Section 8. Pharmaceuticals

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COMPUTER DESIGNED SMALL MOLECULE ACETYLCHOLINERASE INHIBITORS AS POTENTIAL TREATMENT FOR ALZHEIMER'S DISEASE

Abstract. Alzheimer's disease is a chronic illness that most commonly manifests in its victims as a degeneration of their cognitive function and memory. It is also the most prominent cause of dementia. Currently treatment of Alzheimer's disease revolves around two major theories: Amyloid Aggregation Hypothesis and the Cholinergic Deficiency Hypothesis. The Amyloid Aggregation Hypothesis focuses on abnormally processed Amyloid Precursor Protein (APP) protein and neurotoxic Aβ oligomers as a result. In recent years, drugs aimed to eliminate Aβ oligomers from neurons were unable to meet the desired clinical efficiency, and the Cholinergic Deficiency Hypothesis is now seen as a promising potential alternative. This hypothesis focuses the treatment on enhancing the cholinergic pathway centered around the neurotransmitter Acetylcholine (ACh), an important component in cognition, learning and memory retention. This hypothesis attributes the neurodegeneration to the abnormal activity of Acetylcholinerase (AChE), resulting in the dysfunction of the entire pathway. Small molecule AChE inhibitors have been proven effective by multiple clinical trials as well as usage among AD patients, but the process of discovering a new drug is long, complex and costly. Online web servers dedicated to simulate compound-compound interaction and ligand design can serve as a highly cost effective alternative. Using computer algorithms, potential drug candidates can be identified and pre-tested efficiently. In this study, 40 compounds potentially capable of inhibiting AChE are selected using pharmacophore-based virtual screening. 5 compounds that are shown to have the lowest Gibbs free energy (ΔG) values in docking simulations are chosen for further examination. These compounds are: ZINC04716517, ZINC05514424, ZINC19877680, ZINC00754301, ZINC12925747 and ZINC89735569, and they are assessed in terms of absorption, distribution, metabolism, excretion and toxicity. This study follows the process of binding site identification, pharmacophore based virtual screening, docking simulation, and drug-likeness prediction. Thcompounds identified can serve as a starting point for future drug development aimed to create an effective cholinergic drug for Alzheimer's disease, and as an example to test the potential of computer-based drug design.

Keywords: acetylcholinerase inhibitors, drug for Alzheimer's disease, Cholinergic Deficiency Hypothesis, computer-based drug design.

1. Introduction:

Alzheimer's disease and dementia caused by it manifest in patients as chronic losses of memory and degeneration of cognitive abilities that greatly obstruct the livelihood of those affected. It is one of the most prominent multifactorial neural diseases. A report published in 2021 states that more than 1 in 9 Americans (11.3%) suffer from Alzheimer's related dementia [1].

Two prominent hypotheses attempt to explain the pathology of Alzheimer's disease (Fig. 1): the Amyloid Aggregation Hypothesis and the Cholinergic Deficiency Hypothesis. The Amyloid Aggregation Hypothesis is centered around the abnormal cleaving of amyloid precursor protein (APP). Under normal conditions, α-secretase cleaves APP into APP secreted α (APPsα), a neuroprotective compound that drives normal neuron behavior and assists in learning as well as memory retention, and α-C terminal fragments (α-CTF). α-CTF is then cleaved by γ-secretase into APP intracellular domain(AICD), which is also a neuroprotective compound that aids in protein regulation [2] and fragments named p3. However, in an Alzheimer affected brain, genetic mutations cause APP to be abnormally cleaved by β-secretase into APP secreted β (APPsβ) and β-C terminal fragments (β-CTF). β-CTF is then cleaved by γ-secretase into AICD and amyloid β(Aβ), the main neurotoxic compound [3].

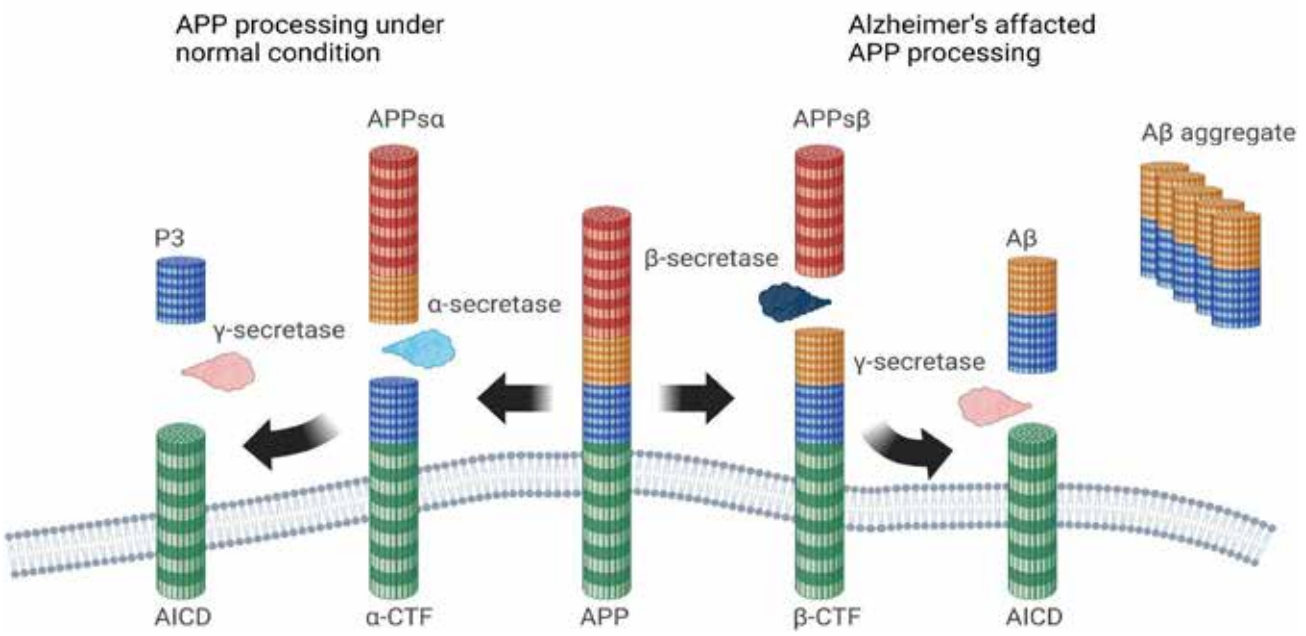

Figure 1. Left: APP processing under normal conditions Right: APP processing in Alzheimer's affected brain that leads to neurotoxic Aβ oligomer formation

A β causes neuronal degeneration in three ways in the Amyloid Aggregation Hypothesis: they can aggregation and form neurotoxic plaques that disrupt neuronal-signalling [4], they can localize on the mitochondrial membrane and disrupt the electron transport chain, causing oxidative stress and in turn glucose hypometabolism and mitochondrial dysfunction [5], and they can cause hyperphosphorylation of the tau protein, a microtubule-associated protein and result in microtubule dissociation in the neurons [6].

Despite its early inception since 1989, treatments based on eliminating Aβ and Aβ aggregates faced numerous setbacks and lead to the

theory being heavily challenged in recent years: In 2018, Boehringer Ingelheim announced that their compound BI 409306 (Fig. 2 A). did not meet the efficiency end point in their phase 2 tests, in February that year, the data released by Boehringer Ingelheim showed no difference between the drugged and the placebo group [7]. Subsequently, the development of this compound was discontinued. Azeliragon is another small molecule drug (Fig. 2B). It was discovered by vTv. Therapeutics (formerly known as TransTech Pharma) and designed to inhibit the receptor for advanced glycation end products(RAGE) responsible for binding Aβ to form neural toxic oligomers. This compound also failed to reach its end point of slowing neural degeneration in the participants in its phase 3 tests, resulting in the company announcing the termination of further development and testing near the end of 2020 [8]. Similar situation is observed in the testing of Verubecestat (Fig. 2C). This is another small molecule drug designed to inhibit BACE1, the β-secretase that cleaves the APP. Verubecestat was discovered by Merck, during its phase 3 testing, the experimental groups scored worse than the control groups on cognition function tests and the development of this compound was stopped [9].

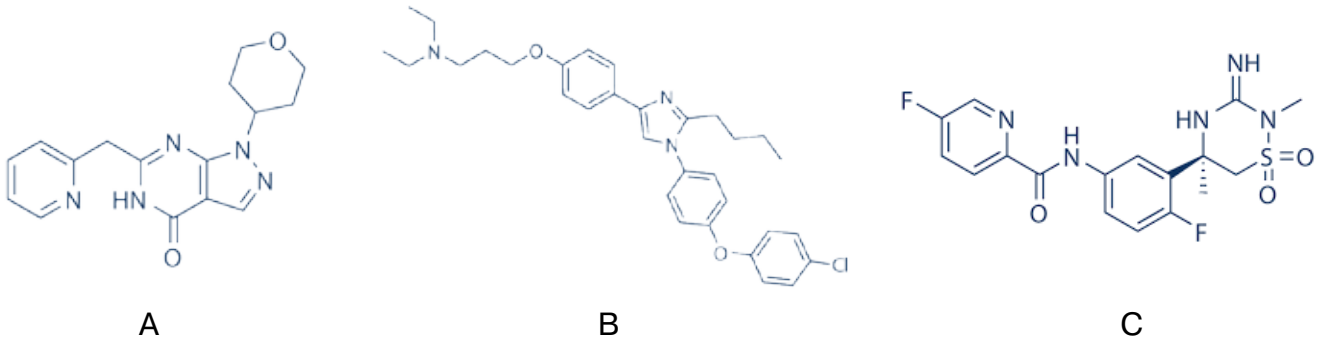

Figure 2. A: BI 409306; B: Azeliragon; C: Verubecestat

This study focuses on another prominent theory: the Cholinergic Deficiency Hypothesis, which is connected to the Amyloid Hypothesis in multiple ways but focuses the treatment on enhancing cholinergic neurotransmission rather than eliminating Aβ. This hypothesis attributes the cognitive decline to the degeneration of cholinergic pathways (Figure 3), a type of pathway crucial for the function of the cerebral cortex and the hippocampus [10]. Cholinergic pathway starts when an action potential reaches the synapse: membrane bound sodium or energy dependent transporters transport choline into the presynaptic cell, the choline then reacts with Acetyl Coenzyme A (AcCoA) to form acetylcholine (ACh), the most crucial neurotransmitter found in this pathway. This reaction is catalyzed by choline acetyltransferase (CAT). The produced acetylcholine molecules are stored in vesicles that prevent them from degrading. As calcium flows into the cell, they interact with the vesicles and the ACh is released into the inter-synaptic space. The neurotransmitter then interacts with various receptors on the postsynaptic membrane, which triggers responses that continue the impulse. Afterward, acetylcholine is hydrolysed by acetylcholinesterase (AChE) back into choline and acetyl, allowing the synapse to reset and the choline to be recycled [11].

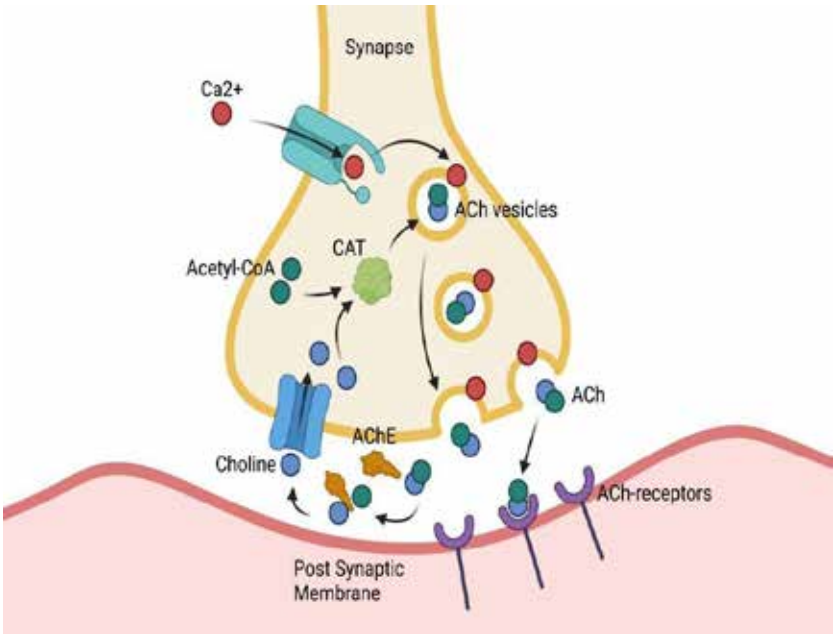

Figure 3. Cholinergic pathway at the synapse under normal conditions

In an Alzheimer affected brain, although the activity of AChE is decreased [12], its ability to withstand lower pH and excess substrate is increased [13]. It is also observed that AChE accelerates the formation of neurotoxic Aβ aggregates [14]. This leads to a lack of ACh and the dysfunction of acetylcholine containing neurons. The resulting degeneration of the cholinergic nervous system has been a long standing observation in the Alzheimer's research community [15], and is understood to contribute to cognitive decline manifested in AD patients [16].

Numerous treatments have been developed based on this hypothesis, with the most prominent ones being Donepezil, Galantamine and Rivastigmine. Donepezil is an AChE inhibitor that is absorbed through the guts and has been shown to have little drug interactions [17]. According to an extensive review that included 28 studies for meta review, patients with mild to severe AD scored better on Alzheimer's Disease Assessment Scale-Cognitive (ADAS-cog, 5 studies), Mini-Mental State Examination (MMSE, 7 studies), Severe Impairment Battery (SIB, 5 studies) and Alzheimer's Disease Cooperative Study activities of daily living score for severe Alzheimer's disease (ADCS-ADL-sev, 3 studies) when administered with 5/10mg capsules or 23mg oral formulation. While no significant difference is observed in terms of Neuropsychiatric Inventory (NPI, 4 studies), Behavioral Pathology in Alzheimer's disease scale (BEHAVE-AD, 1 studies) or Quality of Life (QoL, 2 studies). These studies were all double-blind placebo-controlled, and all but one lasted for six month or less. Donepezil groups also experienced increases on the clinician-rated global impression of change scale (6 studies). However it should be noted that even though patients that were administered 10mg Donepezil scored slightly higher on cognitive tests, they sometimes reported worse QoL (2 studies), experienced more adverse effects and more have withdrawn from treatment. As of those administered 23 mg formulations, they reported no improvement in cognitive test scores, while suffering worse negative effects [18] compared to lower dose groups.

Donepezil can cause nausea, vomiting, diarrhea, muscle cramps, pain, difficulty sleeping and hallucinations [19], it has also been observed that for patients with frontotemporal dementia, Donepezil can worsen their symptoms [20].

Galantamine is another reversible, competitive AChE inhibitor. It also possesses a second mode of action, that being acting as an allosteric modulator for nicotinic acetylcholine receptor (nAChRs) [21], an important group of neuroreceptors crucial for ACh reception on the postsynaptic membrane [22]. Galantamine demonstrated no clinically significant drug to drug interaction and predictable side effects [23]. In several random placebo-controlled, double blind trials that lasted up to six months, Galantamine was administered to patients in doses of 24mg/day. This was observed to slow the progression of the disease, as well as improve their ability to perform activities of daily living (ADL), significantly compared to placebo groups [24]. Galantamine can commonly cause nausea, vomiting, diarrhea, stomach pain, loss of appetite, headache and other common side effects of cholinergic drugs [25].

Rivastigmine is a pseudo-irreversible inhibitor of both AChE and butyrylcholinesterase [26], a non-specific cholinesterase enzyme that is capable of hydrolyzing many choline-based esters. In another review that analyzed data gathered from 13 randomized, double-blind, placebo-controlled trials that had durations ranging from 12 to 52 weeks, the reviewer declared that patients that received 6 to 12 mg/day orally or 9.5 mg/day transdermally returned better score in the ADAS-Cog (6 studies), MMSE (6 studies), Activity of Daily Living (6 studies). In the clinician rated global impression of changes, more in Rivastigmine groups reported improvements (7 studies), while Neuropsychiatric Inventory-Caregiver Distress (NPI-D) returned no change. On the other hand, it is noteworthy that patients who received rivastigmine are 2.01 times more likely to withdraw from the experiment (7 studies) and 2.16 times more likely to experience adverse effects (7 studies) [27]. The most common side effects of rivastigmine include nausea, vomiting and dizziness [28], when administered orally, its chances of causing adverse effects is also higher than other similar drugs [29].

This study's main objective is to identify possible small molecule ligands that can inhibit AChE effectively and are likely safe for human use. The results can serve as a pool of potential candidates future AChE inhibitor studies can be based upon to develop effective cholinergic drugs for Alzheimer's disease.

2. Methodologies

This study first identified the target molecule AChE, after inputting its PDB code 3lii into various binding site identification websites, a map of potential binding sites was obtained. After this, a new PDB code 4QWW that represents the two polypeptide chains of the target molecule is acquired, it is then entered into the PocketQuery website to produce a list of potential pharmacophore features that can interact with AChE. A certain number of these features are selected in the website ZINCpharmer, and a list of molecules containing these features is given. The most reliable of these potential molecules are then put into the docking simulator SWISSdock. Finally, molecules that displayed the most favorable results are checked by SWISSADME for their safety and other properties.

2.1. PrankWeb

PrankWeb is a state-of-the-art online web server that employs template-free machine learning methods to predict ligandability of com-

pounds [30]. The algorithm views binding sites as points placed on reachable protein surface, and draws from lagandability results of compounds in local chemical neighborhoods to produce a map of point clusters on the target molecule [31]. The website can also provide information such as the amino acid sequence that composes these potential binding sites and their reliability displayed as probability scores.

The PDB code of AChE "3lii" was entered into the search box and the results were generated after clicking "search" on default setting.

2.2. ProteinPlus-DoGSiteScorer

ProteinPlus is an advanced web server developed by the University of Hamburg that provides an interface for analyzing binding sites, druggability and protein-protein interactions [32]. The interface include a variety of tools for preprocessing tasks, and for druggability of binding sites, the website contain DoGSiteScorer: an algorithm capable of analyzing the geometric and physico-chemical properties of the binding sites ProteinPlus identified and estimate druggability for each of them [33]. ProteinPlus can also provide information regarding the volume and surface area of potential binding sites.

The same PDB code "3lii" was entered into the search box of ProteinPlus and after clicking "Go!", the compound was mapped. DoGSiteScorer was then used by clicking on the option and then "calculate".

2.3. PocketQuery

PocketQuery is a web interface that allows users to simulate protein-protein interactions [34]. It estimates the druggability of a potential binding site based on estimated maximum cluster distance (Dist), the change of solvent accessible surface (SASA) area upon complexation (ΔSASA), percentage of the estimated change in respect to the total possible SASA(ΔSASA%), estimation of the change in free energy in case of an interaction between the target complex and an alanine mutation (Rosetta Energy (ΔΔG), the change of free energy of a residue upon complexation (ΔG) and a sequence conservation score. The website will provide a list of amino acids arrangement with the appropriate pharmacophore features that allow them to interact with the most druggable binding sites.

The new PDB code 4QWW was entered into the PDB ID box and results are generated by hitting the enter key on default setting. The results were automatically arranged from the highest score to lowest. The results were then exported to other websites to be built upon by pressing the export button and selecting the desired receiver.

2.4. ZINCpharmer

ZINCpharmer is an online interface that can accept results from PocketQuery, it can search the ZINC database for purchasable compounds using the Pharmer pharmacophore search technology [35]. A pharmacophore map describes the 3D placement of pharmacophore features necessary for interaction with the target compound. Once the result is imported from PocketQuery, the user can select at least three pharmacophore features to preserve and ZINCpharmer will return a list of all molecules with these properties, and the ones with the lowest root-mean-square deviation (RMSD) can be chosen for further analysis.

Desired pharmacophore features were selected in the pharmacophore tab, and the list was generated after clicking on "submit query". If no molecule can be identified, the website will return "no hits".

2.5. SWISSdock

SWISSdock is an online web server that simulates the docking of small molecules on target compounds [36] developed by the Swiss Insti-

tute of BioInformatics. Utilizing the EADock DSS engine [37], it is able to predict the Full Fitness of the docking as well as the Gibbs free energy Δ (kcal/mol) of the process. To do this, the target molecule must be selected under the target selection box, this was done by entering the PDB code "3lii" or uploading the protein file directly by clicking the "upload file" button. When the setup was complete, the ligand was then selected by entering the ZINC IDs obtained from ZINCpharmer or uploading a mol2 file directly. The simulation was initiated by clicking "Start Docking" and results were returned after the process ended.

2.6. SWISSADME

Potential candidates for drugs need to undergo extensive virtual screening to be considered qualified for testing, SWISSADME can serve as a good starting point of this process. Also a web server developed by the Swiss Institute of BioInformatics, SWISSADME assesses potential drug candidates in terms of absorption, distribution, metabolism and excretion by modeling their physicochemical properties, pharmacokinetics, drug-likeness and medicinal chemistry friendliness [38]. The website can then provide extensive information including lipophilicity, solubility, gastrointestinal, Blood-Brain Barrier permeance, etc.

To use this website, a list of simplified molecular-input line-entry systems (SMILES) of the ligand provided by the ZINC database was entered into the SMILES box, and the specifics were returned after clicking the "Run" Button.

2.7. ADMETlab 2.0

ADMETlab 2.0 is a recently released webserver for the predictions of pharmacokinetics and toxicity properties of drug candidates. It analyzes 17 physicochemical properties, 13 medicinal chemistry properties, 23 ADME properties, 27 toxicity endpoints and 8 toxicophore rules of any given compound [39]. ADMETlab 2.0 employs a multi-task graph attention framework to predict the chemical's absorption, distribution, metabolism, excretion and toxicity properties, and can serve as a follow up filter after SWISSADME.

To use this website, the same SMILES used in SWISSADME is entered into the search box under the "ADMET screening" option and the website will look for the compound after the "submit" button is clicked. When the result is found, clicking on the "View" button will display the specifics.

3. Preliminary results

3.1. PrankWeb

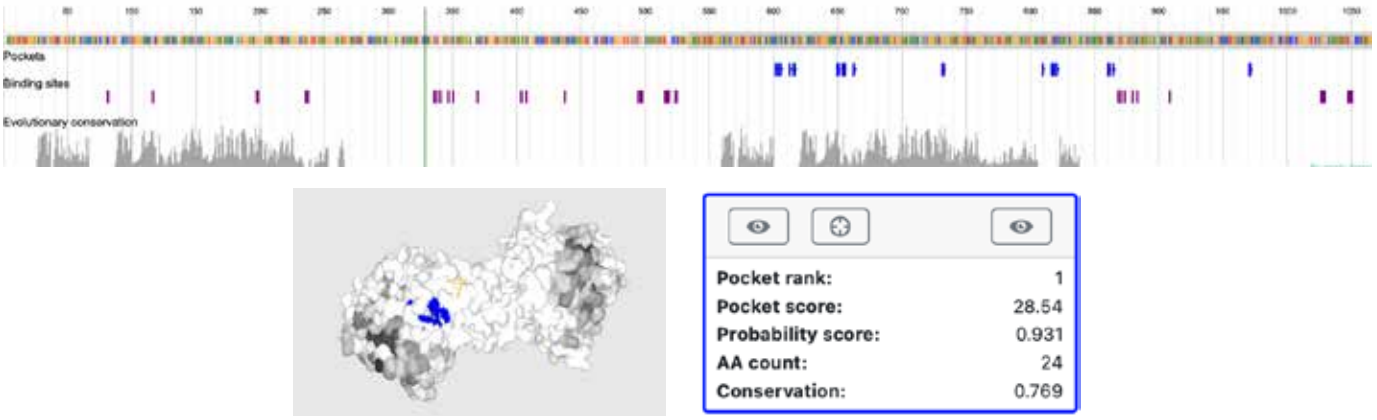

Figure.4: Results of PrankWeb investigation using PDB code "3lii": potential binding site displayed in blue

Results returned from PrankWeb showed several possible binding sites, the one with the highest pocket score of 28.54 is displayed in blue (Fig. 4). This pocket have the following residue: Tyr B72, Asp B74, Leu B76, Thr B83, Trp B86, Asn B87, Gly B120, Gly B121, Tyr B124, Ser B125, Gly B126, Tyr B133, Glu B202, Ser B203, Trp B286, Ser B293, Val B294, Phe B297, Tyr B337, Phe B338, Tyr B341, His B447 and Gly B448.

3.2. ProteinPlus-DoGSiteScorer

ProteinPlus and DoGSiteScorer returned a list of potential binding sites (Fig. 5) ranked by their druggability score. This score as well as the simple score is estimated based on their volume/depth, surface area and amino acid composition, binding sites that are optimized in these aspects for small molecule interaction are considered more "druggable" and can serve as a basis when selecting ligands. Large pocket volume, high depth and high apolar amino acid ratio is considered favorable in the scoring.

	Name	Volume Å³	Surface Å²	Drug Score	Simple Score
	P_1	771.85	643.47	0.83	0.49
	P_0	796.24	650.39	0.82	0.44
	P_2	399.6	623.86	0.81	0.19
	P_3	355.84	537.6	0.74	0.13

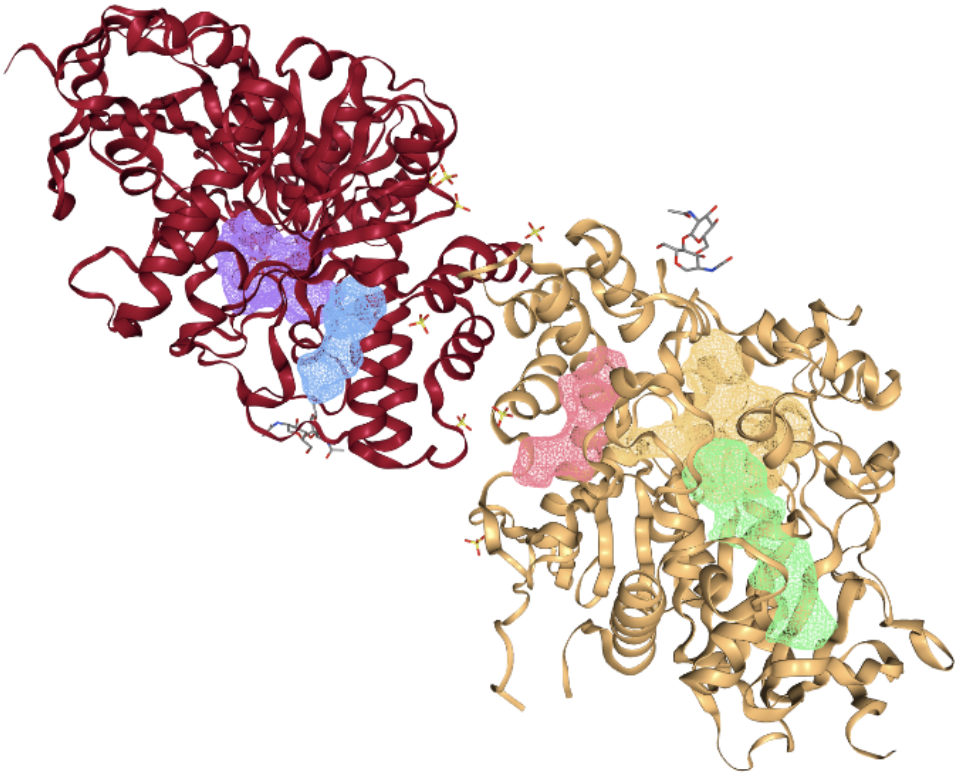

Figure 5. Results of ProteinPlus investigation using PDB code "3lii": potential binding site displayed in colors corresponding to the chart

Binding site P_1 has the highest druggability score and simple score, it has 13 hydrogen bond donors, 60 hydrogen bond acceptors, 39 hydrophobic interactions with a hydrophobicity ratio of 0.35, an apolar amino acid ratio of 0.38 and a polar amino acid ratio of 0.51. The amino acid descriptors of this site are the following: 2 Ala, 1Arg, 1Asn, 1Asp, 1Gln, 1Glu, 6Gly, 1His, 1Ile, 1Leu, 3Phe, 1Pro, 3Ser, 1Thr, 4Trp, 7Tyr, 2Val. The similarity in both location and composition between results returned by PrankWeb and ProteinPlus is very noteworthy, it strongly implies that this binding site can be drugged reliably.

3.3. PocketQuery

In order to account for the two chains that comprise an AChE molecule, a new PDB code must be obtained in order for Pocket Query to return a comprehensive list that includes ligand for both chains. Although the composition of the two chains, C and E, is the same, the features' placement are inverted spatially, this will require different ligands. This new PBD code is "4QWW". After the list is generated, two results with the highest score for both chains are selected for further development. Their specifics are displayed in (Table 1) and their image in (Figure 6). The distance indicates the longest distance in Angstroms between the centroids of any two residues in the cluster. The Avg ΔG indicates the change in Gibbs free energy upon interaction, a more negative value is associated with exergonicity and thus higher affinity. The Avg ΔΔG indicates the per-residue change in free energy of complexation between this complex and an alanine mutant, this is an indicator for the stability of the target molecule upon interaction, a more positive value implies stronger stabilizing effect of the ligand. Avg ΔSASA indicates the change in solvent accessible surface area upon complexation, and Avg ΔSASA% is the percentage of said change. The score is a per-residue conservation score, a higher score suggests a residue is more conserved. This study ranked results based on the score.

Table 1.– Specifics of top 2 results targeting both C and E chains of AChE obtained from PocketQuery using the new PDB code "4QWW"

Cluster	**Chain**	**Size**	**Residue**	**Dist (Angstroms)**	**Avg ΔG (kcal/mol)**	**Avg ΔΔG**	**Avg ΔSASA**	**Avg ΔSASA%**	**score**
1	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***
1	C	2	Tyr 90	7.3544	–4.115	0	111.715	69.5	0.986921
			His 93						
2	C	3	Tyr 90	7.3544	–3.64	0	93.8933	58.7333	0.984095
			His 93						
			Met 95						
3	E	2	Tyr 90	7.3511	–3.975	–1.40485	110.58	68.85	0.981743
			His 93						
	E	3	Tyr 90	7.3511	–3.54333	–0.87113	93.3433	58.4333	0.981391
			His 93						
			Met 95						

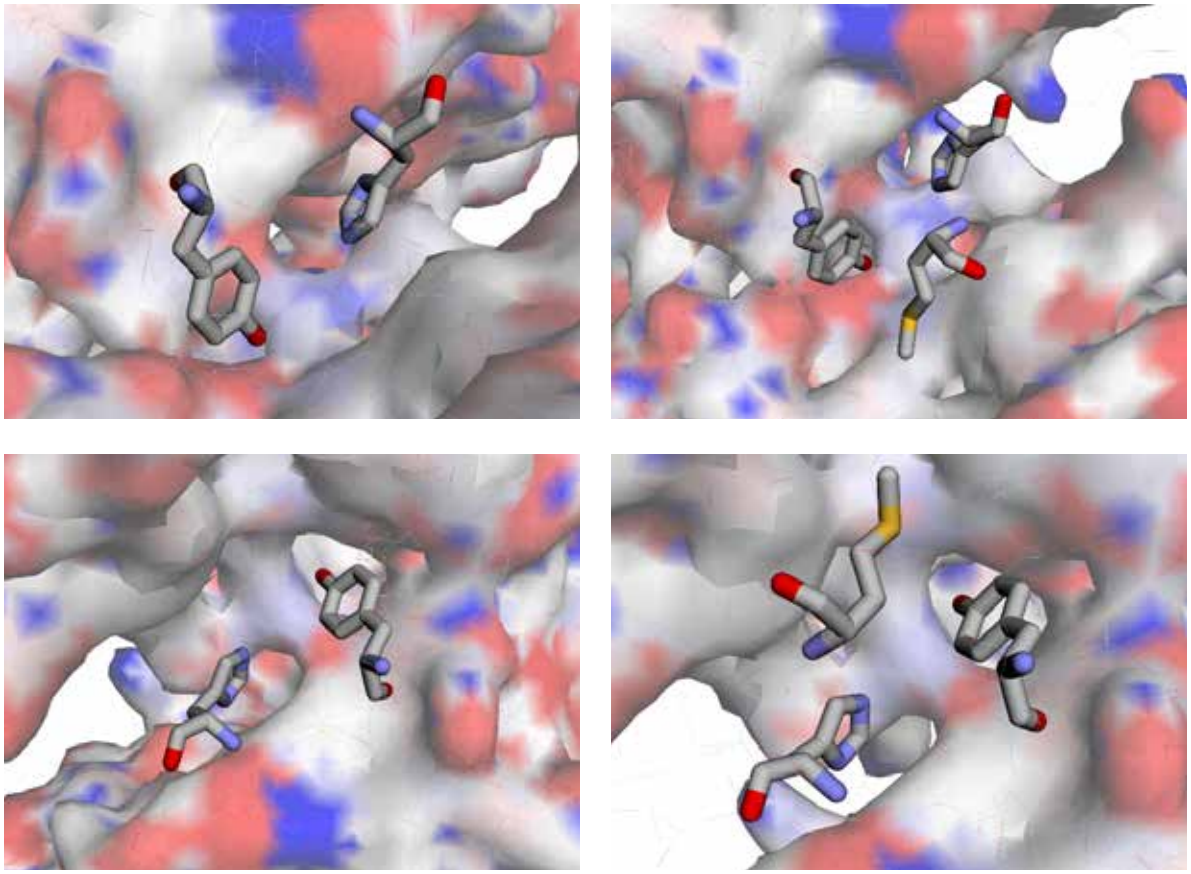

Figure 6. Images of top 2 results targeting both C and E chains of AChE obtained from PocketQuery using the new PDB code "4QWW"
(Top Left: cluster 1, Top Right: cluster 2, Bottom Left: cluster 3, Bottom Right: cluster 4)

3.4. ZINCpharmer

A total number of 40 potential ligands are selected from the list of hits returned by ZINCpharmer, 20 from each chain. All hits with root mean square deviation (RMSD) lower than 0.15 are considered. The hit groups and their respective pharmacophore features are displayed in the following table (Table 2). During the molecule selection process several molecules possess identical main structure with differing side chains, these molecules are marked with a special symbol next to their name (*,%, #, **, ##, @, &). Furthermore, due to the similar nature of the C and E chain within AChE, molecules that appeared in both the C and E chain's hit group are also considered due to their more versatile bonding capabilities. This is reflected in the "1&3 shared" and the "2&4 shared" sections of the table. This table includes the molecule's Root Mean Square Deviation(RMSD), which indicates the level of similarity between the hit result's pharmacophore features and the original pharmacophore features' positions. It also includes mass and the number of rotatable bonds(RBnd). Lastly, some hit molecules are not recognized by SWISSdock, the next website used to evaluate the compounds, they are marked with a "–" in front of their code name. Replacements were found for the unrecognized molecules, and they are marked with a "+" in front of their code name. All the hit results are displayed in (Table 2).

Table 2.– Pharmacophore Features preserved for each cluster and the respective hit groups returned by ZINCpharmer

Cluster	Pharmacophore Feature	Name	RMSD	Mass	RBnds
1	*2*	*3*	*4*	*5*	*6*
1	Aromatic (x: 17.90 y: –94.68 z: –15.15)	ZINC32795055	0.001	403	9
	Radius: 1.10	ZINC02121625*	0.001	382	8
	Hydrophobic (x: 17.90 y: –94.68 z: –15.15)	–ZINC90058378	0.001	373	15
	Radius: 1.00	–ZINC00526453*	0.001	337	4
	Aromatic (x: 18.15 y: –99.07 z: –10.26)	ZINC00704447*	0.001	367	8
	Radius: 1.10	–ZINC05504427*	0.001	367	5
	Hydrophobic (x: 18.15 y: –99.07 z: –10.26)	ZINC12520179%	0.001	435	8
	Radius: 1.0	ZINC04716517#	0.001	358	10
	Hydrogen Acceptor (x: 15.15 y: –98.65 z: –11.07)	ZINC04716528#	0.001	344	9
	Radius: 0.50	ZINC02559431#	0.001	284	5
		+ZINC05514427*	0.001	367	5
		+ZINC00754339*	0.001	352	6
		+ZINC12564214%	0.001	387	8
2	Aromatic (x: 17.90 y: –94.68 z: –15.15)	ZINC00426771**	0.004	317	6
	Radius: 1.10	ZINC04733773**	0.004	382	5
	Hydrophobic (x: 17.90 y: –94.68 z: –15.15)	ZINC35992586##	0.004	429	14
	Radius: 1.00	ZINC36006321##	0.004	414	13
	Aromatic (x: 18.15 y: –99.07 z: –10.26)	ZINC35988983##	0.004	414	13
	Radius: 1.10	ZINC00947297	0.005	387	7
	Hydrophobic (x: 18.15 y: –99.07 z: –10.26)	–ZINC71803034	0.005	461	11
	Radius: 1.00	ZINC12752034	0.006	491	10
	Hydrophobic (x: 18.82 y: –103.03 z: –13.78)	–ZINC91334479	0.006	339	10
	Radius: 1.00	ZINC02072375	0.006	392	4
		+ZINC58008912@	0.007	418	8
		+ZINC72048214@	0.007	368	9
3	Aromatic	ZINC13595266	0.001	394	7
	(x: 18.44 y: –19.89 z: –22.08)	ZINC57503878	0.001	342	10
	Radius: 1.10	ZINC00754301*	0.001	352	6
	Hydrophobic	ZINC19877680	0.001	497	12
	(x: 18.44 y: –19.89 z: –22.08)	ZINC00704450*	0.001	367	8
	Radius: 1.00	-ZINC94714412	0.001	301	5
	Aromatic	ZINC05514423*	0.002	367	5
	(x: 18.88 y: –15.52 z: –26.96)	ZINC02121626*	0.002	382	8
	Radius: 1.10	ZINC05514424*	0.002	367	5
	Hydrophobic	ZINC05464835*	0.002	380	8
	(x: 18.88 y: –15.52 z: –26.96)	+ZINC02978390	0.002	403	4
	Radius: 1.00				

1	*2*	*3*	*4*	*5*	*6*
3	Hydrogen Acceptor				
	(x: 16.25 y: –15.95 z: –26.24)				
	Radius: 0.50				
4	Aromatic	-ZINC12300610	0.005	416	10
	(x: 18.44 y: –19.89 z: –22.08)	ZINC84746894	0.005	304	9
	Radius: 1.10	-ZINC73260184	0.006	360	6
	Hydrophobic	-ZINC08320022	0.006	386	7
	(x: 18.44 y: –19.89 z: –22.08)	ZINC69518069	0.006	333	8
	Radius: 1.00	ZINC17295117	0.006	484	9
	Aromatic	-ZINC08809977	0.007	308	5
	(x: 18.88 y: –15.52 z: –26.96)	ZINC91839326&	0.007	375	10
	Radius: 1.10	ZINC79504196&	0.007	374	10
	Hydrophobic	ZINC26534127	0.007	448	10
	(x: 18.88 y: –15.52 z: –26.96)	+ZINC12925747	0.008	452	10
	Radius: 1.00	+ZINC12072317	0.008	333	3
	Hydrophobic	+ZINC35973602	0.008	339	9
	(x: 19.47 y: –11.50 z: –23.40)	+ZINC14963127	0.008	375	8
	Radius: 1.00				
1 & 3 Shared		ZINC95358168	0.073	381	6
		ZINC89735569	0.092	340	6
		ZINC90851227^	0.109	352	6
		ZINC90852790^	0.11	338	6
2 & 4 Shared		-ZINC95084748	0.068	426	10
		-ZINC69917631$	0.071	344	4
		-ZINC94145307!	0.078	314	8
		-ZINC94145311!	0.078	328	9
		ZINC69919088$	0.085	324	6
		ZINC69919500$	0.086	310	5
		+ZINC72430491	0.088	379	11
		+ZINC92729473	0.096	328	4
		+ZINC72278670	0.097	310	4
		+ZINC80578006	0.102	319	9

3.5. SWISSdock

Hit results from ZINCpharmer were entered into SWISSdock, and the website returned the estimated Full Fitness as well as ΔG of the interaction; they are listed in the following table with their corresponding molecule names. This study primarily focused on ΔG, which measures the binding affinity from an ergonomics perspec-

tive. The lower this value is, the more exergonic the reaction is, and the more likely it will happen spontaneously. All the results are displayed in (Table 3), molecules with desirable ΔG are marked with a "+" after their name, these will be the molecules for further screening. Images of the simulated docking are compiled in (Figure 7), the final candidate molecules' structures are shown in (Figure 8).

Table 3. – The results of the docking simulations for every hit molecule returned by SWISSdock with the pdb file "3lii" assigned as target

Cluster 1 Name	**Full Fitness (kcal/mol)**	**Estimated ΔG (kcal/mol)**	**cluster 2 Name:**	**Full Fitness (kcal/mol)**	**Estimated ΔG (kcal/mol)**
ZINC04716517+	– 1847.76	– 9.27	ZINC12752034	– 1647.07	– 8.25
ZINC02121625	– 1812.98	– 8.24	ZINC58008912	– 1873.39	– 8.22
ZINC04716528	– 1897.98	– 8.12	ZINC00426771	– 1877.2	– 8.16
ZINC00754339	– 1827.51	– 7.94	ZINC02072375	– 1814.71	– 8.07
ZINC12520179	– 1861.25	– 7.79	ZINC36006321	– 1844.57	– 7.95
ZINC05514427	– 1803.95	– 7.78	ZINC72048214	– 1847.41	– 7.9
ZINC02559431	– 1932.59	– 7.75	ZINC04733773	– 1813.45	– 7.75
ZINC00704447	– 1824.89	– 7.59	ZINC35988983	– 1849.92	– 7.64
ZINC32795055	– 1852.12	– 7.55	ZINC00947297	– 1817.68	– 7.84
ZINC12564214	– 1860.45	– 7.41	ZINC35992586	– 1848.28	– 7.53
ZINC19877680+	– 1800.04	– 9.21	ZINC12925747+	– 1825.17	– 9.13
ZINC05514424+	– 1811.58	– 9.17	ZINC12072317	– 1871.05	– 8.75
ZINC00754301+	– 1829.6	– 9.03	ZINC91839326	– 1902.17	– 8.37
ZINC02121626	– 1806.79	– 8.62	ZINC14963127	– 1829.82	– 8.02
ZINC05514423	– 1810.65	– 8.51	ZINC26534127	– 1796.52	– 8
ZINC05464835	– 1832.54	– 8.18	ZINC69518069	– 1836.26	– 7.99
ZINC13595266	– 1847.22	– 7.77	ZINC17295117	– 1892.00	– 7.95
ZINC02978390	– 1824.7	– 7.65	ZINC79504196	– 1903.64	– 7.91
ZINC00704450	– 1833.37	– 7.42	ZINC84746894	– 1878.97	– 7.61
ZINC57503878	– 1820.24	– 7.31	ZINC35973602	– 1856.42	– 7.54
ZINC95358168	– 2072.35	– 8.35	ZINC72430491	– 1893.38	– 8.05
ZINC89735569+	– 1866.8	– 9.28	ZINC69919500	– 1667.42	– 7.69
ZINC90851227	– 1898.1	– 8.69	ZINC69919088	– 1671.89	– 7.66
ZINC90852790	– 1892.89	– 8.94	ZINC92729473	– 1864.17	– 7.54
			ZINC72278670	– 1660.62	– 7.54
			ZINC80578006	– 1961.11	– 7.52

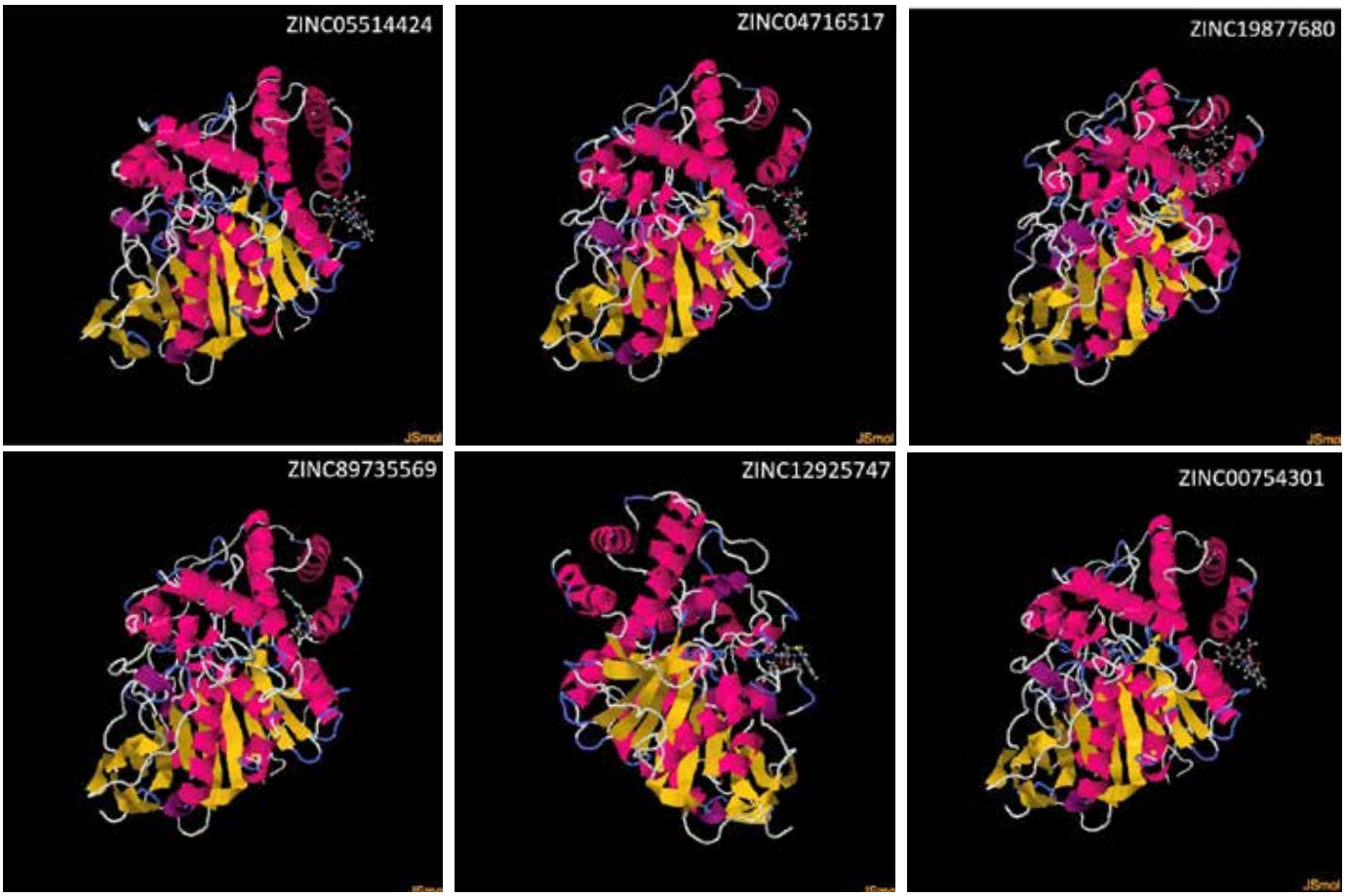

Figure 7. Images of the predicted docking interaction between the target molecule and the selected compounds with the lowest resulting ΔG (>-9 kcal/mol)

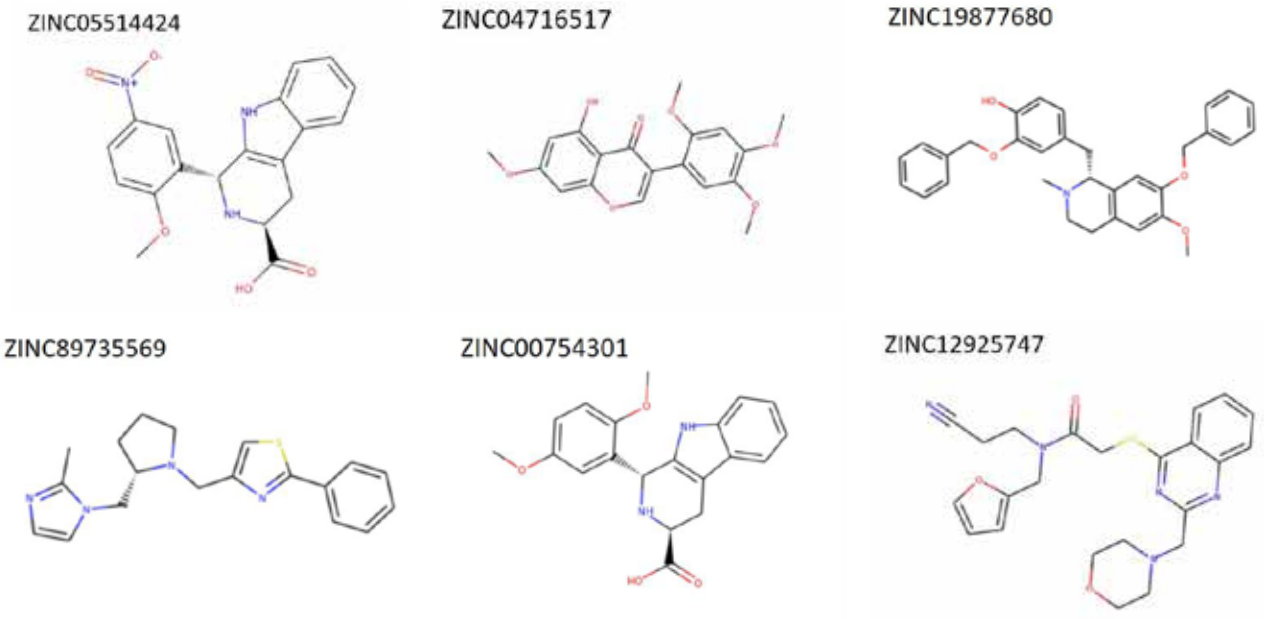

Figure 8. The structures of the final candidate molecules that are selected for further virtual screening

3.6. Swissadme

SWISSADME returns 7 characteristics of the compounds: its molecular weight, its Lipophilicity in MLogP, its number of hydrogen bond donors and acceptors, and its water solubility calculated by Estimated Aqueous Solubility(ESOL) and two other methods: one developed by Jogoth Ali, Patrick Camilleri, Marc B Brown, Andrew J Hutt, and Stewart B Kirton [41; 42] (Ali), and another developed by SILICOS-IT. SWISSADME assesses the compound's conformity to Lipinski's rule of five, which gives requirements in five areas. Any potential drugs must meet them to be considered acceptable; this is reflected in the "Requirements" row. SWISSADME also gave estimated gastrointestinal absorption rate and Blood Brain Barrier(BBB) permeance. This study aims to develop drugs with goals that require them to reach the brain, so being Blood Brain Barrier permeant is considered favorable. All characteristics are displayed in (Table 4) and (Table 5).

Table 4. – compound characteristics returned by SWISSADME after entering their respective SMILE codes

Name:	**Molecular Weight (g/mol)**	**Lipophilicity (MLOGP)**	**No. of Hydrogen Bond Donors**	**No. of Hydrogen Bond Acceptors**	**No. of Rule Violations**	**Log *S* (ESOL)**	**Log *S* (Ali)**	**Log *S* (SILICOS — IT)**
ZINC04716517	358.34	0.40	1	7	0	Moderately soluble	Moderately soluble	Poorly soluble
ZINC05514424	367.3	–1.34	3	6	0	Soluble	Soluble	Moderately soluble
ZINC19877680	495.6	3.97	1	5	0	Poorly soluble	Poorly soluble	Insoluble
ZINC00754301	352.38	–0.74	3	5	0	Soluble	Very soluble	Moderately soluble
ZINC12925747	451.54	0.47	0	7	0	Soluble	Soluble	Poorly soluble
ZINC89735569	338.47	0.37	0	3	0	Moderately soluble	Moderately soluble	Moderately soluble
Requirements:	< 500	< 4.15	< 5	< 10	< 2			

Table 5. – Additional predictions returned by SWISSADME

Name:	**Lipinski Drug-likeness:**	**Gastrointestinal Absorption:**	**Blood Brain Barrier(BBB) permeant:**
1	***2***	***3***	***4***
ZINC04716517	Yes	High	No
ZINC05514424	Yes	High	No

1	*2*	*3*	*4*
ZINC19877680	Yes	High	Yes
ZINC00754301	Yes	High	No
ZINC12925747	Yes	High	No
ZINC89735569	Yes	High	Yes

3.7. ADMETlab2.0

ADMETlab2.0 gives comprehensive predictions in all five areas of pharmacokinetics. In absorption, the server predicts the compound's Caco-2 permeability in log cm/s. The human colon adenocarcinoma cell lines (Caco-2) is a cell line found in the human intestines, and is commonly used to assess the gastrointestinal absorption of drug compounds. The optimal range for this section is > –5.15. Madin–Darby Canine Kidney cells (MDCK) is an in vitro model for assessing potential drug compounds' uptake efficiency, and is measured in cm/s. High passive MDCK permeability is considered favorable for this study. Pgp inhibitor and substrate refers to the P-glycoprotein, a crucial efflux transporter in the ATP-binding cassette (ABC) transporters superfamily. Pgp-inhibition could lead to drug accumulation inside the cell and cause adverse effects, so being an inhibitor is considered unfavorable. On the other hand being a substrate could lead to too little compounds present in the cells and weaken potency, so it is also considered unfavorable. These sections are measured in possibilities out of 1. The probability of Human Intestinal Absorption (HIA) being lower than 30% is also predicted, and lower possibility is considered to be favorable. Lastly, The probability of the human oral bioavailability F% being lower than 20% is predicted, and lower possibility is considered to be favorable. All predictions in the absorption section are displayed in (Table 6).

Table 6. – Absorption characteristics of the compounds predicted by ADMETlab 2.0

Names:	**Caco-2 Permeability (log cm/s)**	**MDCK Permeability (cm/s)**	**Pgp-inhibitor (possibility out of 1)**	**Pgp-substrate (possibility out of 1)**	**Human Intestinal Absorption (HIA) < 30% (possibility out of 1)**	**The human oral bioavailability F% < 20% (possibility out of 1)**
ZINC04716517	– 4.814	3.5×10^{-5}	0.1–0.3	0.7–0.9	0–0.1	0–0.1
ZINC05514424	– 5.470	2.1×10^{-5}	0–0.1	0–0.1	0–0.1	0–0.1
ZINC19877680	– 5.323	1.6×10^{-5}	0.9–1	0–0.1	0–0.1	0.5–0.7
ZINC00754301	– 5.489	1.2×10^{-5}	0–0.1	0–0.1	0–0.1	0–0.1
ZINC12925747	– 5.693	1.5×10^{-5}	0.3–0.5	0–0.1	0.9–1	0.9–1
ZINC89735569	– 5.020	3.3×10^{-5}	0.9–1	0–0.1	0–0.1	0.7–0.9
Optimal Range:	> –5.15	Low: $< 2 \times 10^{-6}$ Medium: $2–20 \times 10^{-6}$ High: $> 2 \times 10^{-5}$	low	low	low	low

In the distribution section, ADMETlab 2.0 predicts the Plasma Protein Binding rate of the compounds, which reflects the molecule's ability to bind to proteins in the plasma, and is measured in percentage. The optimal range is =< 90%. ADMETlab also predicts the volume distribution, which is measured in L/kg. This parameter connects the administered dose with the actual initial concentration present in the circulation, with the optimal range being 0.04 ~20. The probability of the compound penetrating the Blood Brain Barrier is also predicted as possibilities out of 1, higher possibility is considered favorable, in line with SWISSADME. Lastly, ADMETlab 2.0 predicts the amount of molecules unbound in the plasma in percentage form. The optimal range for this parameter is >= 5%. All predictions in the absorption section are displayed in (Table 7).

Table 7.– Distribution characteristics of the compounds predicted by ADMETlab 2.0

Names:	Plasma Protein Binding (PPB)	Volume Distribution (VD) (L/kg)	Blood Brain Barrier Penetration LogBB > –1 (possibility out of 1)	Fraction unbound in plasma
ZINC04716517	80.637%	0.668	0–0.1	18.236%
ZINC05514424	91.474%	0.448	0.1–0.3	5.863%
ZINC19877680	98.751%	1.735	0.1–0.3	1.165%
ZINC00754301	88.287%	0.791	0.7–0.9	11.535%
ZINC12925747	88.828%	1.358	0.7–0.9	10.825%
ZINC89735569	81.768%	2.317	0.9–1	22.456%
Optimal Range:	=< 90%	0.04 ~ 20	high	>= 5%

In the metabolism section, ADMETlab 2.0 predicted the compounds' interaction with 10 isozymes from the human cytochrome P450 family. These are CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4; they metabolize most known drugs. Inhibition of these isozymes are considered unfavorable while being a substrate is considered favorable. The prediction is measured in possibilities out of 1. All the results are displayed in (Table 8).

Table 8.– Metabolism characteristics of the compounds predicted by ADMETlab 2.0 (Possibility out of 1)

Names:	CYP1A2 inhibitor	CYP1A2 substrate	CYP2C19 inhibitor	CYP2C19 substrate	CYP2C9 inhibitor	CYP2C9 substrate	CYP2D6 inhibitor	CYP2D6 substrate	CYP3A4 inhibitor	CYP3A4 substrate
ZINC04716517	0.7–0.9	0.9–1	0.5–0.7	0.3–0.5	0.5–0.7	0.9–1	0.5–0.7	0.9–1	0.7–0.9	0.3–0.5
ZINC05514424	0.1–0.3	0.7–0.9	0.1–0.3	0–0.1	0.5–0.7	0.7–0.9	0–0.1	0.3–0.5	0.1–0.3	0.5–0.7
ZINC19877680	0.1–0.3	0.9–1	0.5–0.7	0.7–0.9	0.1–0.3	0.5–0.7	0.7–0.9	0.9–1	0.1–0.3	0.9–1
ZINC00754301	0.1–0.3	0.9–1	0.1–0.3	0.5–0.7	0.3–0.5	0.7–0.9	0–0.1	0.7–0.9	0.1–0.3	0.5–0.7
ZINC12925747	0.1–0.3	0.1–0.3	0.7–0.9	0–0.1	0.7–0.9	0.3–0.5	0.1–0.3	0.1–0.3	0.9–1	0.7–0.9
ZINC89735569	0.7–0.9	0.3–0.5	0.7–0.9	0.3–0.5	0.1–0.3	0–0.1	0.9–1	0.7–0.9	0.7–0.9	0.5–0.7

In the excretion section, ADMETlab 2.0 predicted the compounds' clearance from the body, measured in ml/min/kg, higher value in this section is considered favorable. The results are displayed in (Table 9).

Table 9.– Excretion characteristics of the compounds predicted by ADMETlab 2.0

Names:	Clearance (ml/min/kg):
ZINC04716517	7.731
ZINC05514424	1.554
ZINC19877680	13.199
ZINC00754301	2.118
ZINC12925747	8.183
ZINC89735569	7.481
Optimal Range:	High: > 15 ml/min/kg
	Medium: 5–15 ml/min/kg
	Low: < 5 ml/min/kg

In the toxicity section, ADMETlab 2.0 assessed the selected compounds and scored them in 8 categories. First, the algorithm assessed the possibility of the compound being a human ether-a-go-go-related gene (hERG) blocker. This gene encodes a voltage gated potassium pump that plays a role in cardiac depolarization and repolarization. Lower possibility is considered to be favorable as hERG blockers can obstruct cardiac function. The human hepatotoxicity(H-HT) indicates risk of liver injury, lower possibility is considered to be favorable. Drug-induced liver injury (DILI) also tests for risk of liver injury, and lower possibility is also desired. AMES Toxicity indicates risk of mutagenicity, lower possibility is considered to be favorable. Rat Oral Acute Toxicity section predicts the chance of less than 500 mg/kg drug causing acute toxicity in mammals, FDA maximum daily dose(FDA MDD) predicts the chance of the maximum recommended daily dosage being less than 0.011 mmol/kg. These two assessments could serve as references for dosage calculations in the future. Lower possibility is favored in these two sections. Lower possibility is considered to be favorable for the Carcinogenicity and the Respiratory Toxicity section. All toxicity predictions are displayed in (Table 10).

Table 10.– Toxicity characteristics of the compounds predicted by ADMETlab 2.0 (Possibility out of 1)

Names:	hERG Blockers	H-HT	DILI	AMES Toxicity	Rat Oral Acute Toxicity < 500 (mg/kg)	FDAMDD ≤ 0.011 (mmol/kg-bw/day)	Carcinogenicity	Respiratory Toxicity
ZINC04716517	0–0.1	0.1–0.3	0.7–0.9	0.1–0.3	0.1–0.3	0.1–0.3	0–0.1	0.3–0.5
ZINC05514424	0.1–0.3	0.7–0.9	0.9–1	0.9–1	0.7–0.9	0.9–1	0.5–0.7	0.9–1
ZINC19877680	0.7–0.9	0.1–0.3	0.3–0.5	0.3–0.5	0.3–0.5	0.9–1	0.3–0.5	0.3–0.5
ZINC00754301	0–0.1	0.9–1	0.9–1	0–0.1	0.5–0.7	0.9–1	0.1–0.3	0.9–1
ZINC12925747	0.1–0.3	0.1–0.3	0.7–0.9	0–0.1	0.3–0.5	0–0.1	0.5–0.7	0.9–1
ZINC89735569	0.1–0.3	0.9–1	0.3–0.5	0.7–0.9	0.1–0.3	0.7–0.9	0.7–0.9	0.5–0.7

4. Discussion

Acetylcholinesterase (AChE) based treatment has been proven to be an effective way to treat Alzheimer's disease by several clinically proven drugs. Its inhibition in patients with mild to severe Alzheimer's has been observed to improve their short (up to 24 weeks) and long (up to 1 year) term cognitive abilities [40]. However, AChE inhibitors available on today's market commonly suffer from limitations that include gastrointestinal toxicity, which significantly limits their administration and thus effects. Furthermore, reversible inhibitors such as Donepezil and Galantamine are also associated with short effect durations, as their binding with AChE hydrolyzes within minutes. While irreversible or pseudo-irreversible inhibitors such as Rivastigmine are observed to have a considerably

higher rate of causing adverse effects in patients. It is due to these limitations and today's growing Alzheimer's-affected population that new, better inhibitors are urgently needed.

However, finding a compound that can serve as a starting point for the development of new treatment methods can be a challenging task, as it is extremely labor intensive and time consuming. Traditional manual search methods can fail to deliver candidates in the needed quantity and quality. Furthermore, the high costs that commonly came with long periods of research made therapeutic drug developments unaffordable for many institutions. Web server based computational methods and virtual screening tools present an excellent alternative to this challenge. They are fast, precise and benefit from a vast array of online databases, and as a result can quickly isolate molecules with the desired characteristics. This study employed several such online tools, combining pharmacophore based virtual screening, docking simulation, and ADMET predicting tools to return a pool of candidate molecules for new AChE inhibitors. Pharmacophore-structure-based tools are especially practical in this context due to their ability to isolate structures that can bind to features on the surface of the target molecule.

Potential ligand binding sites on AChE are initially identified by ProteinPlus-DoGSite-Scorer and PrankWeb, two unique web servers with different algorithms. The two results combine to obtain locations of potential binding sites with improved precision and reliability. PocketQuery is then used to obtain structures that can best bind to the target molecule. As AChE has multiple chains in its structure, two chains, C and E, are considered in this process. PocketQuery returns a score that indicates the suitability of the structures returned as a starting point of inhibitor design, and C and E chain based structures have the highest score, thus they are chosen. The structures are then exported to ZINCpharmer for further development. Each structure comes with a set of pharmacophore structures that allow their interaction with the target molecule, and they are selected in ZINCpharmer to be preserved in the final molecules. ZINCpharmer then attempts to find molecules in the ZINCdatabase that contains these features and returns a list. Molecules with the lowest RMSD from that list are then selected for docking. A total of 50 molecules are found and docked, including 10 that occurred in both the C and the E chain list. In the subsequent docking simulation, ZINC04716517, ZINC05514424, ZINC19877680, ZINC00754301, ZINC12925747 and ZINC89735569 displayed the lowest ΔG values (<-9 kcal/mol). This indicates that they potentially can form strong bonds with AChE and could therefore be stronger inhibitors.

The most common causes of failure in today's therapeutic drugs are undesirable pharmacokinetics and toxicity. Therefore it is important for the drug candidates to undergo extensive virtual screening that examines their physicochemical, pharmacokinetics, and safety characteristics. This study employs two web servers, SWISSADME and ADMETlab, to as sess the drug-likeness of the candidates. These tools assess the molecules in terms of their absorption, distribution, metabolism, excretion, and toxicity, therefore ADMET. All the molecules are first examined under Lipinski's rule of five: molecular weight (MW) $\leq$ 500; logP $\leq$ 5; H-bond donors $\leq$ 5 and H-bond acceptors $\leq$ 10, with at most one violation. All the candidates passed this test, and they are then tested for their ADMET characteristics. For absorption, the molecules are tested for their

gastrointestinal absorption, oral bioavailability, and Pgp interactions. For distribution, the molecules are tested for their plasma protein binding rates, volume distribution, blood brain barrier penetration, and fraction unbound in plasma. For metabolism, the molecules are tested for their interaction with enzymes from the human cytochrome P450 family. For excretion, the molecules are tested for their clearance. For toxicity, the molecules are tested for hERG blocking, human hepatotoxicity, drug-induced liver injury, mutagenicity, rat oral acute toxicity, maximum recommended daily dose, carcinogenicity, and respiratory toxicity.

All of the six candidates returned different sets of strengths and weaknesses.

ZINC04716517 has a ΔG of –9.27, it displayed good gastrointestinal and oral absorption characteristics, but it is a P-glycoprotein (Pgp) substrate which may hinder its absorption into cells. It has good distribution characteristics but can not penetrate the blood brain barrier. It also has medium excretion clearance, but is potentially positive for liver toxicity.

ZINC05514424 has a ΔG of –9.17, it also has good gastrointestinal and oral absorption characteristics, and is neither an inhibitor nor substrate of Pgp. It also has good distribution characteristics but cannot penetrate the blood brain barrier. However, it is low in terms of clearance and may be positive for hepatotoxicity, mutagenicity, carcinogenicity, and respiratory toxicity. So its potential dosage is severely limited and there are large rooms for future optimization.

ZINC19877680 has a ΔG of –9.21, it showed acceptable gastrointestinal and oral absorption characteristics, but may be a Pgp inhibitor so its dosage is limited, it also may have low oral bioavailability. ZINC19877680 has acceptable distribution characteristics but cannot penetrate the blood brain barrier, its fraction unbound in plasma is also low. It has medium excretion clearance but the value is the highest among the candidates. In terms of toxicity, it is potentially positive for cardiac toxicity and hepatotoxicity, and has a low maximum recommended dosage.

ZINC00754301 has a ΔG of –9.03, the highest among the candidates, meaning it potentially has the lowest binding affinity. It displayed acceptable gastrointestinal and oral absorption characteristics, and is neither an inhibitor nor substrate of Pgp. It has excellent distribution characteristics and can potentially penetrate the blood brain barrier. But it has low excretion clearance and may be positive for hepatotoxicity and respiratory toxicity, which heavily limits its potential dosage.

ZINC12925747 has a ΔG of –9.13, it displayed bad gastrointestinal and oral absorption characteristics, but is neither an inhibitor nor substrate of Pgp. It showed good distribution characteristics and can penetrate the blood brain barrier. It also has medium excretion clearance. ZINC12925747 is potentially positive for hepatotoxicity, carcinogenicity, and respiratory toxicity.

ZINC89735569 has ΔG of –9.28, the lowest among the candidates, meaning it potentially has the highest binding affinity. It displayed good gastrointestinal absorption characteristics but may have low oral bioavailability, it is also an inhibitor of Pgp. However, it showed outstanding distribution characteristics and can penetrate the blood brain barrier. It also has medium excretion clearance. In terms of toxicity, ZINC89735569 is potentially positive for hepatotoxicity, mutagenicity, carcinogenicity, and respiratory toxicity. Its maximum recommended dosage is potentially extremely low, and there are large rooms for future optimization.

Overall, the pool of 6 candidate molecules showed unique pharmacokinetics and ADMET characteristics, and each could serve as a starting point for future treatment development. They could be optimized and/or used in compound therapeutics, and their computer predicted advantages and disadvantages can be a valuable reference. These candidates are suitable for both developments that aim to focus on safety and those that aim for potency, and they are a versatile set of options any pharmaceutical developer could choose from.

5. Limitations

This study is based on computational methods, although they are highly sophisticated and are proven in numerous cases toaccurate and reliable, their results should not be 100% relied upon, and they can not fully replace in vitro and in vivo experiments. Furthermore, it should be noted that cholinergic enhancement treatments cannot fully cure Alzheimer's, although it is capable of suppressing symptoms.

6. Conclusion:

Alzheimer's disease is the most common cause of dementia. It severely degrades its patient's cognitive abilities and memory functions, and it threatens a growing population today. Cholinergic enhancement treatment has been clinically proven to be capable of easing Alzheimer's symptoms, and the core concept is the inhibition of the Acetylcholinerase (AChE) enzyme that breaks down the neurotransmitter acetylcholine. However, Designing a new inhibitor using traditional methods can be costly, lengthy, and labor-intensive. This study employs computation based methods and tools to isolate a pool of new AChE inhibitor candidates. It follows the workflow of site identification, pharmacophore virtual screening, docking simulation, and ADMET screening. The resulting molecules are ZINC04716517, ZINC05514424, ZINC19877680, ZINC00754301, ZINC12925747, and ZINC89735569. They each possess a unique set of characteristics and can provide a starting point for any institution aiming to develop a new therapeutic drug or treatment. ZINC04716517 has high binding affinity, good absorption, distribution, excretion characteristics, and relatively low toxicity, but it cannot penetrate the blood brain barrier and is a Pgp substrate. ZINC05514424 has good binding affinity, good absorption and distribution characteristics, but has relatively high toxicity and cannot penetrate the blood brain barrier. ZINC19877680 has good binding affinity, reasonable absorption, distribution characteristics, good excretion characteristics, but has medium toxicity and cannot penetrate the blood brain barrier. ZINC00754301 has acceptable binding affinity, great absorption and distribution characteristics, poor excretion characteristics, medium toxicity, and can penetrate the blood brain barrier. ZINC12925747 has good binding affinity, good absorption, distribution, excretion characteristics, can penetrate the blood brain barrier, and has medium-high toxicity. ZINC89735569 has great binding affinity, acceptable absorption characteristics, great distribution and excretion characteristics, can penetrate the blood brain barrier, but has relatively high toxicity. With future optimizations, and after further evaluation with vitro and in vivo experiments, they all can potentially serve as versatile options as foundations of therapeutic drug development.

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