingested orally as capsules or liquids, Rivastigmine has a bioavailability of 40%. Common side effects are similar to those of Donepezil. The inhibitory potency (IC_{50}) of Rivastigmine towards AChE is 510.0 μM (32). Finally, in June 2021, the Food and Drug Administration (FDA) approved Aducanumab (Aduhelm™) as a treatment for AD (11). As an Aβ-directed monoclonal antibody, Aducanumab delays or reduces the buildup of Aβ by prompting the immune system to break up and remove the plaques, thus delaying the clinical decline in AD patients (11, 17). Common side effects include headaches, amyloid-related imaging abnormalities, confusion, delirium, diarrhea, and so on (11). The inhibitory potency (IC_{50}) of Aducanumab towards Aβ is >1000 nM (33).

The purpose of this work is to focus on the Cholinergic Hypothesis by identifying new inhibitors of AChE that would serve as starting points to designing more efficient inhibitors. As it has been established that a sustained decrease in ACh is directly correlated to neurodegeneration and AD, preventing AChE from binding to ACh may lead to favorable outcomes for patients with AD. The importance of this project cannot be understated, as it can bring unprecedented breakthroughs in the treatment of Alzheimer's disease.

Literature Review

AD was first discovered in 1901 by German psychiatrist and neuropathologist Alois Alzheimer, who treated a patient who claimed to have memory problems, uncontrollable anger, and paranoia (22, 24). Even though Alzheimer proposed his idea of a clinical syndrome related to the pathology of the brain as early as 1906, it was not received well by his peers (22, 25). The research for AD became mainstream only after influential papers published by Sir Martin Roth (Roth et al., 1967) and Gary Blessed (Blessed et al., 1968) demonstrated the pervasiveness of NFT and Aβ in late-onset dementia (26).

There are various drugs that have promising prospects for the treatment of AD, one of which being Lecanemab by companies Biogen and Eisai. Lecanemab is an antibody that binds to the toxic form of Aβ, effectively serving as a "label" so that the immune system can more easily identify and remove that Aβ (30). In the Phase 2 trial of 856 participants that would determine its safety and tolerability, Lecanemab showed the most optimistic results at the twice-monthly (10 mg/kg) dose over 18-months (30). The dose demonstrated a statistically significant lowering of 30% in cognitive decline and a decrease in the rate of buildup of Aβ than that of the placebo group (29). Currently, the drug is in the Phase 3 trial and the researchers are trying to test the safety and efficacy of the drug as a 10 mg/kg dose (29). The study is expected to be completed in 2024 (29).

There are also some drugs in development that are directed at the Tau Hypothesis, with one being Semorinemab (23). This is an antibody developed by Genentech that recognizes abnormal forms of tau and blocks their spread in brain cells (23). In 2017, a Phase 1 trial tested the safety and tolerability of the antibody (23). In the double-blind study, 74 patients with mild to moderate AD received one or multiple injections of Semorinemab or the placebo (21). Though the results were not publicized, Genentech did reveal that there were only minor side effects such as bruising and discomfort at injection sites (21). An ongoing Phase 2 study has recruited about 260 subjects and this study will measure the functional and cognitive abilities of subjects who received Semorinemab compared to placebo after 49 weeks (20). The study is expected to be completed in September 2021 (20).

Finally, there are AChE inhibitors in development to decrease or prevent the hydrolysis of ACh. For example, galangin is a natural inhibitor of AChE derived from *Rhizoma Alpiniae Officinarum* (18, 19). Galangin works by binding competitively to the catalytic site of AChE, and it has an IC_{50} of 120 μm (15, 18). In a research study of the effects of galangin on the spatial memory of rats, doses of 50 mg/kg and 100 mg/kg of galangin were compared to the control of 1 mg/kg of Donepezil (13). Then, the rats' spatial memory was measured by the Morris water maze test (13). The study concluded that galangin could be considered as a possible drug in preventing or delaying AD (13); however, more research is needed to investigate the toxicity of galangin, so there have been no studies done on human participants (19).

Methods

Availability of AChE Binding Sites

In the first experiment, three methods were used to determine available binding sites of AChE: Geometric, Energy-Based, and Machine Learning. Firstly, the Geometric method was being focused on. The website https://proteins.plus/ (35) was used to determine all the binding sites of AChE. The PDB code of AChE, which is "3LII," was entered into the "PDB-code" section. Then, "Go!" was pressed, leading to the ProteinsPlus server (35), which displayed an AChE model on the left and a list of options on the right. In the list of options to the right, "DoGSiteScorer Binding site detection" was selected. "DoGSiteScorer" was then selected and a list of options for settings appeared. The settings were set to as follows: for the "Analysis detail" section, "Pocket(s)" was chosen; for the "Binding site prediction granularity" section, "Druggability" was chosen; for the "Ligands" section, it was left blank; for the "Chain" section, "A" was chosen. After that, "calculate" was selected.

Binding sites of AChE were also determined by using the Energetic method. The website https://ftsite.bu.edu/ (36) was used to find the binding sites of AChE. Putting the website link into the search engine led to the FTSite Server's (36) homepage. Then, a random job name was inputted, and below that "3LII" was entered as the "PDB ID." "A" was inputted for the "PDB Chain ID" section. Then, "Find My Binding Site" was pressed.

Finally, the available binding sites of AChE were determined by employing the Machine Learning method. The website https://prankweb.cz/ (37) was first entered into the browser. Then, "3LII" was typed into the "PDB code" section. From there, "A" was selected in the "Restrict to Chain" section. The server was allowed to run conservation analysis by checking the box beside "Run conservation analysis," and after doing that, "Submit" was pressed and the results were recorded.

Virtual Screening of Potential Inhibitors of AChE

In the second experiment, virtual screening was used to narrow to a smaller number of compounds that can potentially bind and inhibit AChE. Virtual screening was based on pharmacophore maps, which are a map of the interactions between two compounds. First, http://pocketquery.csb.pitt.edu/ (38) was used to determine the strength of the interaction between the compound tested and AChE. After landing on the PocketQuery (38) web page, "Search" was clicked and then "4QWW" was put into the "ID" section. After that, "Enter" was clicked, yielding a list of clusters. Three clusters of chain C and three clusters of chain D with the highest scores were selected.

For each cluster, the "export" button and then the "send to ZincPharmer" button was clicked. After landing in ZincPharmer (43) for each cluster, the "Viewer" tab was clicked and then the "Receptor Residues" was made invisible. After that, the "Pharmacophore" tab was selected. Within the "Pharmacophore" tab, different cluster groups were enabled and/or disabled, and then the "submit query" button was selected to yield the number of hits found. Three hits that had the lowest RMSD scores were clicked and recorded.

Evaluation of Selected Compounds Binding to AChE

In the third experiment, the molecular docking approach identified the degree of binding of each of the 18 compounds from PocketQuery (38) to AchE.
http://www.swissdock.ch/docking# (39) was used to perform this procedure. After landing on the SwissDock (39) website, there are three sections to be filled: Target Selection, Ligand

Selection, and Description. For the "Target Selection" section, "upload file (max 5MB)" was selected. Then, the PDB file for 3LII, which is the PDB code for AChE, was uploaded to SwissDock (39). If a signal indicating "Successful setup" appeared, then the work needed for that section was completed. For the "Ligand Selection" section, the ZINC code for each compound was inputted into the text box. "Search" was then clicked, yielding a new page containing all the ZINC AC hits found. The ligand that corresponded to the ZINC code was selected, and then the "Dock 1 selected ligand" button was clicked. If a signal indicating "Successful setup" appeared, then the work needed for that section was completed. If a signal indicating "ZINC ID not found" appeared, then an alternative approach was taken. http://zinc12.docking.org/ (40) was entered into the search browser. The ZINC code for the specific compound was then inputted into the "Quick Search Bar" of the website. Once inside the page for the specific compound, the SMILES code was copied and pasted into the "INPUT" section of a separate website known as http://www.cheminfo.org/Chemistry/Cheminformatics/FormatConverter/index.html (41). Inside the ChemInfo website (41), the input format was set to "smi -- SMILES format" and the output format was set to "mol2 -- Sybyl Mol2 format." Under the "Generate Coordinates" section, "3D" was selected. Under the "Add or Delete hydrogens" section, "Add" was selected. Finally, the "pH to add hydrogens" section was left blank. "Convert" was clicked, generating an "OUTPUT." The entirety of the output was copied and pasted into a mol2 standard file. From there, in the "Ligand selection" section of SwissDock (39), "upload file (max 5MB)" was selected, and the mol2 file was uploaded. If a signal indicating "Successful setup" appeared, then the work needed for that section was completed. Finally, in the "Description" section, the ZINC code for each compound was entered under the "Job Name (required)" section. Then, an E-mail address was inputted under the "E-mail address (optional)" section. "Start Docking" was selected.

After a few hours, messages containing the link to each output of SwissDock (39) were sent to the inputted E-mail address. The link to the output was opened. The total number of clusters, the most negative estimated Gibbs Free Energy (ΔG), and the pictures of the Figures were recorded in the Results and Discussion.

Identification of the Best Compound as Inhibitor of AChE

In the fourth experiment, SwissADME (42) was used to evaluate how five of the selected compounds based on the most negative ΔG complied with Lipinski's Rule of Five. To start, http://www.swissadme.ch/ (42) was inputted into the search engine. Then, the SMILES code for each of the selected compounds is entered into the "Enter a list of SMILES here" section. Finally, "Run!" was clicked.

Then, a detailed report about each compound was generated. The molecular weight, number of H-bond acceptors, number of H-bond donors, and Log $P_{o/w}$ (LogP) were recorded to determine if any aspects of Lipinski's Rule of Five were violated. Finally, the ΔG of the five compounds were compared to the ΔG of all previously approved drugs by the FDA.

Results and Discussion

Availability of AChE Binding Sites

In the first experiment, three methods were used to determine the available binding sites

for AChE; doing so would ensure that AChE has binding sites for identified inhibitors to bind. The Geometric method determines the binding sites based on the size and shape of AChE. The website https://proteins.plus/ (35) was used because it incorporates the Geometric method when determining the binding sites to AChE. Only Chain A was chosen because Chain A and Chain B are nearly identical. Adding in Chain B would make data more complicated without added value. Thus, including only Chain A makes the data clear and concise for analysis. Figure 2 shows the binding sites of AChE determined using the Geometric method, and Table 1 shows the surface area and volume of binding sites.

Figure 2: The binding sites of AChE determined using the Geometric method.

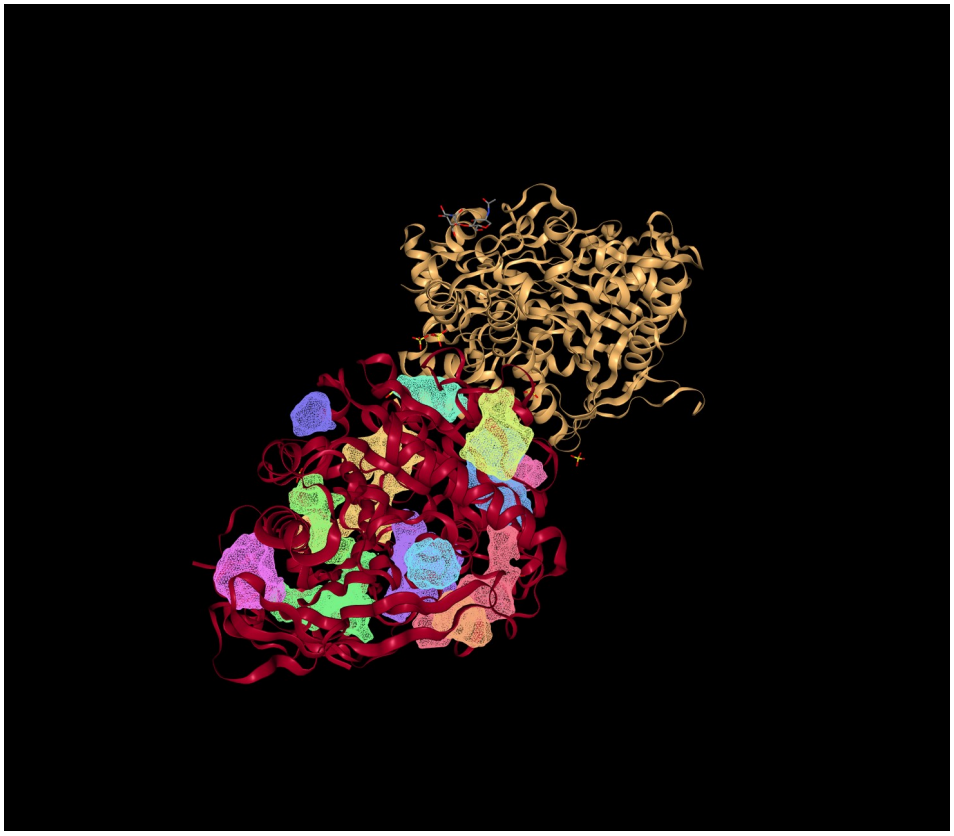


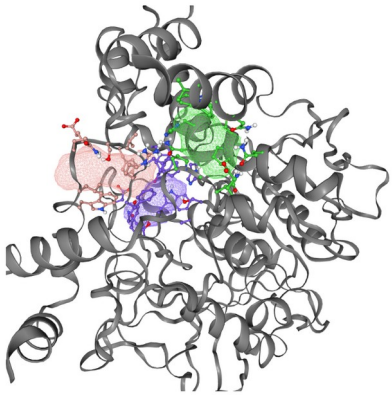
Table 1: The Surface Area and Volume of Binding Sites Determined by the Geometric Method.

Name of binding site	Displayed color	Surface area(Å^2)	Volume (Å^3)
P0		788.91	766.8
P1		664.79	763.41
P2		357.18	430.36
P3		557.46	393.77
P4		496.1	365.91
P5		378.01	255.34
P6		386.69	246.74
P7		202.34	240.04
P8		295.87	157.53
P9		201.82	120.65
P10		133.64	115.22
P11		150.9	103.8
P12		217.42	103.45

From these results, there are 13 available binding sites. It is notable that the binding site with the greatest surface area and volume is P0, which has a surface area of 788.91 Å^2 and a volume of 766.8 Å^3. The binding site with the smallest surface area is P10, with 133.64 Å^2. The binding site with the smallest volume is P12, with 103.45 Å^3.

The Energetic method determines binding sites by energy released when the substrates bind to the enzyme. Since the website https://ftsite.bu.edu/ (36) determines the binding sites using the Energetic method, it was used to generate the data. Figure 3 shows the binding sites of AChE determined using the Energetic method.

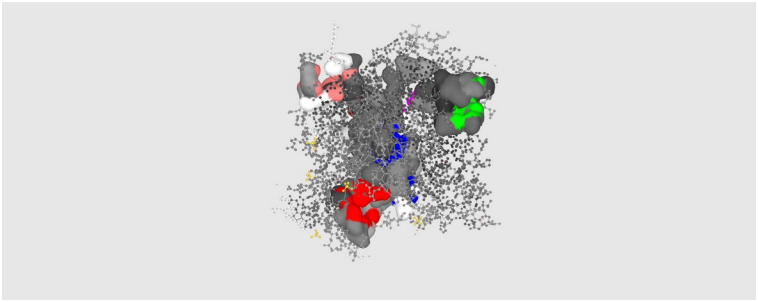
Figure 3: The binding sites of AChE determined using the Energetic method.



The Figure shows that AChE has three binding sites, based on the three distinct colors indicating the sites.

Finally, the Machine Learning method incorporates both the size and the energy released when determining the binding sites of AChE. As the website https://prankweb.cz/ (37) uses both size and energy expenditure when determining the binding sites, it was used to obtain the data. Figure 4 illustrates all the binding sites of AChE determined by this method.

Figure 4: The binding sites of AChE determined using the Machine Learning method.



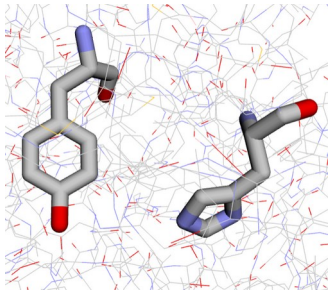
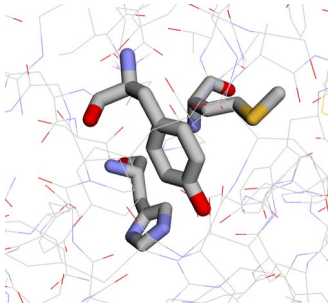
According to the data generated, there are six binding sites to AChE represented by distinct colors.

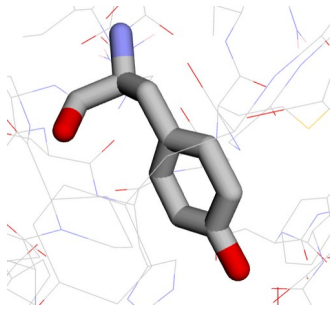
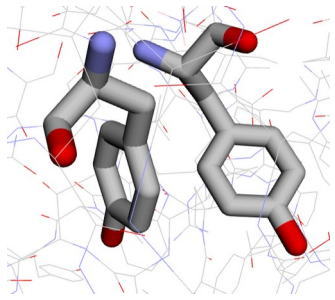
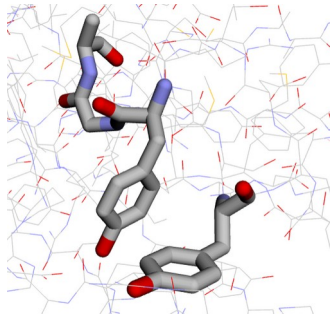
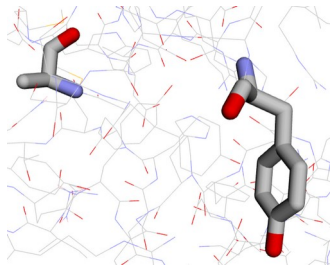
From the experimental results, it was concluded that AChE has numerous binding sites, meaning that there are likely many opportunities to inhibit this enzyme, be it competitively or noncompetitively. However, there are only a very limited number of inhibitors approved by the FDA. This leads to the question of why there are not more inhibitors in use given that there are ample binding sites for inhibitors to decrease or cease the activity of AChE.

Virtual Screening of Potential Inhibitors of AChE

In the second experiment, http://pocketquery.csb.pitt.edu/ (38) was first used to show the strength of the interaction between the compound and AChE, and that strength is represented by a score. PocketQuery (38) determines the strength of interaction by virtual screening and matching the compounds that may bind to AChE through pharmacophore maps. A pharmacophore map is a 3D representation of features that are critical for a ligand to interact with the target receptor of a specific binding site. The strongest interaction has a score of 1, with decreasing strengths leading to lower scores. Virtual screening was completed and three clusters of Chain C and three clusters of Chain D with the highest scores were selected. Chain C and Chain D were used because all pairs of chains to select from were nearly identical. If the other pair of chains were chosen, the outcome would have miniscule differences, if any, that would not affect the outcome of the virtual screening. The scores, along with the models, amino acids, and sizes are showcased in Table 2.

Table 2: The Results of Virtual Screening of Potential Inhibitors of AchE.

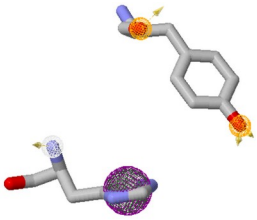
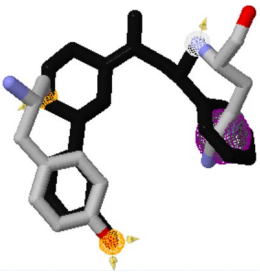
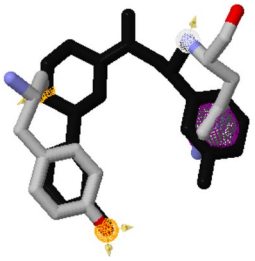
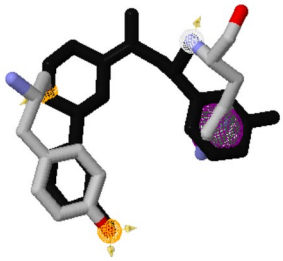
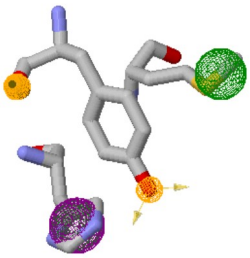
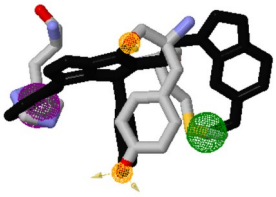
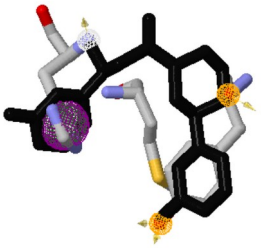
Chain	Size	Amino Acid	Score	Model
C	2	TYR 90 HIS 93	0.986921	
C	3	TYR 90 HIS 93 MET 95	0.984095	

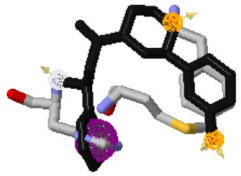
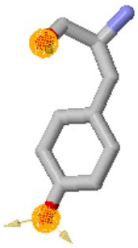
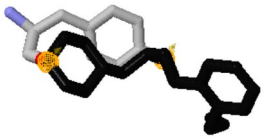
C	1	TYR 90	0.983386	
D	2	TYR 104 TYR 209	0.926622	
D	4	TYR 104 ALA 109 GLY 110 ALA 111	0.925631	
D	2	TYR 104 ALA 111	0.925029	

The table shows that Chain C, consisting of TYR 90 and HIS 93, has the highest score of 0.986921. This indicates the compound and AChE have the strongest interaction. In comparison, Chain D consisting of TYR 104 and ALA 111 has the lowest score of 0.925029. This indicates the compound and AChE have the weakest interaction.

Each cluster was then focused on individually to determine the hits that had the lowest RMSD scores, which indicate the hit that has the greatest overlap with the binding site of AChE. The results are displayed in table 3. The structure in black represents the paired compound while the structure in gray represents the cluster. Table 3 shows each compound's RMSD score, name of hit, and model.

Table 3: The RMSD Scores and the Model of 18 Selected Hits.

Cluster Score + Model	Name of Hit	RMSD Score	Model
0.986921 	ZINC893282241	0.272	
	ZINC893282334	0.272	
	ZINC893282334	0.272	
0.984095 	ZINC36359097	0.226	
	ZINC893282334	0.272	

	ZINC89328241	0.272	
0.983386 	ZINC64726063	0.001	
	ZINC19228765	0.001	
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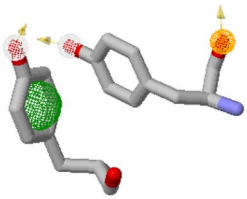
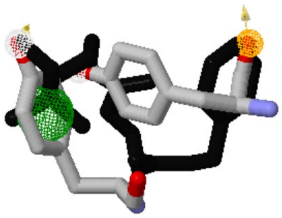
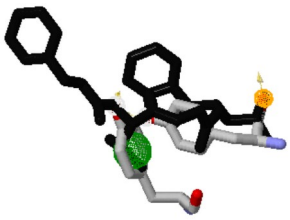
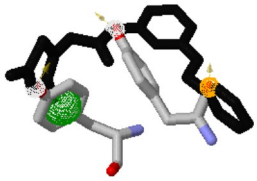
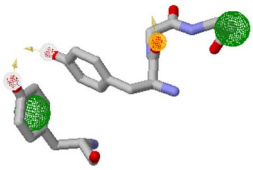
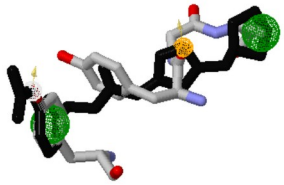
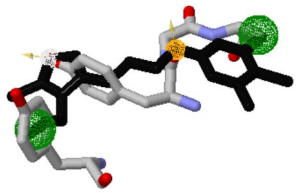
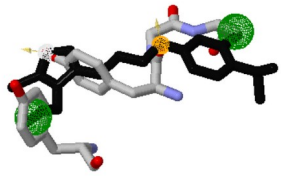
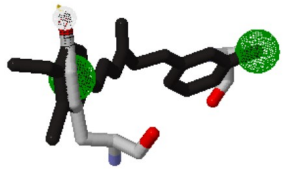
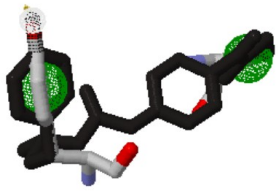
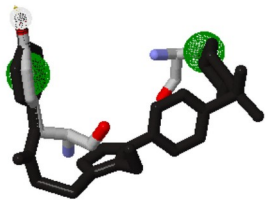
0.926622 	ZINC73981305	0.305	
	ZINC12660148	0.334	

	ZINC42057013	0.376	
0.925631 	ZINC79970264	0.139	
	ZINC64872111	0.158	
	ZINC64872438	0.162	
0.925029 	ZINC05740548	0.005	
	ZINC40104582	0.007	

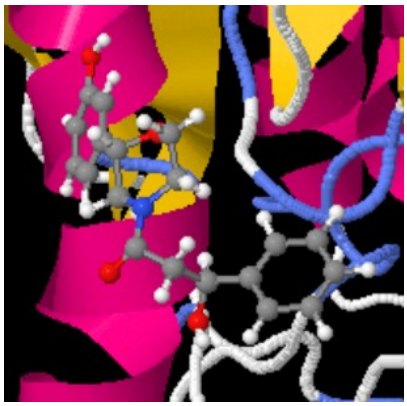
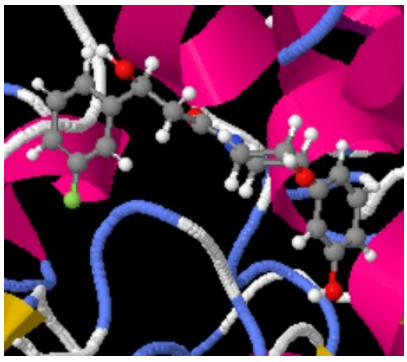
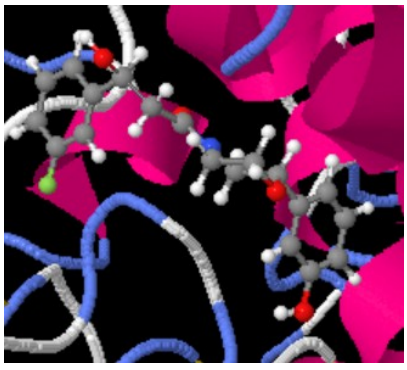
	ZINC36295996	0.007	

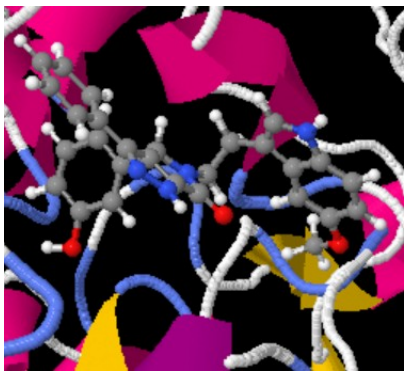
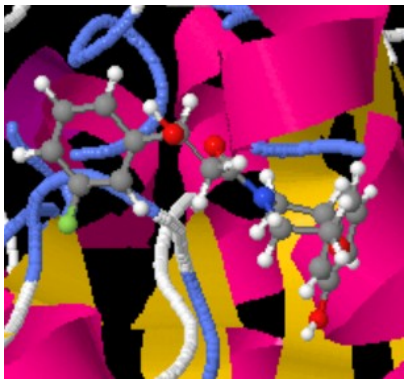
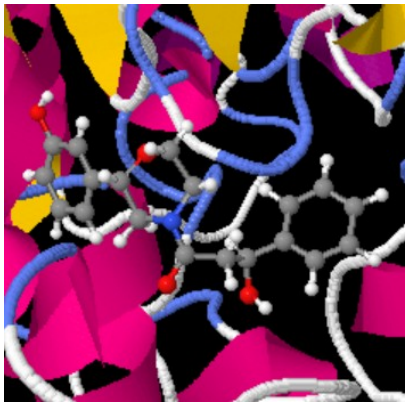
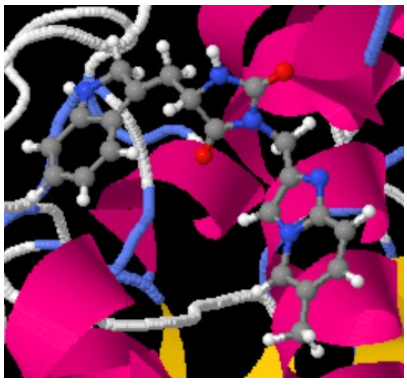
According to this table, the hits ZINC64726063 and ZINC19228765 from the cluster with a score of 0.983386 both have the lowest RMSD scores of 0.001. This represents an almost perfect overlap between the binding site and the hits. In contrast, the hit ZINC42057013 from the cluster with a score of 0.926622 has the highest RMSD score of 0.376. This means that the overlap is the least identical between the hit and AChE. Furthermore, the cluster with a score of 0.983386 has on average the lowest RMSD score, while the cluster with a score of 0.926622 has on average the highest RMSD score.

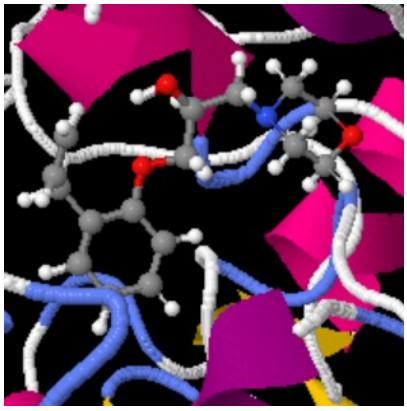
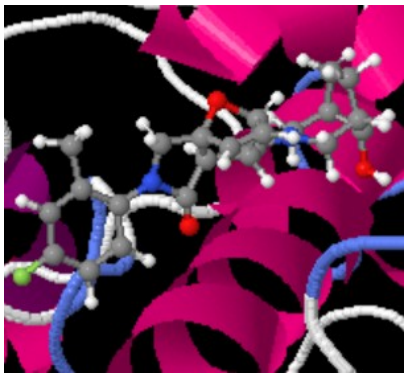
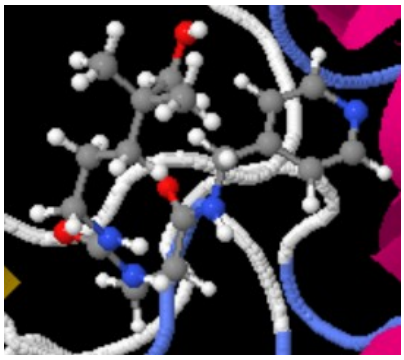
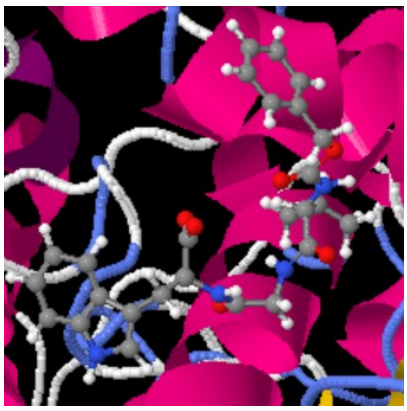
Evaluation of Selected Compounds Binding to AChE

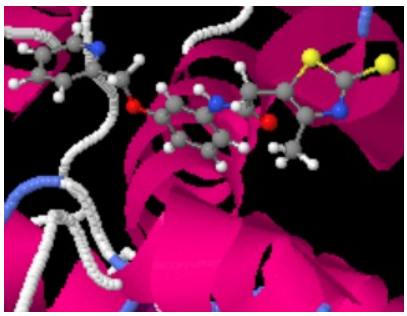
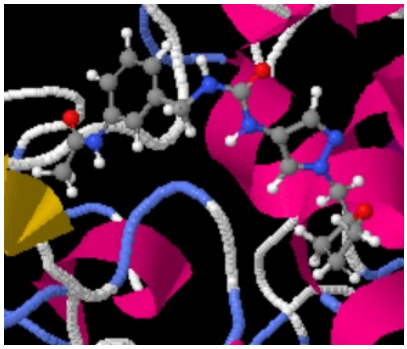
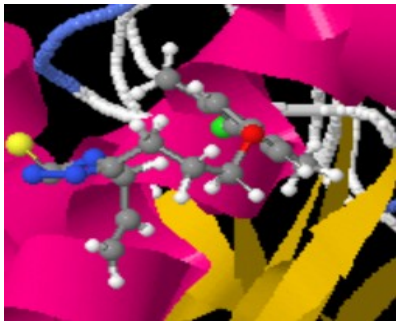
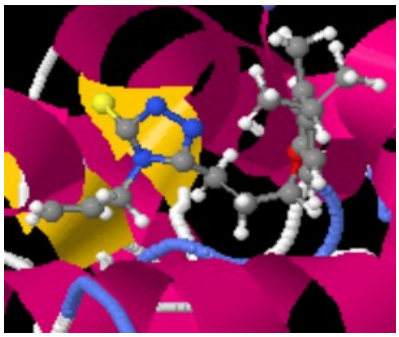
In the third experiment, the SwissDock server [http://www.swissdock.ch/docking#] (39) was incorporated to determine which of the 18 identified compounds is best able to bind to AChE. SwissDock (39) took each of the 18 compounds and AChE and allowed them to freely interact with each other. From this, SwissDock (39) identified the degree of interaction in all available binding sites. The output that SwissDock (39) provided is the number of clusters, which indicates the number of binding sites; the number of elements, which indicates the number of positions the compound can interact with a specific binding site; the Full Fitness and Gibbs Free Energy (ΔG), which both quantify the favorability of the interaction. ΔG will be focused on instead of Full Fitness as it is more universally used by the scientific community. A more negative ΔG indicates a more favorable interaction between the compound and AChE. The results gathered are displayed in Table 4, which shows the ZINC ID of each compound, its number of clusters, estimated ΔG (kcal/mol), and the model. Table 4 shows the models and estimated ΔG values for the 18 selected compounds.

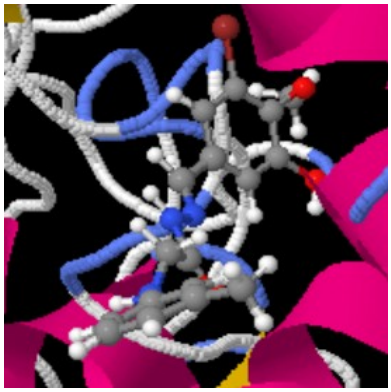
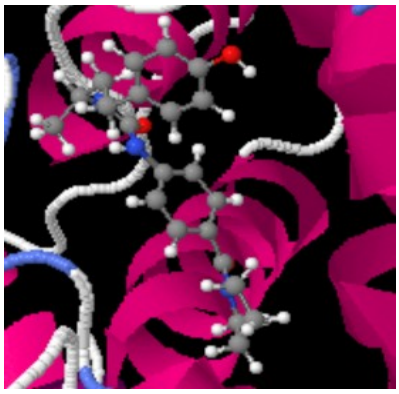
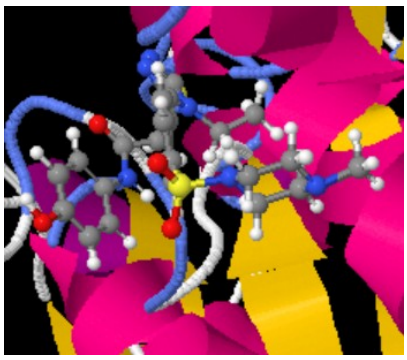
Table 4: Models and Estimated ΔG values for the 18 Selected Compounds.

ZINC ID	Number of Clusters	Estimated ΔG (kcal/mol)	Model
ZINC89328241	44	-7.48	
ZINC89328234	55	-7.61	
ZINC89328234	47	-8.02	

ZINC36359097	35	-7.80	
ZINC89328234	46	-7.60	
ZINC89328241	40	-7.61	
ZINC64726063	40	-10.16	

ZINC19228765	53	-7.44	
ZINC77439010	48	-8.45	
ZINC73981305	48	-8.11	
ZINC12660148	35	-10.11	

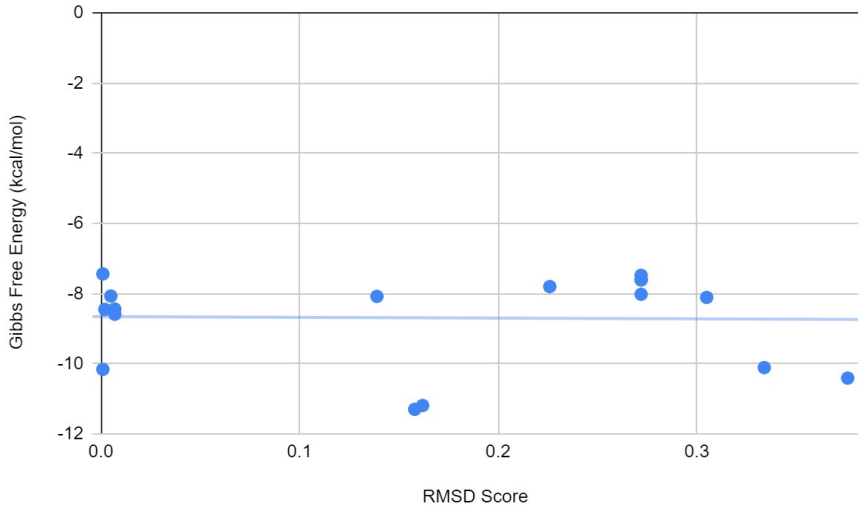
ZINC42057013	40	-10.41	
ZINC79970264	47	-8.08	
ZINC64872111	48	-11.30	
ZINC64872438	43	-11.19	

ZINC05740548	43	-8.07	
ZINC40104582	52	-8.44	
ZINC36295996	38	-8.59	

From the results, the compound ZINC64872111 had the most negative estimated ΔG of -11.30 kcal/mol. This suggests that ZINC64872111 binds to AChE the most favorably. The compound ZINC89328234 has the lowest estimated ΔG of -7.60 kcal/mol. This suggests that ZINC89328234 binds to AChE the least favorably. From this data, it was concluded that we do have compounds that are capable of binding to AChE and the most promising prospect is ZINC64872111. Furthermore, the range of clusters between the 18 compounds is 35 to 55. This may seem like a discrepancy from the first experiment regarding the predicted availability of AChE binding sites, which was only from 3-13. However, both chains of AChE were used in this experiment, whereas only Chain A was used in the first experiment. As a result, the discrepancy is expected and shows that the Geometric method is likely the most accurate method compared to the Energetic method and Machine Learning method in the first experiment. Another aspect to address is the

potential relationship between the ΔG and RMSD scores of compounds. This is shown in Figure 5.

Figure 5: The Relationship between RMSD Score and Gibbs Free Energy (kcal/mol).



From Figure 5, there appears to be no correlation between the RMSD Score and the Gibbs Free Energy, as seen by the relatively small slope of the line of best fit. This makes sense because the RMSD score is an overlay score: it indicates the degree of overlap between the pharmacophore map of the selected compound and AChE. In contrast, the Energy Method allows the selected compound to bind to AChE in any alignment. Therefore, since the two measures are completely different and they use different evaluation techniques, a correlation between these two measures is not expected.

Identification of the Best Compound as Inhibitor of AChE

From the previous experiment, each compounds' affinity to the binding site of AChE was quantified using ΔG. From the results, the five compounds with the most negative ΔG were selected for the last experiment because they bind to AChE the best. In this experiment, each compound's adherence to Lipinski's Rule of Five is evaluated to determine the best compound to bind to AChE. Lipinski's Rule of Five was used to evaluate a drug's viability in terms of absorption and permeation. The four requirements of Lipinski's Rule of Five are as follows: no more than five hydrogen bond donors, no more than ten hydrogen bond acceptors, calculated LogP of no greater than five, and molecular mass of fewer than 500 Daltons. Specifically, LogP determines the range of aqueous character and the lipid character, with a score having a positive relationship with the former and a negative relationship with the latter. Each compound was screened in the SwissADME server (42), which generated all the data needed to determine the conformity to Lipinski's Rule of Five. In Table 5, each component of Lipinski's Rule of Five is quantified by SwissADME (42) and the conformity to Lipinski's Rule of Five is determined.

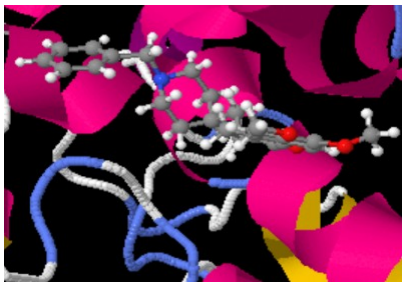
Table 5: Analysis of Five Selected Compounds by SwissADME.

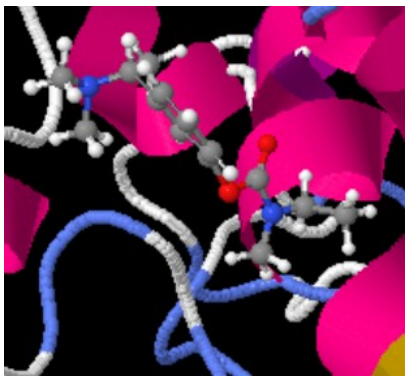
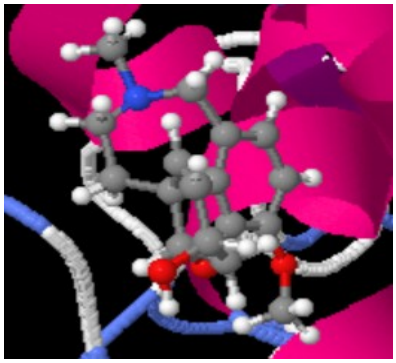
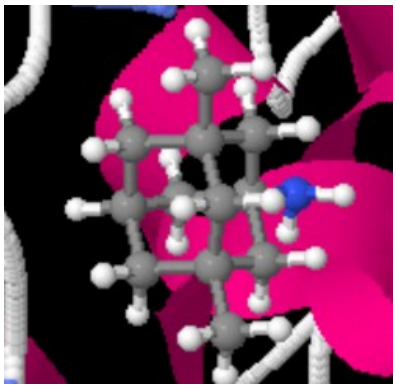
ZINC Code	Molecular Weight (g/mol)	Number of H-bond Acceptors	Number of H-bond Donors	Log $P_{o/w}$ (iLOGP)	Conformity to Lipinski's Rule of Five	Estimated ΔG (kcal/mol)
ZINC64872111	336.86	3	0	3.41	Yes	-11.30
ZINC64872438	330.47	3	0	3.62	Yes	-11.19
ZINC42057013	370.47	4	1	2.80	Yes	-10.41
ZINC64726063	374.42	3	2	3.12	Yes	-10.16
ZINC12660148	493.53	6	4	2.70	Yes	-10.11

According to the table, all five compounds adhere to Lipinski's Rule of Five. In this case, logically, the compound with the most negative estimated ΔG would serve as the best inhibitor for AChE because it has the most favorable interactions with AChE. The compound that is the best inhibitor for AChE, therefore, is ZINC64872111, and it has a ΔG of -11.30 kcal/mol.

Furthermore, all five compounds exceed the ΔG of any of the AChE inhibitors already approved by the FDA. Table 6 shows each approved drug's clusters, ΔG, and model.

Table 6: Analysis of FDA-Approved Acetylcholinesterase Inhibitors.

Name	Number of Clusters	Estimated ΔG (kcal/mol)	Model
Donepezil	46	-7.58	

Rivastigmine	72	-7.24	
Galantamine	30	-7.12	
Memantine	38	-6.42	

A comparison of Tables 5 and 6 indicates that all five newly screened inhibitors have greater ΔG in comparison to previously approved drugs. Thus, from the viewpoint of molecular docking studies, there is promise for these new potential drugs to supersede previous inhibitors in delaying or curing AD.

Conclusion

In this research paper, the purpose was to identify new inhibitors of AChE that would serve as starting points to making more efficient inhibitors. These inhibitors would serve as therapeutics to prevent or cure AD. The approach used was a series of computational studies. First, three methods were used to determine if there were sufficient binding sites for compounds to bind and inhibit AChE. Then, a list of inhibitors was gathered through virtual screening in the PocketQuery (38) and ZINCPharmer (43) servers. Finally, the compound that would serve best as the inhibitor to AChE on the basis of energy of interaction and adherence to Lipinski's Rule of

Five was identified using SwissDock (39) and SwissADME (42) servers. The outcome from this computational study is the compound ZINC64872111. From all the compounds screened, ZINC64872111 had the most negative ΔG of -11.30 kcal/mol, indicating that it had the most favorable binding interactions with AChE. Furthermore, ZINC64872111 satisfies Lipinski's Rule of Five, signifying that it has the chemical and physical properties to serve as a potential therapeutic for human use. It is also important to note that some other compounds listed in the table above such as ZINC64872438 are also suitable candidates as therapeutics for AD given that they have a relatively negative value of ΔG and comply with Lipinski's Rule of Five.

One of the limitations of the study is the inability to test ZINC64872111 in a laboratory environment due to the COVID-19 pandemic. Thus, a future direction would be to study ZINC64872111 in a laboratory setting in order to validate the results obtained in this study as well as to further assess ZINC64872111 as a viable therapeutic for human use. Furthermore, due to software limitations, it was not possible to calculate the ADME properties and toxicity risk parameters of any of the compounds. Finally, while the inhibition of butyrylcholinesterase on the treatment of AD was not investigated, a future step of this work would be to identify dual inhibitors for these enzymes, and it is hoped that this work will facilitate such an approach.

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