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DRUG DISCOVERY: DEVELOPING NOVEL COMPOUNDS FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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DRUG DISCOVERY: DEVELOPING NOVEL COMPOUNDS FOR THE TREATMENT OF ALZHEIMER'S DISEASE

by

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ABSTRACT

DRUG DISCOVERY: DEVELOPING NOVEL
COMPOUNDS FOR THE TREATMENT
OF ALZHEIMER'S DISEASE

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The University of Texas at Arlington, 2016

Faculty Mentor: Frank Foss, Walter Schargel

Alzheimer's disease (AD) is a neurological condition that accounts for 60 to 70% of dementia. The neurotransmitter acetylcholine is thought to play a fundamental role in the propagation of memory and is a neurotransmitter of great importance in AD. Another neurotransmitter involved in the treatment of AD is glutamate via the NMDA receptor. While cholinergic medications and NDMA antagonists are available for the treatment of AD there remains a need to develop different medications that are more effectual for certain individuals My objective is to design pharmacologically active molecules to treat AD by utilizing knowledge of neuropharmacology, computational techniques, and the literature in order to generate molecules that have applications to better treat Alzheimer's disease by modifying functional groups to increase receptor efficacy and affinity. Computational models demonstrated greater receptor affinity compared to currently available AD drugs.

This shows that these compounds might be potentially useful in treating AD.

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CHAPTER 1

BACKGROUND

1.1 Drug Discovery and Neurotransmission in Alzheimer's Disease

Alzheimer's disease (AD) is a neurological condition that accounts for 60 to 70% of dementia (Burns & Iliffe, 2009). The neurotransmitter acetylcholine is thought to play a fundamental role in the propagation of memory and is a neurotransmitter of great importance in AD (Francis et al., 1999). Another neurotransmitter involved in the treatment of AD is glutamate via the NMDA receptor (Reisberg et al,. 2003). While certain cholinergic medications and NDMA antagonists are available for the treatment of AD there remains a need to develop different medications that may be more effectual for certain individuals. Memantine is an NMDA receptor antagonist and is one of several medications currently available for the treatment of moderate to severe Alzheimer's disease in the United States (Reisberg et al,. 2003). A molecule with similar molecular structure to that of memantine is Huperzine A, which has been shown to be an NMDA antagonist. Huperzine A is an alkaloid found in the moss *Huperzia serrata* (Zangara, 2003). Although this molecule by itself has not been shown to be effective in the treatment of AD, functional group modification in attempts to make the medication have greater receptor affinity and efficacy could elucidate a new medication that could replace Memantine.

1.2 The Genesis of Alzheimer's Disease

There are several theories in place as to how Alzheimer's disease onsets in the brain. One such theory is known as the Amyloid hypothesis in which neuronal disruptions

caused by the beta-amyloid protein impair axonal connections (Tanzi & Bertram, 2005). Another such postulate involves the formation of Tau tangles caused by the protein Tau which break down the cell bodies of neurons as they accumulate, leading to the death of the neurons (Lester et al., 2004). As these conditions progress, breakdown in cholinergic neurons as well as other neurons can lead to reduced capacity for memory and other cognitive functions. Currently pharmacological interventions for Alzheimer's disease involve the application of cholinergic medications that stimulate acetylcholine receptors therefore allowing greater activation of these synapses leading to an increase in their function (Francis et al., 1999). A major challenge is that not all such drugs are selective to the to the specific acetylcholine receptor alpha7, and greater efficacy is desired in new molecules to better treat a wider variety of patients with Alzheimer's disease. In addition many patients experience side effects that make many of these current treatments intolerable (Reisberg et al., 2003).

Several different approaches for pharmacological interventions exist in the treatment of Alzheimer's. One such treatment currently available includes acetyl cholinesterase inhibitors such as galantamine (Scott & Goa, 2000). Acetyl cholinesterase inhibitors reduce the breakdown of acetylcholine at the level of the synaptic cleft thereby increasing the strength and duration of effect of acetylcholine at the synapse. Galantamine was initially found in the species of flower found in *Galanthus caucasicus* (Scott & Goa, 2000). Other such interventions include antagonists Of the NMDA rreceptor. NMDA receptors are involved with the release of the excitatory neurotransmitter glutamate. By antagonizing the NMDA receptor the release of glutamate is blocked. Access glutamate release causes toxic effects and brain damage in the brain. The major NMDA antagonist

currently used in practice is Memantine (Reisberg et al., 2003). Currently some interventions exist that combine such acetylcholinestase inhibitors and NMDA antagonists creating a combination that better stabilizes some patients with AD. Agonists specific to the nicotinic receptor alpha7 are currently being developed. Nicotinic acetylcholine receptors are so named because nicotine was found to selectively bind to this receptor. Interestingly, this makes the study of the structure of nicotine itself, which is chemically similar to acetylcholine an important starting point for potential agonists. One such molecule currently being researched is DMXBA. DMXBA utilizes a structure not dissimilar to nicotine itself in its base form and incorporates a series of functional groups that allow for more specific efficacy at the 7aplha receptor (Kem et al., 2004).

1.3 Actylcholinesterase Inhibitors

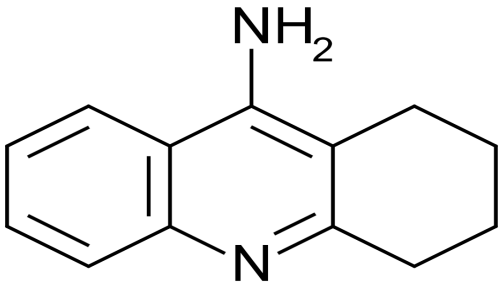

Figure 1.1: The Molecular Structure of Tacrine

Tacrine was the first compound to be approved for treating AD (Mehta et al., 2012). While Tacrine has been shown to be effective in improving quality of life of patients with AD, it has a high rate of adverse effects and limitations. Tacrine has a half life of 2 to 4 hours which requires the drug to be administered 4 times per day (Mehta et al., 2012). A high rate of side effects including gastrointestinal disturbances, seizures, dizziness, and

liver toxicity were experienced. (Mehta et al., 2012) Tacrine's potential for liver toxicity lead to its discontinuation in the United States in 2013 (Mehta et al., 2012).

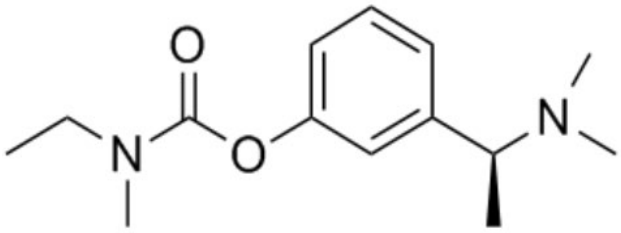

Figure 1.2: The Molecular Structure of Rivastigmine

Rivastigmine is an actylcholinesterase inhibitor with a relatively low molecular weight (Mehta et al., 2012). When taken orally side effects including gastrointestinal disturbances and anorexia were experienced frequently (Mehta et al., 2012). Rivastigmine's small size has enabled a novel way of overcoming these side effects by infusing the drug in a transdermal patch which bypasses the GI system. Possessing a short half-life of 1.5 hours, the transdermal patch also provides a tactic for overcoming this hurdle by allowing the drug to be delivered over 24 hours with a single patch (Mehta et al., 2012). The patch has also been shown to have a higher rate of patient compliance due to both convenience and reduced side effects (Mehta et al., 2012).

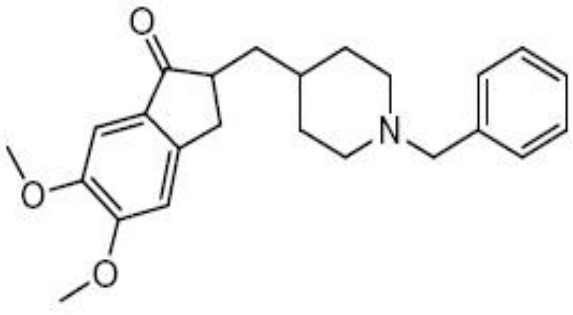

Figure 1.3: The Molecular Structure of Donepezil

Donepezil is a piperidine derivative acetylcholinestase inhibitor, which was initially designed for the treatment of AD in Japan (Rogers et al., 1998). Donepezil's high selectivity and reversibility contribute to its effectiveness as an intervention for AD (Rogers et al., 1998). The drug reaches peak plasma concentrations 3 to 4 hours after oral administration. Donepezil has exceptional oral relative bioavailability at 100% and an extended half life of 70 hour allows its effects to be experienced throughout the day. When compared to Tracrine and Revastigmine, Donepezil has higher selectivity, specificity and is thought to produce fewer adverse effects (Rogers et al., 1998). Despite its tendency to produce fewer side effects relative to other interventions, some patience experience insomnia, gastrointestinal discomfort, and muscle cramps (Rogers et al., 1998).

1.4 NMDA Antagonists

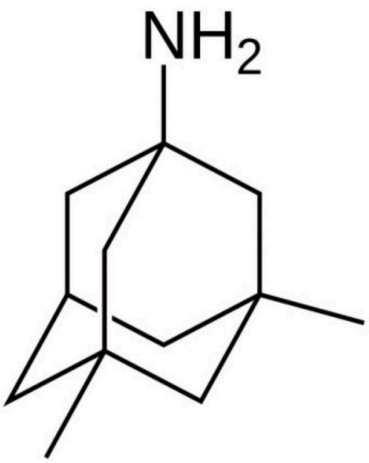

Figure 1.4: The Molecular Structure of Memantine

Memantine is an uncompetitive NMDA receptor Antagonist that has been used in the treatment of moderate to severe AD (Reisberg et al., 2003). It is thought that over stimulation of glutamatergic neurons, specifically the NMDA subtype receptors causes deleterious excitotoxicity that has been implicated in contributing to the cognitive

impairments associated with AD and dementia (Reisberg et al., 2003). By inhibiting the stimulation of the NMDA receptor, Memantine acts as a neuroprotective agent against further deterioration caused by excitotoxicity. Side effects reported in patients taking Memantine include insomnia, diarrhea, headache, and agitation (Reisberg et al., 2003).

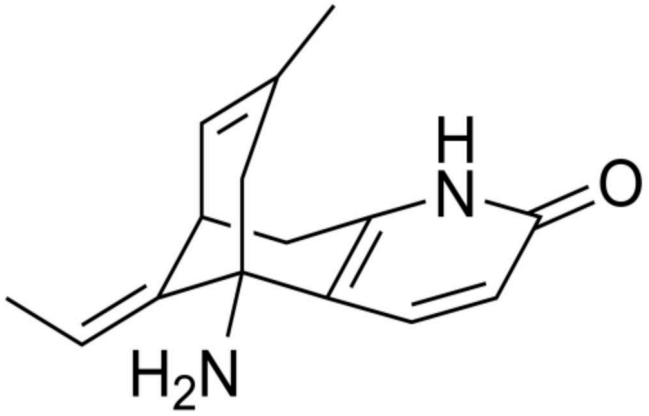

Figure 1.5: The Molecular Structure of Huperzine A

Huperzine A is a naturally occurring alkaloid of the Chinese herb *Huperzia serrata* (Yang et al., 2013). The alkaloid has been explored as a potential agent in the treatment of AD. Uniquely, Huperzine A has both NMDA receptor antagonist activity as well as acetylcholinestase inhibiting properties. Currently there is insufficient evidence to justify its clinical use for the treatment of AD (Yang et al., 2013). Several analogues of Huperzine A have been synthesized with the aim of elucidating a unique AD medication with potentially both NMDA receptor antagonist and acetylcholinestase inhibiting properties (Yang et al., 2013). Adverse effects observed in patients treated with Huperzine A in clinical trials included nausea, dizziness, constipation, excitability, insomnia, sweating, and abdominal pain (Yang et al., 2013).

CHAPTER 2

AIM AND APPROACH

The central goal of this research is to generate novel molecules that are intended to be potential candidates as future medications for the treatment of Alzheimer's Disease (AD). The past several decades of medicinal chemistry research and drug design have elucidated a range of molecules that have been used to treat AD, with many more still under development. This research hopes to aid in the process of drug discovery by presenting a variety of different potential remedies. A wide range of the techniques used includes rational drug design, molecular hybridization, and computational modeling. Applied knowledge of medicinal organic chemistry as well as structural observations of other successful drugs also aid in the process of proposing new molecules.

The aim of rational drug design is modification of functional groups on a known drug or suspected candidate molecule with the aim that the product will have greater receptor affinity, efficacy, or a unique pharmacological profile acting on different receptor subtypes. By examining key positions on a particular molecule, functional group substitutions or additions can be made, yielding a unique molecule with potential activity. One possible way to test a new molecules affinity for the target receptor or enzyme is to use computational protein molecules that simulate the molecular docking of the drug to its receptor. Such programs provide us with information regarding the affinity of this molecule for the target.

Hybridization is a useful technique in drug discovery. By comparing structures of several different molecules that either act on one particular target or act on multiple targets associated with the treatment of the ailment in question, it is possible to produce new molecules with either enhanced activity at a specific receptor, or affinity for multiple receptors. With the AD currently being treated by both Acetylcholinestase inhibitors and NMDA receptor antagonists, hybrids aiming at filling both these niches with one effectual molecule would be highly desired.

CHAPTER 3

MATERIALS AND METHODS

3.1 Molecular Design

By examining the currently available pharmacotherapies for the treatment of AD, the most promising compounded were studied in order to propose analogs that have greater binding affinity for the Acetylcholine esterase (ACHE) active site. The drugs Donepezil and Rivastigmin, both ACHE inhibitors were used as templates for the analogs. In the case of Donepezil analogues, modifications to the phenyl rings of the structure including chlorination, fluorination, and installation of methoxy groups at several locations on the phenyl rings were preformed. For Rivastigmine analogues carbon modifications were utilized to both alter the carbon chain and propose alternative installations on the compounds two nitrogen atoms.

3.2 Computational Modeling

A variety of software tools were utilized to take two-dimensional renderings of the analogues and create computer generated three-dimensional structures that can bind to the modeled active site of ACHE. Using the Protein Data Bank the ACHE structure (PBD ID: 1GQR) was downloaded and instilled in the program Autodock. Analogues were then drawn using the program Chemdraw which creates the initial two-dimensional structures. The Chemdraw files were then loaded to the program Chem Bio 3D which allows for 3D rendering of the 2D structures and allows the 3D molecules to be oriented in the most energetically favorable way, minimizing the total energy caused by intramolecular

interactions. Once both these processes have been undergone, the files are then saved in Protein Data Bank format PDB. The Autodock software was utilized the program PyRX which allows for the 3D structures of the analogues to be digitally bound to the previously downloaded ACHE structure. By selecting the enzyme ACHE and the desired analogues and running the docking function, the binding energies of the compounds to the active site were obtained as were the binding energies for the initial compounds studied Donepezil and Rivastigmine. The binding energies obtained and compared against the original structures were the method to quantify potential improvements in binding affinity of the new analogues.

CHAPTER 4

RESULTS

4.1 Analogues

In total, seven analogues of Donpezil and six analogues of Rivastigmine were designed and tested. Six of the seven Donpezil analogues designed had binding energies lower than the original molecule and therefore greater affinity for the ACHE active site. Three Rivastigmine analogues had lowering binding energy and greater affinity for the ACHE active site, while two of the analogues had virtually the same binding energy.

4.2 Molecular Structure and Binding Energy

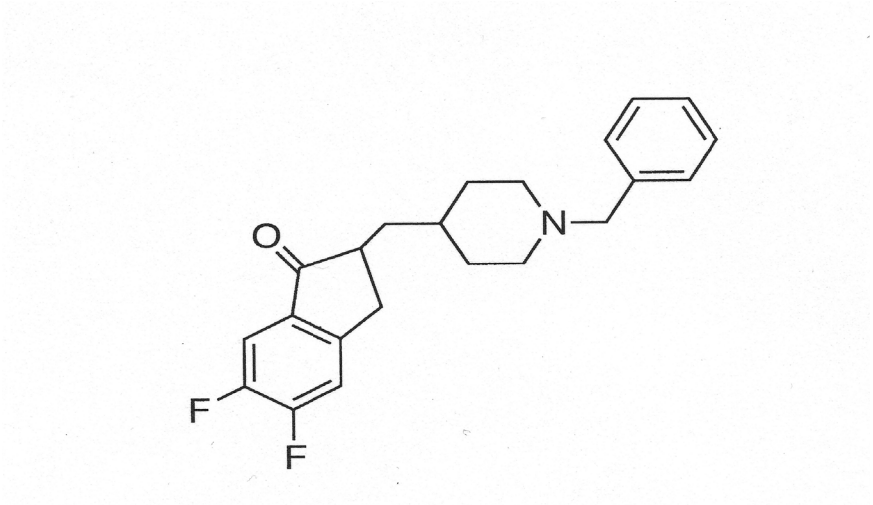

Figure 4.1: KL701

Compound KL701 is an analogue of Donpezil which was motified to replace the two methoxy groups with Fluorine groups. The binding energy of Donpezil is -10.4 kcal/mol.KL701 when tested had a binding energy of -11.3 kcal/mol representing an 8.65% decrease in binding energy, which represents an increase in receptor affinity.

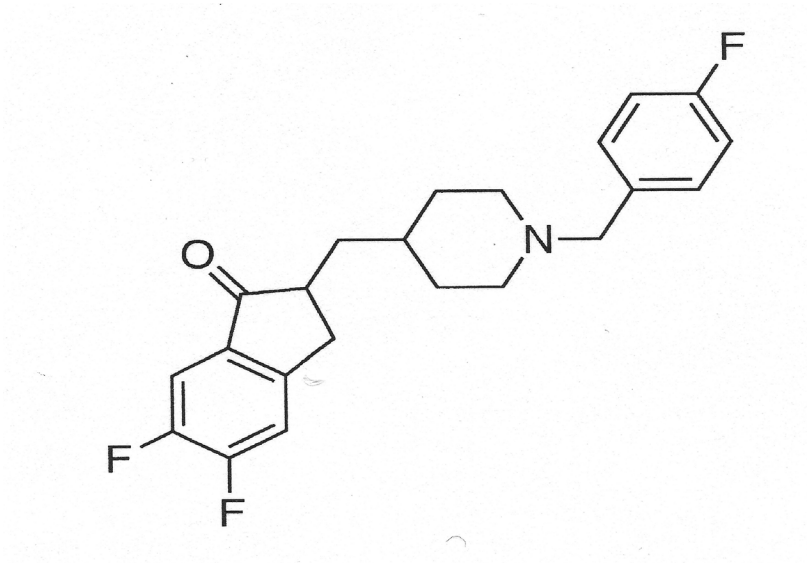

Figure 4.2: KL702

KL 702 is an analogue of Donpezil which failed to display binding affinity that rivals the original molecule. This compound was fluorinated at 3 key positions on the 2 phenyl rings as displayed in figure 4.2. The binding energy for KL702 was found to be -8.4 kcal per mol which is 23.80% greater than Donpezil and therefore has less binding affinity

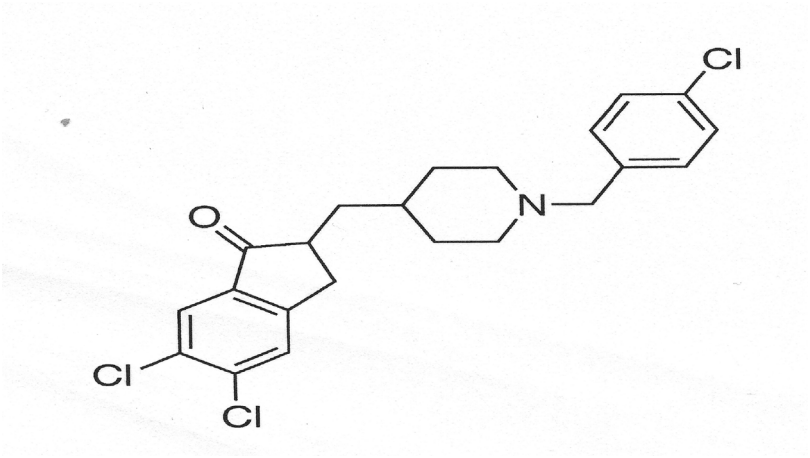

Figure 4.3: KL703

Compound KL703 is a Donpezil analogue that has been chlorinated at 3 key locations on the structures 2 phenyl rings. KL 703 was found to have greater binding

affinity for the ACHE active site than Donpezil. A binding energy of -11.9 kcal/mol was obtained representing a 14.42% decrease in binding energy and an increase in receptor affinity.

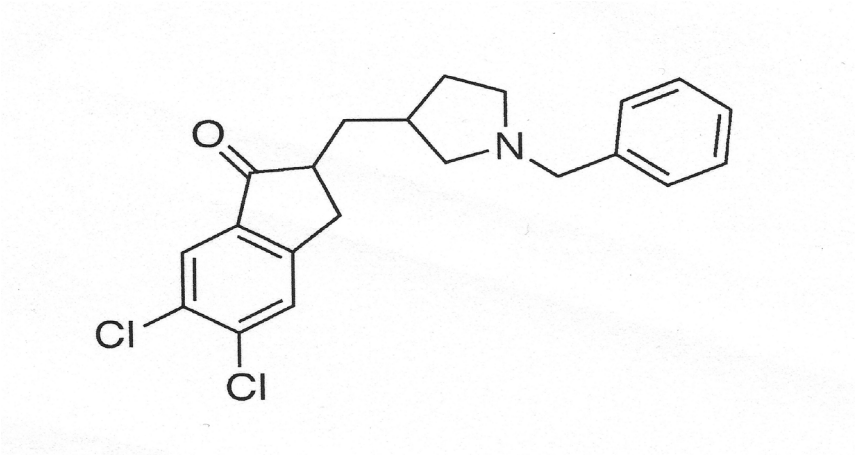

Figure 4.4: KL704

KL704 is a Donpezil analogue with two chloro groups replacing the methoxy groups. A binding energy of -11.1 kcal/mol was obtained representing a decrease of 6.73% and an increase in binding affinity over Donpezil.

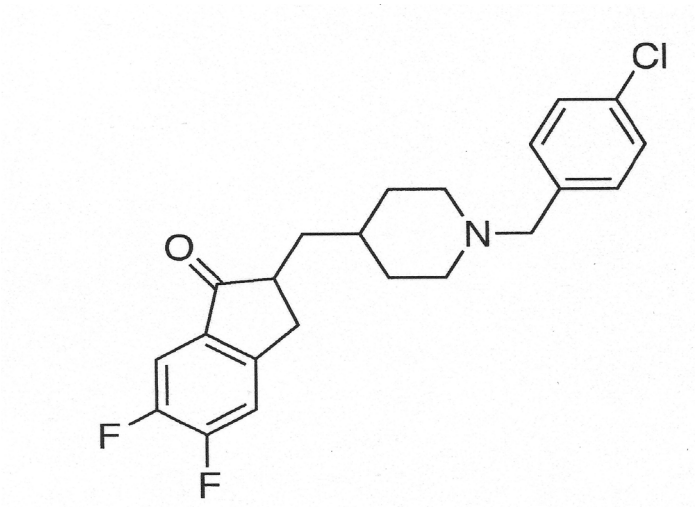

Figure 4.5: KL705

KL705 is a Donpezil analogue with Fluorine replacing the methoxy groups and an additional chlorination at the other terminal phenyl location. The binding energy at the ACHE active site is -11.9 kcal/mol. This represents a 14.42% decrease in binding energy relative to Donpezil and therefore KL705 has greater affinity for the ACHE active site.

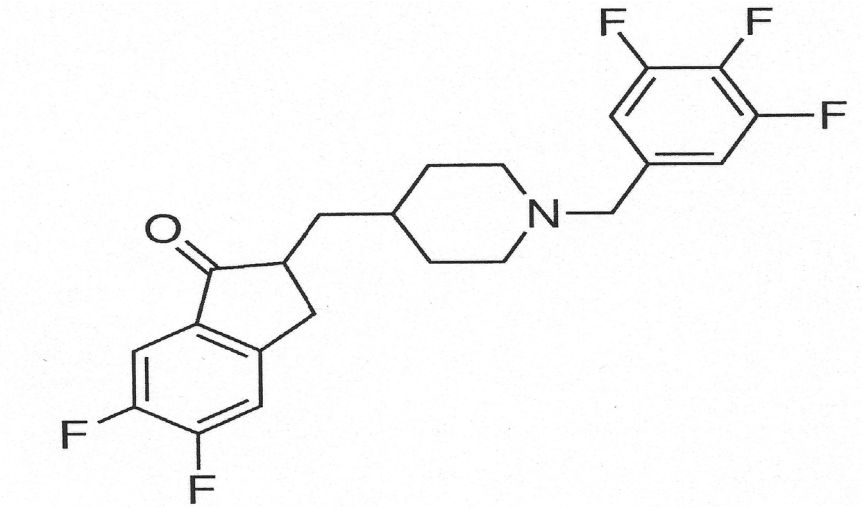

Figure 4.6: KL706

KL706 is a Donpezil analogue with the greatest binding affinity observed among analogues studied. Five Fluorine molecules were installed on the compounds 2 phenyl rings as depicted in Figure 4.6. The binding energy for KL706 at the ACHE active site is -12.1 kcal/mol. This represents a 16.35% decrease in binding energy and therefore an increase in binding affinity.

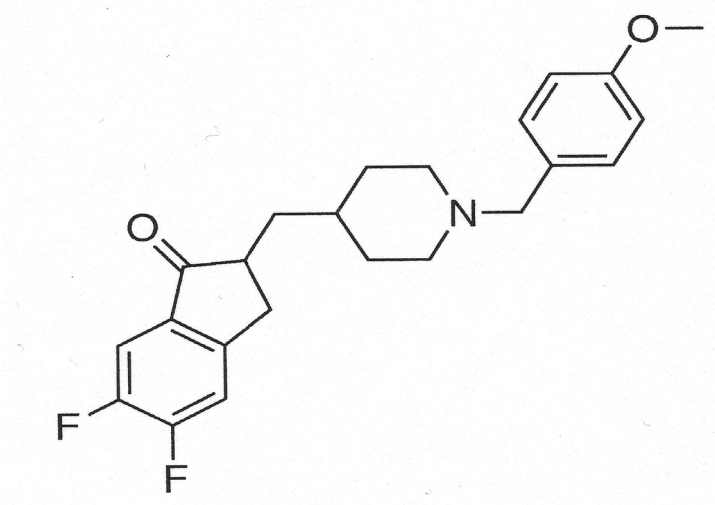

Figure 4.7: KL707

KL707 is a Donpezil analogue with Fluorine replacing the Methoxy groups and an additional Methoxy group installed at the other terminal phenyl as depicted in Figure 4.7. KL707 has a binding energy of -11.2 kcal/mol with respect to the active site of ACHE. This represents a 7.69% decrease in binding energy, and therefore greater binding affinity for the ACHE active site.

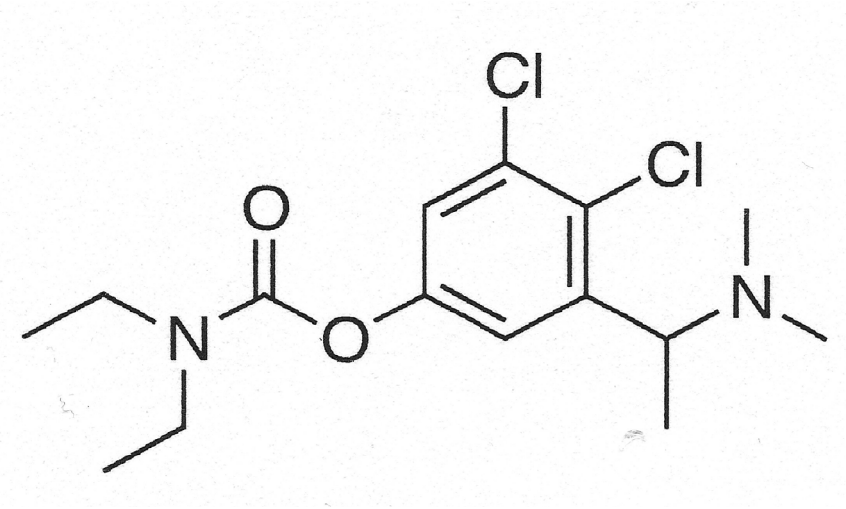

Figure 4.8: KL801

KL801 is a Rivastigmine analogue with two chlorinations on the phenyl ring and a diethylamide in place of a ethylmethylamide. KL801 was found to have less binding energy

with respect to the ACHE active site compared to Rivastigmine. The binding energy observed was -7.9 kcal/mol, while Rivastigmine's binding energy was -7.8 kcal/mol. This represents a 1.2% decrease in binding energy, and therefore a slightly greater affinity for the ACHE active site.

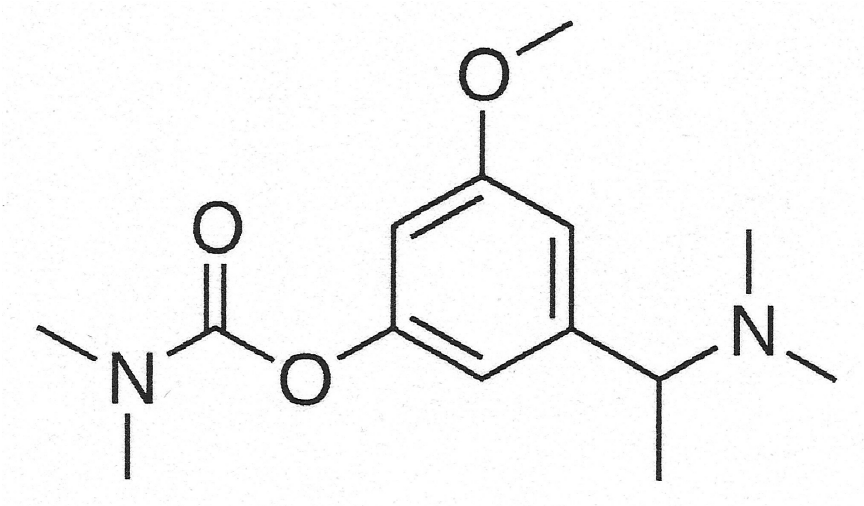

Figure 4.9: KL802

KL802 is a Rivastigmine analogue that failed to produce a greater binding affinity compared to the parent molecule. A Methoxy group was installed on the phenyl ring and dimethylamide was installed. The binding energy of KL802 at the ACHE active site is -7.4 kcal/mol, which is a 5.41% increase in binding energy and therefore a decrease in binding affinity compared to Rivastigmine.

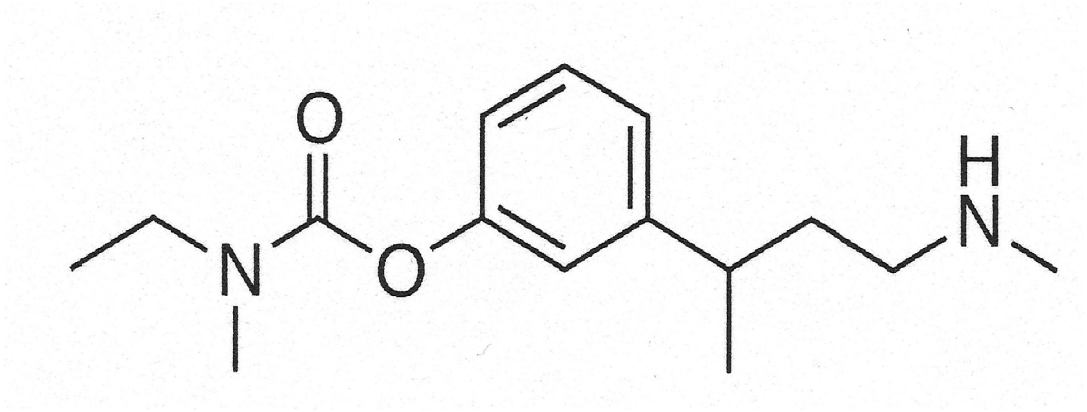

Figure 4.10: KL803

KL803 is a Rivastigmine analogue with virtually identical binding affinity for the ACHE active site as the parent molecule. A methylamine group replaces the dimethylamine and the carbon chain is increased by one as depicted in Figure 4.10.

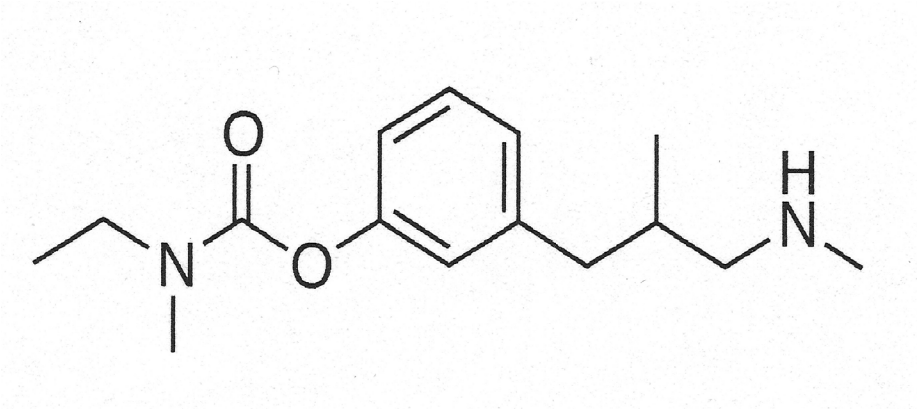

Figure 4.11: KL804

KL804 is a Rivastigmine analogue with a modified carbon chain and methylamine in place of dimethylamine. KL804 was found to have decreased binding energy relative to the parent molecule, and therefore greater binding affinity for the ACHE active site. The binding energy observed was -7.9 kcal/mol, a 1.28% decrease over Rivastigmine.

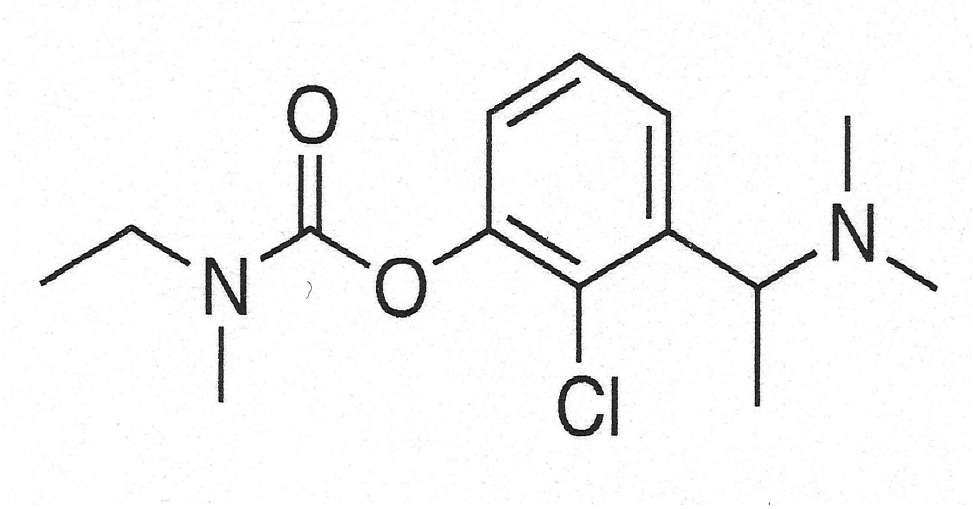

Figure 4.12: KL805

KL805 is a Rivastigmine analogue that displayed the lowest binding affinity of any Rivastigmine analogue tested. The binding energy was observed to be -8.0 kcal/mol, or a 2.56% decrease in binding energy, increasing the compounds binding affinity for the ACHE active site.

CHAPTER 5

DISCUSSION

After systematically altering the functional group composition of the parent molecules, increases in binding affinity for many of the analogues were observed. The most significant improvement noted was compound KL706 as illustrated in Figure 4.6. It appears that installations of fluorine functional groups on both Donpezil's phenyl rings had the most notable increase in binding energy. Similarly, compound KL805 represents the greatest decrease in binding energy at the ACHE active site among the Rivastigmine analogues. While this decrease for compound KL805 was fairly small at 2.56%, the location of the particular functional group addition of the Chlorine on the phenyl ring may be of interest in the generation of future analogues. Similarly, KL706 reveals multiple locations for useful functional group modification along its phenyl rings.

Many of the analogues generated are likely to be effective agonists at the ACHE active site. This activity at the ACHE active sight inhibits the breakdown of acetylcholine at the level of the synapse, making these compounds potentially useful for the treatment of AD. More exploration is necessary, including toxicity tests, before in vivo experimentation can be considered. These molecules may also hold information that could lead to the creation of compounds with even greater affinity for the ACHE active site.

Donpezil analogues worth testing would include alternative halogenation structures such as chlorination at the same positions we see halogens on compound KL706 as depicted in Figure 4.6. Bromination may too yield potentially worthwhile compounds. In

addition, looking at replacing Donpezil's methoxy groups with ethoxy groups is worth testing in future experiments. Several Donpezil analogues studied are worth considering for future investigation.

Rivastigmine analogues proved more difficult to achieve significant breakthroughs in increasing receptor affinity for the ACHE active site. With modest decreases in bonding energy of 2.56%, chlorination on Rivastigmine's phenyl ring offered the most promising of the analogues generated as depicted in Figure 4.12. Alternative installation of other halogens at this position is worth exploring as these may yield more significant results.

With this method of computational drug development every generation of molecules has the potential to suggest even greater results with every generation of new molecules. This research involes several key functional group locations on Rivastigmine and Donpezil that are worthy of continued research efforts. By increasing receptor affinity, the aim is that molecules can be generated that require smaller doses to achieve greater therapeutic results. This may lead to medicines with fewer side effects, or issues with metabolism. With continued efforts, this line of research could elucidate a life changing medicine for the treatment of Alzheimer's disease

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BIOGRAPHICAL INFORMATION

Kevin Luciani spent his undergraduate carrier immersed in studying the biological sciences with particular passion for neuroscience and pharmacology. His research background includes work in Biophysics and Physiology, Regenerative Neurobiology, and Medicinal Chemistry. While at the University of Texas Arlington, Kevin won such prestigious scholarships as the I-engage research scholarship and the Honors College research fellowship.

Research has been among Kevin's greatest joys and he looks forward to a life rich in exploration. Kevin has plans to continue conducting research once enrolled in medical school. Kevin wishes to peruse a carrier in drug development and pharmacology in the years to come. Kevin has a particular passion for ocular neuroscience and seeks to become a neuro ophthalmologist, practice academic medicine and continue research.