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## A COMPUTATIONAL STUDY ON THE EFFICACY OF SMALL MOLECULES AS DUAL INHIBITORS FOR β-SECRETASE 1 AND ACETYLCHOLINESTERASE AS ALZHEIMER'S DISEASE THERAPEUTICS

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**Abstract**

Alzheimer's disease is characterized by the progressive decline of many cognitive functions that involve numerous parts of the brain. Due to the complex nature and multi-faceted pathogenesis of neurodegenerative diseases, AD's pathology has been correlated with a loss of cholinergic function as well as the overproduction of A$\beta$42. Because of this, multitargeted ligands have great potential as therapeutics. In this paper, we assessed the "bindability" of the proteins using various computational methods and online software. Then, we virtually screened through millions of potential small molecules using pharmacophore maps to find potential small molecule candidates. We then used software developed by the Swiss Institute of Bioinformatics (SIB) to both assess the binding energy of the small molecules to the compound (of which all compounds had a $\Delta G < -7.00$ kcal/mol) as well as assess the druggability of the small compounds through their ADME profiles. By the end, we were left with 4 organic compounds that showed the most promise as dual inhibitors. Ranked from most to least promising, they are: ZINC68569271, ZINC41367268, ZINC67202317, ZINC05611209. They all show strong binding affinities for both AChE and BACE1, with the majority of each compound having a Gibbs free energy value of $\Delta G < -7.00$ kcal/mol.

**Keywords:** *Alzheimer's Disease, Acetylcholinesterase, β-secretase 1, Molecular Docking, ADME*

## 1. Introduction

### *1.1. Alzheimer's disease*

Alzheimer's disease (AD) is characterized by the progressive onset of cognitive, functional, and behavioral impairment. Currently, AD is the sixth leading cause of death and is one of the only top ten causes of deaths that is continually increasing in prevalence (Atri, 2019). AD is fatal due to a multitude of reasons, however one of the biggest prob-

lems with AD is identifying its existence. Unlike other diseases, AD's symptoms can often be attributed to regular aging. This causes symptoms to be left undiagnosed, untreated, and ignored, losing valuable time, and causing harmful delays in receiving appropriate therapeutics. For example, while lapses in memory and judgment are frequent symptoms among aging, complete irreversible loss of memory is a common side effect of AD (Atri, 2019). This demonstrates how AD often accentuates the symptoms of normal aging, often making it more severe.

As it stands right now it is estimated that by 2030, the number of Americans with AD would increase by 35%, with the potential to skyrocket up to tripling in prevalence by 2050. Current projections and estimates show that risk of dementia doubles roughly every 5 years from the age of 65 to 85. At 65 years old, the risk of developing AD is roughly 1% – 2%; by 85, the risk of developing neurodegenerative disease is 30% – 50% (Atri, 2019; Alzheimer's association, 2019).

### *1.2. AD Risk factors*

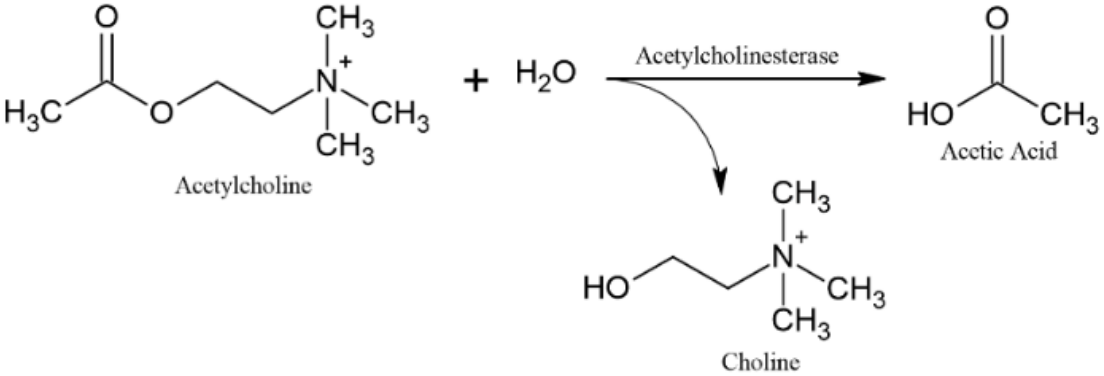
Unlike other diseases, AD is thought to be multi-faceted, meaning its pathogenesis requires a combination of factors. So far, the greatest known risk factor for AD is age, followed closely by family history and one's genotype (Alzheimer's disease, 2019). Previous twin and family studies indicate that genetic factors play a major role in roughly 80% of AD cases (Tanzi, R. E., 2012; Gatz, M. et al., 2006).

There are two categories of genes that can influence the pathogenesis of a disease: risk genes and deterministic genes. A risk gene simply increases the risk of developing a disease, while a deterministic gene all but guarantees the development of a disease. Rare mutations in the APP, PSEN1 and PSEN2 genes have been shown to be deterministic genes for AD, whereas common gene polymorphisms for the APOE gene such as ε4 and ε2 have been shown to influence but not cause the pathogenesis of AD (Tanzi, R. E., 2012).

### *1.3. Acetylcholine/Acetylcholinesterase*

Neurons are the fundamental component of communication within an organic system. They communicate through electrochemical signals that are driven by charged particles. Communication occurs at tiny gaps between the neuron receptors called synapses, where the presynaptic neuron sends a neurotransmitter to the postsynaptic neuron which binds to receptor proteins on its surface (Lovinger, D. M., 2008). One of the most important neurotransmitters is called acetylcholine (ACh) (Kondziella, D., 2016), which is associated with AD and is one of the two proteins of focus of this study. Though AD has no cure, therapeutics can help delay symptoms or even completely halt the onset of dementia, one of the many symptoms of AD. Currently, the main class of drugs used to treat AD are acetylcholinesterase / cholinesterase inhibitors (ChEIs).

**Figure 1.** *Catalyzed hydrolysis of ACh into HAc and Choline (Cavalcante, et al., 2020; Trang, A. et al., 2020)*




Maintaining a healthy equilibrium of neurotransmitter systems such as ACh is essential to cognitive function. Although the cholinergic nervous system isn't the only neurological system affected by cognitive disorders such as AD, a lacking cholinergic

system has been attributed to the progressive impairment in learning and memory (Marucci, G. et al., 2020). For example, a premature loss of cholinergic neurons in the *basal forebrain* was observed for those afflicted with AD. Acetylcholinesterase is an enzyme with the capability to catalyze the hydrolysis of acetylcholine into acetate and choline (see fig. 1), thus reducing its concentration at the synapse and consequently, its ability to help conduct a synapse (Trang, A. et al., 2020).

### *1.4. β-secretase*

*β*-secretase (BACE1) is responsible for the aggregation of amyloid-β (Aβ) and is the second of the two proteins that we are studying (Ma, W.-H. et al., 2021). BACE1 is responsible for the aggregation of $A\beta_{42}$, which has been correlated to increased toxicity in AD (Hampel, H., 2020). BACE1 has been measured in higher concentration and heightened activity in AD patients' brains and body fluids; corroborating the idea that BACE1 plays a critical role in the pathogenesis of AD (Hampel, H., 2020; Breijyeh, Z et al., 2020;).

Familial AD (FAD) cases are linked to mutations in the gene for amyloid precursor protein (APP) production. Patients who exhibit "increased dosage" of the APP gene, which is located on chromosome 21, are at higher risk to develop early-onset AD and overproduce the $A\beta_{42}$ peptide (Vassar, R. et al., 2009). Thus, the BACE1 enzyme is a prime drug target to develop inhibitors that inhibit the amyloid aggregation of $A\beta_{42}$.

### *1.5. Tau and Amyloid Aggregation*

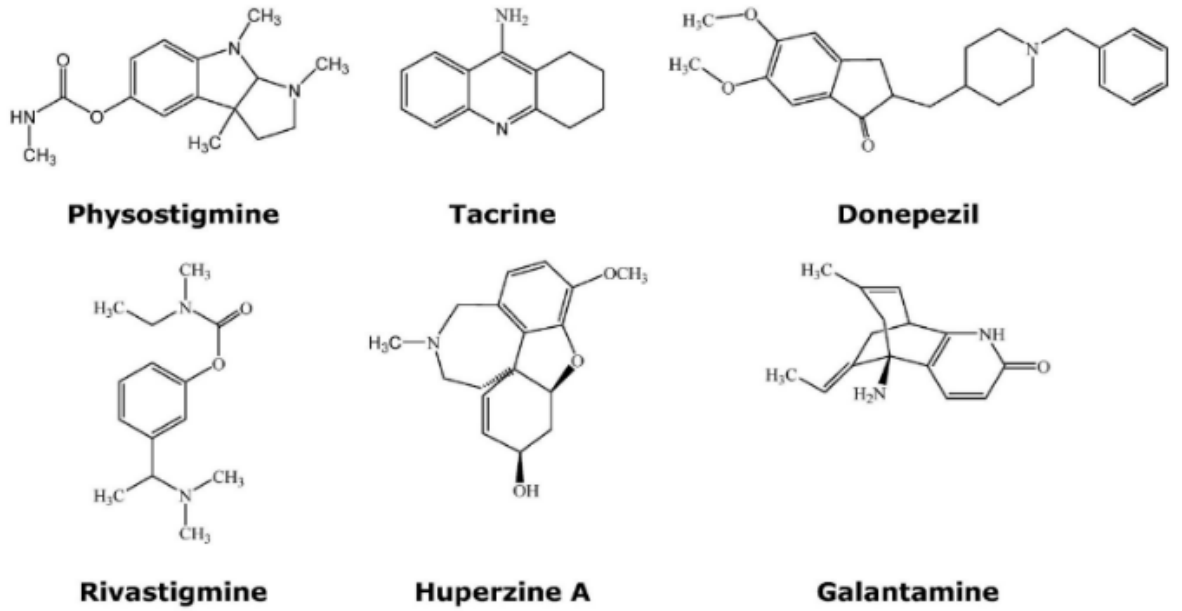
Another field of AD therapeutic research is the inhibition of tau and amyloid aggregation. Amyloid aggregation is the aggregation of the protein called amyloid in organs which can cause organ failure. As mentioned before, patients with FAD have a genetic mutation on their 21st chromosome which allows the overproduction of $A\beta_{42}$ (Ma W.-H. et al., 2021; Hardy, J., 2006; Breijyeh, Z et al., 2020;).

Under normal physiological conditions, the tau (τ) protein monitors and regulates the structural stability of microtubules and microfibers. However, in a diseased brain such as an Alzheimer's brain, tau becomes abnormally hyperphosphorylated. Then, the tau proteins aggregate together to form paired helical filaments, which then form neurofibrillary tangles which get in the way of the nervous system (Medeiros, R. et al.,)

### *1.6. FDA-approved therapeutics:*

AChE has proven itself to be a viable target for therapeutic drugs and symptomatic improvement against the development of AD. Inhibition of AChE has real clinical results because of its proven efficacy as a treatment for previous ailments such as myasthenia gravis (Mehta M. et al., 2012).

**Figure 2.** *Chemical diagrams of known AChEIs. Note physostigmine is no longer in use (Marucci, G. et al., 2020)*



Currently, AChEIs are the main class of drugs to treat AD, with other drugs making it onto the list (Marucci, G. et al., 2020). Currently, the FDA-approved drugs available to treat AD are galantamine, rivastigmine, donepezil, and memantine (Medications for Memory, Cognition and Dementia-Related Behaviors, 2021). Note that while the first three drugs are AChEIs, memantine is not, rather than inhibiting acetylcholinesterase, it inhibits glutamate, a neurotransmitter that brains which are afflicted with AD produce too much of; which can cause harmful side effects (NHS, 2021).

### *1.7. Dual inhibitors of BACE1 and AChE*

Both the amyloidal aggregation caused by the BACE1 protein and lack of cholinergic function caused by AChE has made research into finding dual inhibitors for both enzymes a hot spot for scientists in the field. This process of targeting two or more biomolecular targets with a single molecule is called the "multi-target directed ligands" (MTDL) process.

The MTDL approach is more well-received when attempting to therapize patients suffering from multi-faceted diseases such as AD. Reportedly, the MTDL approach is suggested to have seen better efficacy and safety compared to the original "STDL" approach (Stern, N. et al., 2022). Previous works by researchers discovered only 107 already FDA-approved drugs that can bind to AChE. Of those 107 drugs, 33 of them can also bind to BACE1 (Stern, N. et al., 2022).

## 2. Methods

### *2.1. Preparation of screening process and tools*

Proteins and enzymes operate via binding sites; to properly inhibit their function, small molecules must preferentially bond to these binding sites to obstruct other objects from doing so. The approach we will use for this computational study is first, we identify readily available binding sites on both enzymes using freely available software online. Next, we aim to utilize pharmacophore maps to map out the interaction between our preferred enzyme and a receptor as the base pharmacophore map to compare other compounds too.

### *2.2. Identification of binding sites on AChE and BACE 1*

Computationally, we can identify binding sites on our enzymes of choice using readily available free software online. There are two processes by which we can operate: Firstly, we can preliminarily detect potential binding sites on the protein by using a geometric method to identify the sizes of these binding sites. Note that if the binding site is too large, there is no proper "cup" that can hold the small molecule in place. Conversely, if the binding site is too small, then the small molecule cannot even fit in the binding site. For our experiments, we will be looking at a range of 100–550 Å, preferably looking for sites with volumes around 300 Å. This will be done using online software developed by the University of Hamburg called Proteins Plus (Schöning-Stierand, K. et al., 2022; Schöning-Stierand, K. et al., 2020; Fährrolfes, R. et al., 2017).

The second process of experimentation we will use is an energy-based method. The potential energy of the interaction between our small molecule and the binding site is as critical to our assessment of the binding site as the geometry of it. If there is no favorable energetic interaction between the binding site and small molecule, then nothing will happen. We will assess the energy of our interactions using online software developed by the VAJDA lab at Boston University called FT Site. Our last process of experimentation is a machine learning-based method. Instead of looking at the geometry or the energy of the interactions, machine learning is used to predict the quality of the ligand binding site. We will utilize online software called PrankWeb, which builds upon P2Rank, a stand-alone command line program that predicts ligand binding sites from its structure (Prankweb, 2017; Jackubec, D. et al., 2022; Jendele, L. et al, 2019; Krivák, R., 2018; Cusbg, 2020;).

### *2.3. Virtual screening of small molecules*

Though sources dispute on the exact value, it is commonly agreed that only under 10% of the human proteome is druggable (Kana, O., et al., 2019). This means that potentially either of our enzymes of interest are not druggable, i.e., they do not have any suitable binding sites. After having

identified whether or not BACE1 and AChE are druggable, we will move on to our next phase of experimentation, where we will virtually screen millions of compounds to identify those that show promise when binding to either protein.

We will be accomplishing this through utilizing pharmacophore maps, a pharmacophore is a map of the interaction between two compounds which aims to identify important features for receptor binding. When looking at an interaction, there are three necessary points of information that are need-to-know. Firstly, we need to know the quantity of interactions; we ask, "how many interactions are there?" Secondly, we look at the location of the interactions; we ask, "where do these compounds interact?" Lastly, we must also identify the type of interactions; we ask, "what kind of intermolecular forces are making this interaction happen?" A pharmacophore map answers all these questions graphically.

Computationally, we will be utilizing an online software called PocketQuery (Koes D. R. et al., 2011; Koes, D. R., 2023). Developed by the University of Pittsburgh, Pocket Query looks at the interaction between a protein and a small molecule and generates a pharmacophore map for it based on the conditions we set. These pharmacophore maps is the main tool that we will be using to virtually screen through millions of FDA-approved compounds. If the pharmacophore map of the original interaction overlaps with the pharmacophore map of another compound, then the molecule of interest can bind very similarly to the original overlap and thus is a potential inhibitor of it.

In this study, we will first construct the pharmacophore map of the interaction between BACE1 and a peptide inhibitor, as peptide inhibitors are already rather small; the peptide we will be looking at is OM99–2. The reason we are looking at BACE1 first is because, in comparison to AChE, BACE1 is much less readily druggable. Thus, it is the more challenging target; and, if compounds that can inhibit it are identified, there is a much higher chance that it can also bind AChE. Conversely, had we identified compounds that could inhibit AChE first, there would be a much lower chance that it would have the dual function to also inhibit BACE1.

### *2.4 Molecular docking*

Up to this point, while we can determine how well compounds can match the pharmacophore of OM99–2 with BACE1, with our current methods we cannot determine how well these compounds can actually bind to BACE1 and AChE. This is where we will utilize molecular docking; molecular docking is a key tool in structural molecular biology and computer-assisted drug design (Morris G. M. et al., 2008).

Molecular docking is a process where the energy of an interaction between a ligand and a biomolecular target is estimated. In this computational study, we will be utilizing an online docking software called SwissDock (Grosdidier, A. et al, 2011a; Grosdidier, A. et al., 2011b). Since some of the compounds potentially are not within SwissDock's database, we generated mol2 files detailing the composition of the ligands and small molecules using the ZINC IDs we found from Pocket Query and ZINCPharmarcy.

### *2.5 Pharmacokinetics, and ADME*

Pharmacokinetics is a subbranch of pharmacology where the interaction between an administered drug and the human body is studied. Generally, there are four main parameters to be examined in this field, which includes absorption, distribution, metabolism, and excretion (aptly named ADME) (Grogan, S. et al., 2023).

Absorption is the process by which a drug, either in tablet or capsule form, enters the circulation system of the human body. This parameter determines how quickly the drug is able to enter the bloodstream and reach its desired location. Absorption itself has a sub-parameter, called liberation; liberation is the process by which the drug is released from its pharmaceutical form (i.e. tablet/capsule) (Grogan, S. et al., 2023).

Distribution is a parameter that describes how a substance can distribute itself throughout the human body. This parameter varies from substance to substance as substances have differing biochemical properties. Put simply, the process of distribution has two main factors: diffusion, and convection. These can be affected by the size, polarity, or binding ability of the substance (Grogan, S. et al., 2023).

Metabolism is the process by which the body processes and decomposes the drug. If

a drug is metabolized by the body too quickly before it reaches its designated compartmental destination, it is ineffective and therefore has an inadequate ADME profile; however, if the drug is difficult to metabolize, it has the potential to have potently toxic effects from being in the body for too long. Most of the metabolism done by the body onto drugs is through the phase I (CYP450) and PHASE II (UGT) reactions in the liver. The phase I reaction oxidizes the molecules and converts them into polar metabolites that can then be bound to polymers in the phase II reaction through glucuronidation (Miyauchi, Y. et al., 2021). Most importantly, the combined efforts of these processes generally inactivate the medical capabilities of the drugs processed. This demonstrates that a balance between being metabolized quickly or slowly is an essential part of the ADME profile (Grogan, S. et al., 2023; Miyauchi, M. et al., 2021).

**E**xcretion is the process by which the drug is flushed and eliminated from the body. Generally, this process is done by the kidneys, however, excretion can also occur via the lungs, skin, or the gastrointestinal tract. In the kidney, drugs can be excreted by the passive glomerular filtration or the active tubular secretion (Grogan, S. et al., 2023; Madison J. E. et al., 2008).

### *2.6 Lipinski's Rule of 5*

There are many biochemical properties that predict whether a substance would have an adequate ADME profile for draggability. For example, the number of hydrogen bond donors and acceptors, molecular weight, calculated LogP (CLogP), etc. The number of hydrogen bond donors and acceptors dictates how easily the drug can bond to receptors. A higher amount of bond donors/acceptors means that the drug will attempt to bind to every available protein on its way to the designated compartmental destination and reduce its effectiveness. Additionally, the molecular weight of the drug must be relatively light to allow skin absorption, as larger molecules cannot pass the corneal layer (Bos J. D. et al., 2020).

For Lipinski's set of rules, aptly named *Lipinski's rule of 5*, it requires that the compound has no more than 5 hydrogen bond donors and no more than 10 hydrogen bond acceptors. It also requires that the molecular weight of the molecule stays under 500 Daltons, which is equivalent to one atomic mass unit. And finally, a CLogP no more than 5 (Benet L. Z. et al., 2016). The calculated LogP is a measure of how hydrophobic or hydrophilic a compound is. If hydrophobic, then the compound is lipophilic, and vice versa; if hydrophilic, then the compound is lipophobic.

For our purposes, we will utilize an online software called SwissADME, utilizing the top five compounds that we determined from our data from SwissDock (Daina, A. et al., 2017). [41,] We can reconstruct their chemical structure in the software and the software will run an algorithm to determine many factors, namely the hydrogen bond donors and acceptors, molecular weight, CLogP, as well as any other important factors, such as gastrointestinal (GI) absorption, and blood-brain-barrier (BBB) permeant.

## 3. Results

### *3.1 Identifying binding sites on AChE and BACE1 geometrically*

Using the geometric based method, we are given both the volume of the binding site as well as the drug score, which indicates the quality of the binding site and how druggable it is. On the tables below, the volumes and drug scores are detailed for the top five binding sites on both AChE and BACE1

**Table 1.** – *Top 5 binding sites on AChE filtered for size*

| Pocket | me (Å3) | g Score |
|---|---|---|
| P_2 | 399.60 | 0.81 |
| P_3 | 355.84 | 0.74 |
| P_4 | 352.42 | 0.70 |
| P_5 | 285.17 | 0.63 |
| P_6 | 284.94 | 0.61 |

From table 1, we can infer that AChE is a very promising drug candidate. With an average volume of 335.59 $Å^3$, the average binding site sits well within the range decided earlier. Furthermore, the average drug score of the 5 pockets is 0.698. In figure 3, we can see a cartoon visualization of AChE as well as the 5 binding sites identified.

**Figure 3.** *Top 5 identified binding sites on AChE through the geometric method*

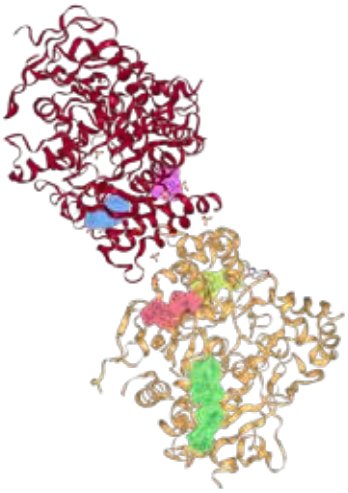
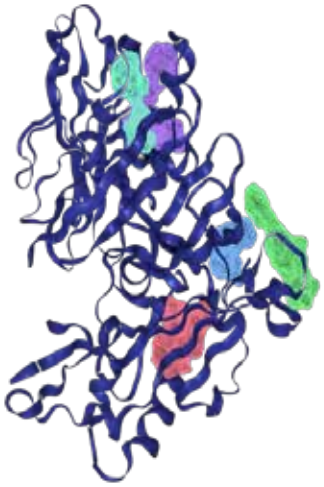


**Table 2.** *Top 5 binding sites on BACE1 filtered for size.*

| Pocket | Volume (Å3) | Drug Score |
|---|---|---|
| P_4 | 305.39 | 0.73 |
| P_5 | 291.68 | 0.66 |
| P_6 | 225.62 | 0.66 |
| P_7 | 292.61 | 0.51 |
| P_8 | 171.87 | 0.50 |

**Figure 4.** *Top 5 identified binding sites on BACE1 through the geometric method*



From table 2, we can see that ftsite predicts that BACE1 is also druggable. There are also multiple binding sites all within the volume range earlier determined. However, note that the average binding site and drug score, with an average volume of 257.43 $Å^3$, and an average drug score of 61.2, is generally lower than that of AChE. From these results, we can reasonably infer that AChE is a more viable binding target than BACE1 is.

***3.3 Identifying binding sites on AChE and BACE1 based off machine learning.***

While the previous method predicted the presence of binding sites solely based off the geometry and the 3D structure of the protein. PrankWeb predicts the presence of binding sites using a machine learning algorithm that has become well adapted to predicting the presence of binding sites. PrankWeb is an extension of the machine learning algorithm P2Rank, which is a ligand binding site

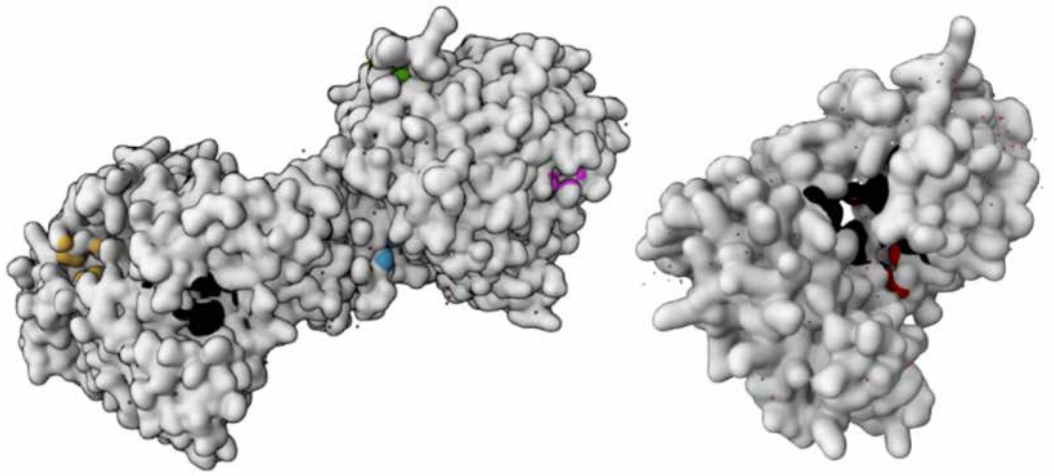
**Figure 5.** *The black binding site pocket profiles for AChE (top) and BACE1 (bottom)*


Figure 5 was generated by the machine learning-based algorithm multiple binding sites were identified. The machine learning algorithm detected only 2 pockets on BACE1 while it detected 12 pockets for AChE, further supporting our claim that BACE1 is less readily bindable relative to AChE.

In figure 5, the colored amino acids represent the detected binding sites on the protein. With the black colored amino acids representing the binding site that is ranked the highest. By being ranked the highest, the algorithm predicts that these binding sites are the most promising binding sites in terms of druggability. Figure 6 displays the pocket profiles of both pockets; the pocket profile is a quick summary of important parameters of the pocket such as its rank, the pocket score, its probability score, etc. We can see that these pockets have scores

of 29.65 and 28.47 for AChE and BACE1 respectively, which is indicative of their capability as binding sites. Overall, this shows us that while AChE is more readily bindable than BACE1 is, both protein targets do show promise of being drug targets.

**Figure 6.** *Images of AChE (left) and BACE1 (right) as generated from the machine learning-based method*

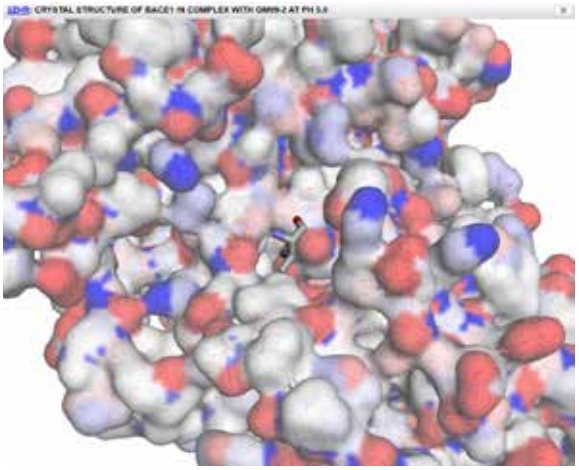


### *3.4 Pharmacophore maps*

Now that AChE and BACE1 have both been assessed to be viable inhibitor targets, we must identify various compounds that have the potential to act as a ligand and inhibitor for these proteins. Since AChE has a "binding advantage," – being more capable of being bound – over BACE1, we will examine BACE1 first. Utilizing the base pharmacophore map of the interaction between BACE1 and OM99–2, we selected the top 5 interactions between the protein and small molecule. Listed below in table 3 is summative data on the information given about the interactions.

**Table 3.** *– Data on 5 different interactions between BACE1 and OM99–2*

| PDB | Chain | Size | Distance | Avg ΔG | Residues | Score |
|---|---|---|---|---|---|---|
| 2ZHR | C | 1 | 0 | –5.86 | VAL | 0.8654 |
| 2ZHR | C | 2 | 4.4547 | –3.02 | VAL, ASN | 0.8118 |
| 2ZHR | C | 3 | 5.925 | –3.15333 | GLU, VAL, ASN | 0.7919 |
| 2ZHR | C | 2 | 7.3035 | –3.565 | ALA, PHE | 0.7849 |
| 2ZHR | C | 3 | 7.3035 | –3.13 | ALA, GLU, PHE | 0.7627 |

| Residue | Figure |
|---|---|
| VAL |  |

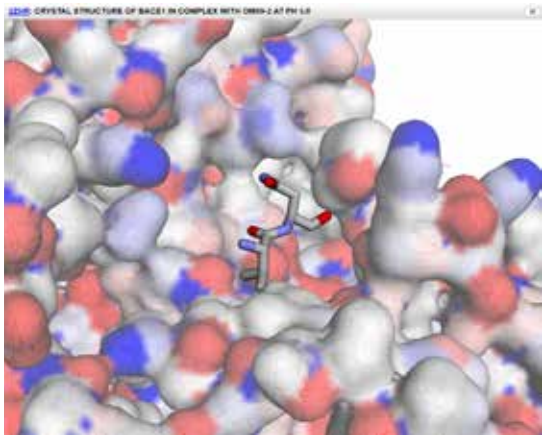
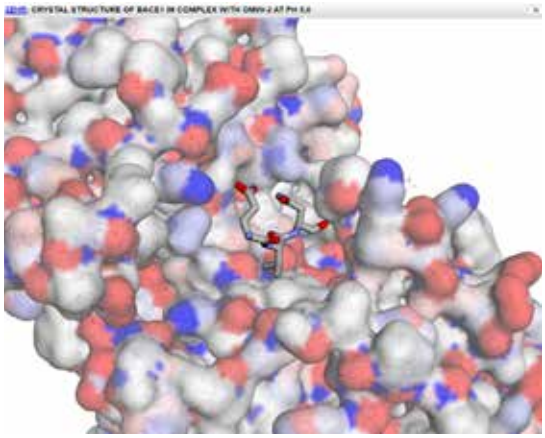
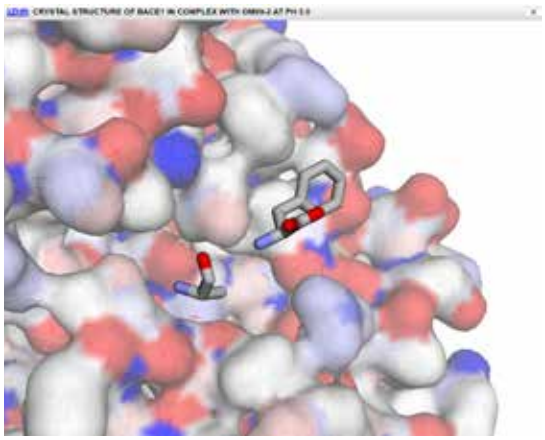
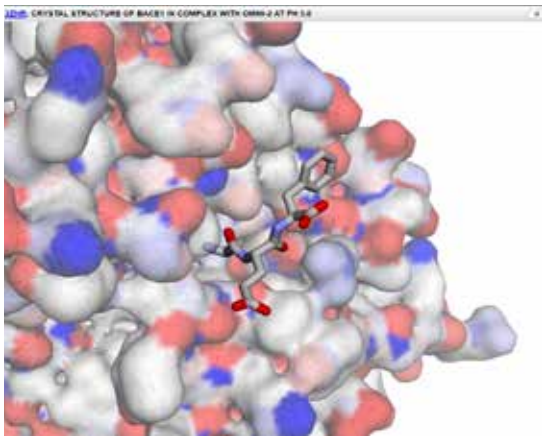
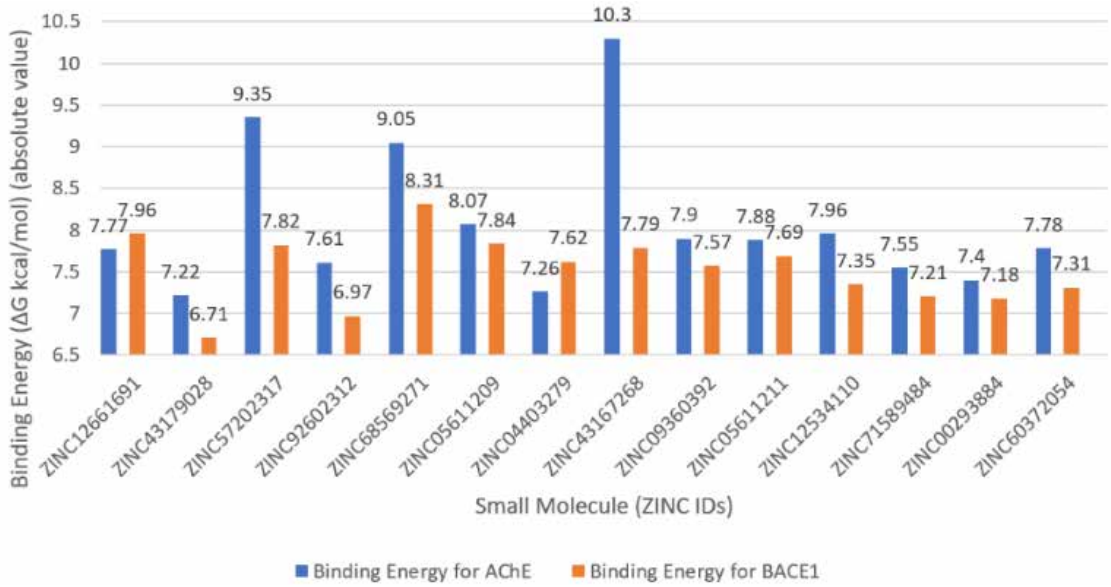
| Residue | Figure |
|---|---|
| VAL, ASN |  |
| GLU, VAL, ASN |  |
| ALA, PHE |  |
| ALA, GLU, PHE |  |

**Table 4.** – *The top 3 compounds that matched the pharmacophore map of its specific interaction the closest for all 5 interactions selected*

| | **1 st Choice** | **2 nd Choice** | **3 rd Choice** |
|---|---|---|---|
| AA Residue: VAL | ZINC12661691<br>RMSD: 0.026 | ZINC05611209<br>RMSD: 0.027 | ZINC05611211<br>RMSD: 0.027 |
| AA Residue: VAL ASN | ZINC43179028<br>RMSD: 0.036 | ZINC04403279<br>RMSD: 0.037 | ZINC12534110<br>RMSD: 0.046 |
| AA Residue: GLU VAL ASN | ZINC57202317<br>RMSD: 0.045 | ZINC43167268<br>RMSD: 0.049 | ZINC71589484<br>RMSD: 0.052 |
| AA Residue: ALA PHE | ZINC92602312<br>RMSD: 0.061 | ZINC09360392<br>RMSD: 0.062 | ZINC00293884<br>RMSD: 0.068 |
| AA Residue: ALA GLU PHE | ZINC68569271<br>RMSD: 0.089 | ZINC78555142<br>RMSD: 0.089 | ZINC60372054<br>RMSD: 0.091 |

When selecting compounds to use for this study, PocketQuery provides us with a parameter called the root mean squared deviation (RMSD) value. The RMSD value is a quantitative measure of the similarity between superimposed atomic coordinates (Kufareva, I. et al., 2012). For our purposes, the RMSD value is used to determine how closely the chosen compound's pharmacophore map matches the overlay of the original pharmacophore map (the one between BACE1 and OM99–2). To ensure accuracy that we choose compounds with the highest likelihood to properly bind BACE1 (and hopefully AChE), we reject any compounds with values of RMSD > 0.10.

**Figure 7.** *Binding energies of identified small molecules with the enzymes AChE and BACE1*




### *3.5 Molecular Docking*

From the data gathered and summarized in figure 7, we can see a few clear candidates as our "best" compound. For example, we can see that 43167268 shows promise as a candidate with its ΔG for AChE of 10.3. For our other compounds, we can determine which are the top five candidates by looking at the largest minimum ΔG value for each pair of values. Using this method, we will rank the top 5 compounds by ranking them from the largest minimum value to the smallest minimum value. This yields the ranking: 1. ZINC68569271, 2. ZINC41367268, 3. ZINC12661691, 4. ZINC67202317, 5. ZINC05611209.

### *3.6 ADME Profiles*

In table 5, we can see the respective ADME profiles of all the 5 compounds chosen from the docking procedures. Of the 5 compounds examined, 4 successfully passed Lipinski's Rules, while ZINC12661691, with a molar mass (MM) = 518.52 g/mol, exceeded the MM limit of 500 g/mol.

**Table 5.** – *ADME profiles of the top 5 identified compounds from SwissDock*

| | H-bond Donors | H-bond acceptors | LogP | Molecular Weight | GI Absorption | BBB permeant | Lipinski's Rules? |
|---|---|---|---|---|---|---|---|
| 68569271 | 3 | 3 | 3.69 | 375.49 g/mol | High | No | Yes |
| 41367268 | 1 | 7 | 2.03 | 337.31 g/mol | High | No | Yes |
| 12661691 | 2 | 4 | 4.92 | 518.52 g/mol | High | No | No |
| 67202317 | 2 | 2 | 3.75 | 379.56 g/mol | High | No | Yes |
| 05611209 | 1 | 5 | 3.08 | 409.25 g/mol | High | No | Yes |

Though SwissADME deems these compounds incapable of crossing the blood brain barrier (BBB), chemical modifications can be made to these compounds in the lab to allow them to cross the BBB. These findings act as a good starting point for the next series of trials and experiments needed to further validate the work of this paper.

## 4. Conclusion

Alzheimer's disease is a disease that afflicts millions throughout the world and has a heavy prevalence in our modern day. Alzheimer's is believed to be a multi-faceted disease, the facets we chose to attack in this study is by inhibiting the functions of the proteins AChE and BACE1. AChE's function in the brain is to catalyze the hydrolysis of ACh, a vital neurotransmitter, thereby reducing its concentration and therefore effectiveness at the synapse. BACE1's function is to cleave the APP gene, which causes the overproduction of Aβ42 peptides, which form long fibrils that interfere with neuronic communication and can lead the pathogenesis of AD.

We applied the computational methods listed above to search for dual inhibitors that could effectively inhibit the functions of both AChE and BACE1, which prevents the hydrolysis of ACh and the production of Aβ42 peptides, respectively. Because different binding targets have varying levels of druggability, we first assessed how capable AChE and BACE1 were of being bound through varying computational methods. Through these methods, we aimed to identify potential binding sites and utilize the data gathered to determine the general druggability of both proteins.

We first looked utilized a method called ProteinsPlus that uses a Difference of Gaussian (DoG) filter to detect potential binding pockets purely based on the physical structure of the protein. The method then predicts various parameters that describe the size, shape, and chemical properties of the pocket detected. We used this method to give a surface-level indication to the druggability of the proteins. Next, we utilized an online software called PrankWeb to further assess the druggability of potential binding sites on AChE and BACE1. PrankWeb utilizes machine learning to predict the location and druggability of binding sites on proteins. From these methods, we were able to determine that BACE1 was much less readily bindable than AChE is.

Due to the fact that AChE has a "binding advantage" over BACE1, it seemed more practical to first identify compounds that could inhibit the function of BACE1 first, then see how well these identified compounds could inhibit AChE. To accomplish this, we utilized pharmacophore maps. Using the interaction between BACE1 and OM99–2 as a base, we identified compounds that overlayed well with the base pharmacophore map to virtually screen through millions of compounds. Then, we chose the top fourteen compounds that bound to BACE1 the most similarly to OM99–2. We then utilized docking software to predict the binding energies (measured in $\Delta G^\circ$) of the interaction of these fourteen compounds with both AChE and BACE1. From the data collected, we chose the top 5 most appealing compounds that showed the capability to bind both BACE1 and AChE. Finally, we assessed the ADME profiles of the top 5 chosen compounds using online methods to determine how effective these compounds could be as a drug. Of our top 5 compounds identified, all but ZINC12661691 had adequate ADME profiles. All 5 compounds bound to both protein targets with binding energies less than −7.00 kJ/mol, with ZINC43167268 binding to AChE with a binding affinity of $\Delta G^\circ$ = −10.30 kJ/mol. Thus, of the millions of compounds initially screened, we are left with only 4 compounds (from most to least promising): ZINC68569271, ZINC41367268, ZINC67202317, ZINC05611209. Note, SwissADME assessed that all 4 small molecules were incapable of crossing the BBB, this is a crucial part of drug discovery for neurodegenerative diseases, as being incapable of crossing the BBB effectively makes the drug useless; however, simple chemical modifications to the compounds can render them BBB-permeable.

Due to the nature of computational studies like this paper, there are several limitations that this paper has. For example, the computational methods used in this paper such as ftsite, PrankWeb, SwissDock, and SwissADME are all based upon prediction and have not been physically observed. The predicted binding affinities of these compounds potentially do

not reflect the real-world binding affinity. To further validate the work of this paper, there are numerous next steps that this line of work can take. Firstly, to alleviate the risk of using such an uncertain and small sample size, this computational experiment can be conducted several more times to identify new compounds that are able to bind to the protein targets as readily or more readily than the currently identified compounds. Once this is accomplished, the binding affinities of these compounds should be experimentally determined within the lab. Then pre-clinical *in vitro* trials can be conducted to determine the efficacy these drugs are within an organic system; note that during these *In vitro* trials, chemical modifications are to be made that can render the small molecules BBB permeable. Finally, clinical *in vivo* trails can be conducted to determine the efficacy of these drugs in the human body.

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