CHAPTER 1

Alzheimer’s Disease: Treatment and Development of Novel Lead Molecules
1.1 Introduction

Neurological disease has a destructive impact on patients, their healthcare providers and economy of the society. Alzheimer’s disease (AD) is one of the prominent neurological diseases. It is a progressive neurological disorder in the elderly people for which no cure exists.\textsuperscript{1} It is a common form of dementia which leads to the functional deterioration in memory and ability to learn, the progressive loss of mental and behavioral ability and deterioration of cognitive functions.\textsuperscript{2,3} According to the WHO report in 2015, an approximately 44 million people worldwide have AD and this number will be increased up to approximately 65 million in 2030 and 131 million in 2050.\textsuperscript{4} From the available data shown in 2015, it is clear that the Asian countries are being the most affected. Approximately 22 million people in Asia suffered from dementia. Out of which 70-80% dementia are due to AD, which is almost half of the worldwide, and this number will be raised to 38 million in 2030 and 67 million in 2050.\textsuperscript{4}

According to the World Alzheimer Report, an approximately 5% of all people who have aged 65 or more have Alzheimer disease, and this number will be increased up to 25-45% for those who aged above 70. This disease is the 6\textsuperscript{th} leading cause of death in USA, and 5\textsuperscript{th} leading cause of death for those who aged above 70.\textsuperscript{4}

As per report published by Alzheimer’s and related disorders society of India (ARDSI) in 2010 (which was based on 2001 census data), there are more than 70 million people in India who aged above 60 years which is almost 7.5% of the population in 2001.\textsuperscript{5} This age group is expected to grow dramatically in the coming decades. The individuals with dementia is expected to double in every 5 years of age, so India will have higher numbers of elderly people with this problem.\textsuperscript{5} With increasing age the prevalence of dementia increases, and it has also been found that older women are more affected than men (Figure 1.1).\textsuperscript{5} The larger percentage of older women than men who is suffering from dementia is because the women live longer in India.\textsuperscript{5} Further, the ARDSI report emphasized that approximately 3.7 million individual have aged over 60 suffering from dementia (approximately 1.5 million individual are men and 2.1 million are women),\textsuperscript{5} and 90% cases of dementia in India is due to AD and this number is expected to double by 2030 (Figure 1.2).\textsuperscript{5}
Figure 1.1: Prevalence of individual with dementia by age and gender in India

Figure 1.2: Number of individual with dementia by age in India, 2010-2050

1.2 History of Alzheimer’s disease (AD)

Alzheimer disease (AD) was discovered in 1906 for the first-time by Alios Alzheimer, a German psychiatrist and neurologist. This disease was first-time observed in 1901 when a 51 year old woman (named Auguste D) was taken to Dr Alios Alzheimer by her family after seeing significant changes in her behavior and personality. Her family noticed she had problem with memory, unable to speak and recognize things, and impairment in awareness. Then she was followed by Dr. Alzheimer for five years and during these periods he noted that she suffered from many abnormal symptoms like difficulty with speech, confusion and agitation. After which he described that she had an aggressive form of dementia which affected her memory, behavior and thinking ability. After her death in 1906, an autopsy of her brain was performed by
Dr. Alzheimer and he found dramatic contraction of the cerebral cortex, and fatty accumulations in blood vessels and atrophied brain cells.\(^1\) Later on neurofibrillary tangles and senile plaques β-amyloid (Figure 1.3), which is now an indicative hallmark of AD, was also discovered by him.\(^6\) This type of condition was discussed and reported for the first time in 1907 after which it was named as Alzheimer disease in 1910.

![Figure 1.3: (a) Amyloid Plaques\(^6\) (b) Neurofibrillary tangles\(^6\)](b)

### 1.2.1 Disease process

AD starts mainly above the age of 35, but the detection of this disease in early stage is not feasible. This disease develops slowly and gradually over several years and lead to severe shrinkage in healthy brain.\(^6\) There are different stages of AD, each one has its own challenges and symptoms.\(^6\) The different stages help to understand progression of this disease and possible course of treatment. Each stages of AD have different unique symptoms, and are characterized in different classes.

**Early stage AD or Preclinical AD:** This stage of AD usually resides 2-4 years. The patient fails to recognize family and friends occasionally and show deterioration in the cognitive function. The most common symptoms during this stage include difficulty in maintaining information, decision making and problem solving, and organizing and expressing new thoughts. Getting lost
or misplacing belongings and changes in personality due to lack of social motivation are also observed (changes in brain shown in figure 1.4a).\textsuperscript{7,8}

**Moderate AD:** This is the longest stage of AD. In this stage, the patient is more confused and forgetful, and needs help to perform activities of daily living. Increasingly confusion and poor judgment in which individuals completely forget to track of where they are: for example, the days of week or season, etc. are the symptoms at this stage.\textsuperscript{7,8} They may also have confusion about family members and close friends. At this stage, individuals lose orientation to place and time and may start wandering in search of surrounding that feel more familiar which makes it unsafe for them to left alone. Individuals also felt difficulty in completing daily task of life and need assistance (changes in brain shown in figure 1.4b).\textsuperscript{7,8}

**Severe AD:** This is the final stage of AD. At this stage, mental decision capability continues to decline and the disease has a growing impact on movement and capabilities. Common symptoms which appearing in this stage includes the loss of ability to communicate coherently in which individuals occasional say word or phrases and can no longer speak coherently.\textsuperscript{7,8} The individuals may unable to walk or sit independently and requires daily assistance with personal care. After diagnosis of sever AD, people can survive 8-10 years only (changes in brain shown in figure 1.4c).\textsuperscript{7,8}

![Figure 1.4: Structure of brain at the different stages of Alzheimers disease\textsuperscript{1}]  
(a) Preclinical AD   (b) Moderate AD   (c) Severe AD

### 1.2.2 Diagnosis and treatments

The cause and progression of AD is not well understood. The only known method for diagnosing AD is brain autopsy. However, physician diagnosed 90 percent of AD cases by mental and behavioral tests and also physical examinations of individuals.\textsuperscript{9} Besides above, brain
scans such as magnetic resonance imaging (MRI),\textsuperscript{10} positron emission tomography (PET)\textsuperscript{11} and computed tomography (CT)\textsuperscript{12} may also be performed for the diagnosis of AD. Each scan involves unique procedure which can be used for getting information regarding the dimension, and volume of the brain. Periodic scan of brain by the physician allows them to determine how effectively brain neurons are working and to monitor the kind of changes occurs during the process of AD.\textsuperscript{12}

On the basis of these observations, several hypotheses are put forward for the treatment of AD. Until now there is no cure for complete treatment of AD; however, several drugs are available which slow down the disease progression and treat symptoms occurring. Most of the available drugs which slow down the progression of this disease are mainly based on the cholinergic hypotheses.

1.2.3 Cholinergic hypothesis for Alzheimer’s Disease (AD)

This is the oldest hypothesis for treating AD at the early stage. This hypothesis arose after seeing the significant loss of cholinergic neurons in the AD patient brain. There is also a decline in activity of choline acetyltransferase enzyme (which plays an important role in the formation of acetylcholine (ACh) in presynaptic neurons) that results in decreased neurotransmission and cognitive dysfunction.\textsuperscript{13-15} According to Francis et al\textsuperscript{16} there is a reduction in the activities of nicotinic and muscarinic receptors in brain for people suffering from AD. Acetylcholine esterase (AChE) and butyrylcholinesterase (BuChE) enzymes play an important role in the reduction of acetylcholine by hydrolyzing acetylcholine to choline and acetate (Figure 1.5). The AChE enzyme which is concentrated in the synaptic cleft rapidly decreases the concentration of ACh. AChE has a very high catalytic activity; about 5000 ACh molecules are hydrolyzed per AChE enzyme per second. Liston et al\textsuperscript{17} in 2004 reported that the level of ACh (an important neurotransmitter which play a role in cognitive function) can be restored by inhibiting cholinesterase enzyme. Several research laboratories usually target AChE for the treatment of AD but later on the researchers have also been focused on developing of BuChE inhibitors.\textsuperscript{18-21} The presence of both cholinesterase in glia as well as in neurons, neuritic plaques and tangles within the AD patient has also been established.\textsuperscript{22,23} The AChE activity decreases continuously from mild to severe stage of AD. On the other hand, the activity of BuChE is either unaffected or
even increased with the progression of this disease.\textsuperscript{24} Thus, in the brain of AD patient, the BuChE takes part in a more major role in cholinergic transmission with already reduced acetylcholine levels resulting in further cognitive decline.\textsuperscript{25} Thus, by inhibiting these two enzymes the amount of free acetylcholine which interacts with neuronal receptors for signaling can be increased.\textsuperscript{26}

Cholinesterase enzyme belongs to a family of serine hydrolases because it has an ability to hydrolyze substrate by using nucleophilic serine residue active site. Serine hydrolases superfamily belong to a broad group of proteins which are involved in several important physiological processes like blood coagulation,\textsuperscript{27} digestion,\textsuperscript{28} as well as in neurotransmission.\textsuperscript{29} Because of this, many of these enzymes are related to various diseases such as AD, thrombosis and pancreatitis. Therefore, cholinesterase is an attractive target for drug discovery. Keeping in view of all these finding, several molecules as cholinesterase inhibitors had been synthesized and many of them are in clinical use today like Rivastigmine (Exelon) and Donepezil (Aricept) are used for the treatment of AD; Dabigatran (Pradaxa) and Rivaroxaban (Xarelto) are used for thrombosis; and Sitagliptin (Januvia) and Saxagliptin (Onglyza) are used for the treatment of type 2 diabetes.\textsuperscript{30} However, there are many serine hydrolases available that need to be characterized as their function and substrate specificity are still unknown.\textsuperscript{31} The cholinesterase enzymes mainly consist of AChE and BuChE which are responsible for the breakdown of cholinergic neurotransmitters and acetylcholine. Both, AChE and BuChE have known structures but only the function of the former has been well-established.

**Acetylcholinesterase (AChE)**

This is known as the main enzyme of cholinesterase family. By post-translational associations of catalytic and structural subunits, different molecular forms of AChE are obtained and alternative mRNA splicing provides its structural diversity. Disulfide-linked dimers and tetramers are formed by the hydrophilic part of this enzyme and they are the main forms of AChE. According to Taylor and Radić, and Massoulié et al\textsuperscript{32,33} AChE can also be attached to the cell membrane by using glycophospholipid anchors. This enzyme is found in most of the tissues like neuromuscular junctions,\textsuperscript{34} brain cholinergic synapses,\textsuperscript{35} autonomic ganglia\textsuperscript{36} and red blood cell membranes.\textsuperscript{37} This enzyme is also known as a modulator of neurotransmission which hydrolyses neurotransmitter acetylcholine (ACh) that is synthesized from acetyl coenzyme A
Acetylcholine is synthesized from choline by the catalytic action of choline acetyltransferase enzyme and stored into synaptic vesicles. From this vesicles, the ACh is released to presynaptic nicotinic (N) and muscarine type 2 (M2) receptors which further release this ACh to postsynaptic M1 receptor. During this ACh transfer to post synaptic neurons, acetylcholinesterase (AChE) breaks down the ACh which is left in the synaptic gap into choline and acetate. These molecules are again transferred into presynaptic neurons for ACh synthesis. Several AChE functions have also been reported such as cellular differentiation and tumorigenesis, apoptosis, etc. Unattended release of ACh results in the continuous stimulation of receptors which causes symptoms like confusion, vomiting, convulsion and respiratory failure. On the contrary, lack of ACh lowers receptor stimulation leading to cognitive impairment (significant symptom of AD). Therefore, it is essential to keep a balance of ACh activity.

**Figure 1.5:** Acetylcholinesterase/Butyrylcholinesterase hydrolyzing acetylcholine

**Butyrylcholinesterase**

Butyrylcholinesterase (BuChE), is a serine hydrolase and also called as pseudocholinesterase, which catalyzes the hydrolysis of choline ester including butyrylcholine, succinylcholine and acetylcholin. In contrast to AChE, which is predominantly present in the brain, BuChE is also present in neurons but it is highly effective in peripheral tissue than in the brain and mostly found in the serum and glial cells. AChE exhibits specificity towards the neurotransmitter acetylcholine, whereas BuChE catalyzes the hydrolysis of a wide variety of choline and non-choline esters such as ACh, succinylcholine, cocaine and aspirin. Due to this kind of enzyme’s involvement, it plays a significant role in neurotransmission, anaesthesia and drug abuse.

AChE and BuChE are major enzymes in the family of cholinesterases. These enzymes are associated with several diseases. Therefore, these enzymes are considered as attractive targets.
in the field of drug discovery for various kinds of diseases. For example, cholinesterase inhibitors are mainly used in the treatment of early stage of AD\textsuperscript{52} and myasthenia gravis.\textsuperscript{53} These drugs are also beneficial for the management of several other disease like chronic pain\textsuperscript{54,55} and type 2 diabetes.\textsuperscript{56} Most of the early drug development efforts against AD targeted cholinesterases.

### 1.2.4 Amyloid hypothesis for Alzheimer’s Disease (AD)

In amyloid hypothesis, AChE forms a secondary non-cholinergic activity which enhance the formation and deposition of senile plaques called β-amyloid (Aβ) and neurofibrillary tangles (Figure 1.3). The neurofibrillary tangles are hyperphosphorylated twisted tau protein in the brain of AD affected individuals.\textsuperscript{57-60} The deposition of Aβ in the form of β pleated sheet conformation and formation of tangles inside the brain are found to play an important role in the initiation and progression of AD.\textsuperscript{60} Aβ is produced by the abnormal and sequential cleavage of amyloid precursor protein (APP) by β- (also named as β-site APP cleaving enzyme, BACE) and γ-secretase enzyme respectively.\textsuperscript{61-63} This shows that for Aβ formation, the cleavage of APP by both β- and γ-secretases is essential, which postulate that either inhibition or modulation of these proteases enzyme in the brain should decrease the level of Aβ in the brain of AD patient.\textsuperscript{64} Since the abnormal cleavage of APP is first initiated by β-secretase enzyme, so research is mainly focused on the synthesis of small molecule as BACE inhibitors.\textsuperscript{61} Based on this hypothesis, small molecule BACE inhibitors have also been synthesized for the treatment of AD. Several small molecule BACE inhibitors have been synthesized and few reach to clinical trial, however others fail at some stage of clinical trial (Table 1.1).\textsuperscript{62,63}

### 1.2.5 MAO hypothesis of Alzheimer’s Disease (AD)

Monoamine oxidase (MAO) is a flavin-adenine dinucleotide enzyme which is extensively dispersed in animal tissue.\textsuperscript{65} It mainly catalyzes the oxidative deamination of amines particularly, primary amines to produce aldehyde, ammonia and hydrogen peroxide (Figure 1.6).\textsuperscript{66} This enzyme preferentially targets a wide variety of neurotransmitters having amine group in the brain, including dopamine (DA), serotonin (5-HT), epinephrine (EP), norepinephrine (NE), and β-phenylethylamine (PEA).\textsuperscript{66,67}
Table 1.1  Potential drug molecules in clinical trial as BACE inhibitors

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Clinical Trial Phase and Current Status</th>
</tr>
</thead>
</table>
| ![AZD3293](image) | **Phase III**  
Study start date Sep. 2014  
Study end date Aug 2019 adopted from  
(accessed on 11 Oct 2017) |
| ![BI 1181181](image) | Discontinued in Phase I in 2015, due to low oral bioavailability and low blood brain barrier penetration.  
http://www.alzforum.org/therapeutics/bi-1181181  
(accessed on 11 Oct 2017) |
| ![E2609](image) | **Phase III** started on 27 April 2017  
(accessed on 11 Oct. 2107) |
| ![LY2886721](image) | **Phase II**  
discontinued due to liver biochemistry in 2013  
http://www.alzforum.org/therapeutics/ly2886721  
(accessed on 11 Oct 2017) |
| ![PF05297909](image) | Studied of Phase I was completed in 2012 and goes for further studies.  
https://clinicaltrials.gov/ct2/show/NCT01462851  
Two isoforms of MAO enzyme have been identified in human, MAO A and MAO B. A large number of studies have demonstrated that MAO also play an important role in neurodegenerative disease like Parkinson disease, AD and other types of dementia. During oxidative deamination of amine by MAO, the formation of H$_2$O$_2$ takes place resulting in oxidative stress which plays a central role in neurodegeneration. Literature also shows that neurotransmitter containing monoamine systems play a important role in cognitive function, like memory, attention, thinking, behavior and emotion. MAO disturb the balance of neurotransmitters by oxidative deamination, which includes glutamatergic action, ChE, serotonin and norepinephrine and these may result in cognitive impairment. The substrate specificity and inhibitor selectivity for MAO-A and MAO-B are different. The MAO-A enzyme preferentially catabolizes the oxidative deamination serotonin and norepinephrine. On the other hand, MAO-B catabolizes 2-phenylethylamine and benzylamine. Oxidative stress in AD patients also contributes in the formation of Aβ-amyloid plaques. Therefore, it has been concluded that MAO enzyme is associated with the production of reactive oxygen species which cause oxidative stress, and is responsible for neuronal damage and neurodegeneration leading to AD. Molecular biology studies have also shown that the modulation of APP by MAO triggers the generation Aβ. Therefore, inhibitors of MAO have also been used as drug for the treatment of AD. But none of them further permanently cure the disease. Several side effects are also observed by using these drugs.

\[ R\text{NH}_2 + \text{FAD} + O_2 \rightarrow R\text{H} + \text{FADH}_2 + H_2O_2 + \text{NH}_3 \]

**Figure 1.6:** Oxidative deamination of amines by MAO proteins

### 1.2.6 Current therapies for Alzheimer’s Disease (AD)

Out of the three important hypothesis mentioned above (Sections 1.2.3-1.2.5), the current therapies follow cholinergic hypothesis for the treatment of AD. The drugs used for treating AD were mostly based on cholinesterase inhibitors (Section 1.2.3). AChE inhibitors were developed initially for the treatment of AD, because it is the main enzyme which hydrolyses ACh to disrupt the neurotransmission. In this regard, Tacrine was the first drug, approved by
FDA in 1993, entered the market for the treatment of AD. It is a non-competitive AChE inhibitor which also inhibits BuChE.\textsuperscript{82} Due to toxicity of this drug,\textsuperscript{83,84} it is not commonly in use. However, many medicinal chemists used its scaffold to synthesize many cholinesterase inhibitors (Section 1.3.1-1.3.2).\textsuperscript{85-87} Later on few drugs like donepezil (1996), rivastigmine (2000) and galantamine (2001)\textsuperscript{80} were introduced as cholinesterase inhibitor in the market. These three drugs were also authorized by European market and are still in use for the symptomatic treatment of AD. These drugs have higher affinity for AChE while rivastigmine is a dual inhibitor with higher potency towards AChE than BuChE.\textsuperscript{24}

Besides cholinesterase inhibitors, a medication involved the use of Memantine was also approved for the treatment of AD, which mainly regulates the activity of glutamate.\textsuperscript{88,89} It is an excitatory neurotransmitter which plays a role in learning and memory, and over stimulation of glutamate may be the reason for neurodegeneration.\textsuperscript{90,91} This glutamate binds to N-methyl-D-aspartate (NMDA) receptors and opens the calcium ion channel leading to hyperpolarization of neurons results in cellular apoptosis.\textsuperscript{91} Memantine mainly blocks the NMDA receptors; therefore, prevents the nerves from excessive glutamate stimulation.\textsuperscript{92} This drug is mainly used for the treating moderate to severe AD (Figure 1.4b, 1.4c). However, the drugs mentioned in table 1.2 are effective in controlling the AD symptoms not to a large extent. Due to their severe side-effects, they have limited efficacy. Oxidative stress and neurodegeneration are considered as a major factor for the side-effects. Therefore, there has been a continuous research to synthesize more potent and highly efficient cholinesterase inhibitors by combining moieties which are known to active against cholinesterase for AD treatment and management.

1.3 Synthetic compounds as cholinesterase inhibitors

Several research groups have synthesized compounds as AChE/BuChE inhibitors and most of them are of heterocyclic origin. Few of them were approved for the treatment of AD at the early stages (Figure 1.4) and some of them are under pre-clinical as well as clinical trials stages. Heterocyclic chemistry deals with heterocyclic compounds which have long history and future prospects in medicine.
Table 1.2: Cholinergic hypothesis based drugs, their action and adverse effects

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Action</th>
<th>Adverse Effects of Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tacrine</td>
<td>It mainly inhibits AChE and prevents the hydrolysis of acetylcholine (Ach).</td>
<td>Headache, seizures, muscle pain, depression, nausea, vomiting, liver problem, diarrhea</td>
</tr>
<tr>
<td><img src="image1.png" alt="" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donepezil (Aricep)</td>
<td>It also inhibits AChE and prevents the hydrolysis of acetylcholine (Ach)</td>
<td>Dizziness, tiredness, muscle cramps, drowsiness, nausea, vomiting, diarrhea, Weight loss, tremor, appetite loss, insomnia</td>
</tr>
<tr>
<td><img src="image2.png" alt="" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rivastigmine (Exelon)</td>
<td>Obstructs the hydrolysis of Ach through inhibition of enzymes that degrade Ach</td>
<td>Headache, confusion, nervousness, paranoia, malaise chest pain, edema back pain, bone fractures Respiratory: bronchitis, seizures, constipation, nausea, vomiting</td>
</tr>
<tr>
<td><img src="image3.png" alt="" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galantamine (Razadyne)</td>
<td>Obstructs the hydrolysis of Ach through inhibition of enzymes that degrade Ach</td>
<td>Chest pain, dizziness, shortness of breath, blurred vision, dry mouth, nausea, vomiting, confusion, anemia</td>
</tr>
<tr>
<td><img src="image4.png" alt="" /></td>
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</tr>
</tbody>
</table>

The earliest compounds with medicinal applications (medicines) known to mankind were of heterocyclic origin. Heterocyclic compounds are cyclic compounds with at least one hetero atom in the ring. These compounds are integral parts of our life which are seen with purine/pyrimidine bases, sustain on carbohydrates, and in case of disease it act as medicine. Today, the heterocyclic compounds finds its application in all field of life, like it can be used as pesticides, reagents, detergents, polymers and in the field of material sciences.
1.3.1 Tacrine and its derivatives

1,2,3,4-Tetrahydroacridin-9-amine (Tacrine) was the first drug approved by the FDA (1993) for the treatment of mild to moderate AD (IC$_{50}$ = 167 nm) in U.S. (Figure 1.7).\textsuperscript{93} It is an aminoacridine compound which is centrally active and is a reversible AChE inhibitor with a moderate duration of action. Hepatotoxicity and serious side effects were the main cause of its withdrawal from the market.\textsuperscript{83,94} This drug is not effective for the treatment of all stages of AD, because it metabolizes to distinct hydroxyl metabolites depending on the activities of the cytochrome P450 isoenzyme family in any individual.\textsuperscript{95} It was found that few tacrine derivative are pharmacologically active but they are toxic also. To improve efficacy and to eliminate its toxicity, several researchers made modifications through substituents at the structure of tacrine and synthesized novel derivatives.\textsuperscript{96} One of the derivative, 9-amino-7-methoxy-1,2,3,4-tetrahydroacridine (7-MEOTA), was found to be a potent and less toxic cholinesterase inhibitor.\textsuperscript{96} This molecule is also free from their adverse side effects which were observed in tacrine.\textsuperscript{96} Several modifications were also performed with either replacing or annulating benzene ring of tacrine by different heterocyclic molecule like pyrazolo[3,4-b]quinoline, coumarin,\textsuperscript{97} benzo[b]pyrazolo[4,3-g][1,8] napthyridine,\textsuperscript{98} Tacrine-benzofuran Hybrids,\textsuperscript{99} and benzochromene.\textsuperscript{100,101} These derivatives are reported as multi-targeted cholinesterase inhibitors for the treatment of AD. Large number of multi-targeted molecules based on tacrine-coumarin, thiazole-tacrine and tacrine-trolox conjugates have also been synthesized and evaluated for the treatment of AD.\textsuperscript{102,103}

![Figure 1.7: Tacrine molecule (I)](image)

1.3.2 Tacrin-phenylthiazole hybrids

Thiazole is a five-membered heterocyclic molecule having molecular formula C$_3$H$_3$NS. It was reported that thiazole helped in the normal functioning of nervous system because it is also present in vitamin B$_1$ (thiamine) and plays an important role in the synthesis of acetylcholine.\textsuperscript{104} It has also reported that modifications of at various position thiazole ring provide a variety of
novel derivatives which have wide range of biological activities namely antioxidant, anti-inflammatory, anti-tubercular and anticancerous. Wang et al synthesized two series of novel phenylthiazole–tacrine conjugates by changing the number of spacer atoms between the two parent molecule compound II (Figure 1.8).  

![Figure 1.8: Phenylthiazole-tacrine conjugates (II)](image)

Numerous changes were incorporated to increase the potency of compounds, by changing the length of spacer atom between the parent fragments (I) and by substitution at 4' position of phenyl thiazole ring. Screening results showed that when the spacer atoms have \( n_1 = 1 \) and \( n_2 = 4 \), then the formed derivative (pIC\(_{50}\) = 7.14 for AChE and 9.45 for BuChE) was found to be the most potent inhibitor against BuChE and AChE enzymes. When \( n_1 = 1 \) and \( n_2 = 2 \), the compound has pIC\(_{50}\) = 6.31 for AChE and 9.22 for BuChE. It was further seen that when the H atom at 4' position of phenyl-2-aminothiazole is replaced by Cl atom, the compound had decreased activity against AChE and BuChE (pIC\(_{50}\) = 5.87 for AChE and 7.78 for BuChE).

The compound III derivatives (Figure 1.9), having the spacer atoms between the parent molecules and substituent incorporation of substituents at the 4' position of the phenyl-2-aminothiazole have also been studied. The screening results revealed that substitution with OCH\(_3\) or Cl at 4' position was less favorable. It was found that these tacrine-phenyl thiazole hybrid derivative inhibited BuChE with pIC\(_{50}\) value ranging from 5.75 to 10.35 which were higher or comparable than tacrine (pIC\(_{50}\) = 8.42). However, the activity towards AChE were less than tacrine (pIC\(_{50}\) = 7.19).  

![Figure 1.9: Phenylthiazole-tacrine conjugates (III)](image)
1.3.3 Coumarins derivatives

Ensaculin\textsuperscript{106} (IV, KA-672), is a coumarin derivative, containing benzopyran and piperazine substituted moieties (Figure 1.10). This molecule was under clinical trial for the treatment of AD. The ensaculin exhibited multiple actions including AChE inhibition with IC\textsubscript{50} value 0.36 \(\mu\)M against AChE.

![Figure 1.10: Ensaculin, KA-672 (IV)](image)

Piauzzi et al\textsuperscript{107} have reported novel series of coumarin derivatives which are multi targeted and potent AChE inhibitors. In their study, coumarin ring with benzyl amino group (important constituent of donepezil) were linked by using phenyl ring as spacer between these two moieties. Studies showed that the coumarin interacted with the peripheral anionic site (PAS) while the benzyl amino group interacted with the catalytic site of AChE. In this series, compound (AP2238, V) was found to be the most potent AChE inhibitor having IC\textsubscript{50} value 44.5 nM (Figure 1.11). This molecule is highly selective towards AChE in comparison to BuChE with IC\textsubscript{50} = 48900 nM. The docking studies of these compounds further confirmed the interactions with both PAS and catalytic sites of AChE. AP2238 was also reported to exhibit A\(\beta\) anti-aggregating property.

Same research group made modification in V. They mainly replaced the methyl group present at N atom of benzyl amine group by ethyl group replacing methyl substituent (Compound VI). It was found that the activity towards AChE increased (IC\textsubscript{50} = 18.3 nM) which was due to its increase in lipophilicity.\textsuperscript{108} When \(-\text{OCH}_3\) group at 6\textsuperscript{th} or 7\textsuperscript{th} position was replaced by bulkier halogenated phenyl group, the AChE inhibitory activity was reduced (Compound VII IC\textsubscript{50} = 7.16 \(\mu\)M and VIII, IC\textsubscript{50} = 4.57\(\mu\)M). This decrease in activity suggests that molecule with bulky groups at 6\textsuperscript{th} and 7\textsuperscript{th} position are not allowed to penetrate into the active site of AChE.
Shen et al\textsuperscript{109} reported frano coumarin derivatives and provided substitution at 4\textsuperscript{th} position of the coumarin with substituted aryl amino group with one atom as spacer. These derivatives displayed moderate AChE inhibitory activity having IC\textsubscript{50} value in \( \mu \text{M} \) range. The compounds having electron donating groups such as \(-\text{OCH}_3, -\text{NH}_2\) and \(-\text{OH}\) on the benzene ring of anilino moiety reported as significant and potent AChE inhibitors as compared to molecules with weak electron donating group such as \(-\text{CH}_3\). The most potent compound of this series was compound IX (IC\textsubscript{50} = 0.19\( \mu \)M) having \(-\text{OCH}_3\) group at the second position of phenyl ring of anilino moiety (Figure 1.12).

Bruhlmann et al\textsuperscript{110} reported 7-benzyloxy coumarin derivatives as multi-targeted dual inhibitors against AChE as well as MAO. It was reported that 3-methyl substituted coumarin derivatives exhibit higher activity towards AChE as well as MAO. Further, the compound having unsubstituted phenyl ring of benzyloxy moieties of coumarin was more active towards AChE as
well as MAO than compounds having substitution at ortho-, meta- and para-positions by any electron donating groups like -CH₃, -OH, -OCH₃ or other electron withdrawing groups. The 3-chlorobenzylxocoumarin (Compound X, Figure 1.12) showed exceptional behavior towards the inhibition of AChE and MAO.

![Chemical Structures](image)

**Figure 1.12:** Coumarin derivatives

Novel AChE inhibitors were reported by Leonetti et al.¹¹¹ by linking 3-hydroxy-N,N-dimethylanilino derivatives at the 7th position of coumarin with an appropriate linker. These derivatives exhibited activity towards AChE in nanomolar to sub-nanomolar range (XII, IC₅₀ = 275 nM). These derivatives were also found to be highly selective over BuChE.¹¹¹ The derivatives with spacer consisting of tetramethylene were found to be the most potent than the corresponding derivatives with trimethylene. Further, modification on this was carried out by the same research group to change position of 3-hydroxy-N,N-dimethylanilino on coumarin moiety. The derivatives having 3-hydroxy-N,N-dimethylanilino group attached at 3rd position of
coumarin were more potent than corresponding derivatives formed by linking 3-hydroxy-N,N-dimethylanilino group at 4\textsuperscript{th}, 5\textsuperscript{th}, 6\textsuperscript{th}, 7\textsuperscript{th} and 8\textsuperscript{th} positions respectively. They were reported compound (XII, IC\textsubscript{50} = 0.236 nM), as the most potent AChE inhibitor. The AChE/BChE selectivity was found to be greater than 300,000 times, and this molecule is in clinical trial.

\section*{1.4 Computational Approaches for Lead Discovery}

Computational approaches facilitate the discovery of novel lead molecules against a target. These approaches also assist the identification of diversified lead molecules against any target. Computational drug discovery method is the cornerstone for development of lead molecules against diverse targets over last three decades. Docking approaches are appeared to be important computational tools for predicting binding modes of small molecule in the active site of protein/enzyme. However, the effectiveness of binding mode prediction is dependent upon accuracy of geometry optimization and calculation (modeling) of docking score. Accurate geometry optimization is generally facilitated by quantum chemistry methods.\textsuperscript{112} The quantum chemistry method predicts/models partial atomics charges more effectively resulting in more accurate polar interaction energy calculation.

\subsection*{1.4.1 Geometry optimization}

In spite of experimental advancements, computational approaches i.e. quantum mechanical calculations are preferred to determine microscopic properties of the molecules. A molecule is represented as combination of electronic wave functions representing each atom forming it. The electronic wave function of a polytatomic molecule depends on several parameters such as radial and angular parts which are dependent upon bond distances, bond angles and dihedral angles of rotation about single bonds. Schrodinger equation (\textit{HΨ}=\textit{EΨ}) is used to determine energy of the molecule. Configurations with different geometries may generally have different energies. Four major methods are used to calculate molecular energy and properties: semi-empirical, ab initio, density-functional theory (DFT) and molecular mechanics methods. Semiempirical method, not so popular today, uses a simpler approximate Hamiltonian operator, and uses empirical parameters whose values are adjusted to fit the experimental data. In
contrast, ab-initio calculations are based on correct Hamiltonian without use of experimental data. The density-functional method (DFT) is based on electron probability density, \( \rho \) and this parameter is used to calculate the molecular electronic energy. This DFT method uses wave function that involves fewer variables and calculates the energy and other properties. The molecular mechanics method considers the molecule as a collection of atoms and expresses the molecular energy as sum of bond stretching, bending, etc. energies.\textsuperscript{113}

**Basis functions**

A basis set is a mathematical function to represent an electronic orbital or electronic wave function in atoms/molecules. These functions are used in Hartree–Fock method or density-functional theory (computational chemistry) methods approaches to convert the partial differential equations generated from a molecule into algebraic equations suitable for effective implementation on a computer. Several atomic orbitals are types of atomic orbitals Slater-type, Gaussian-type, numerical, etc. Different categories of basis sets such as minimal, split-valence, Pople basis, correlation-consistent, polarization-consistent, Karlsruhe, plane-wave, etc. are available with increasing computing time. The Pople basis sets are optimal as they take less time and provide good optimized geometry.\textsuperscript{113}

Determination of configuration with minimum energy from many conformations of a given molecule is defined as geometry optimization. The steepest descent and conjugate gradient algorithms are used for geometry optimization. The former is used at the initial steps of geometry optimization whereas conjugate gradient method is used in the final stages to get global energy minima. All positive vibrational frequencies indicate global minima of the structures. **B3LYP** uses Backe’s three parameters with correlation provided by the LYP expression, and VWN functional III for local correlation.

\[
C^*E_{C^{LYP}} + (1-C)^*E_{C^{VWN}}
\]

VWN is implemented to provide the excess local correlation required as LYP contains a local term equivalent to VWN.\textsuperscript{114} The DFT hybrid functional B3LYP with the basis set 6-31G*, is used to calculate individual atoms’ electron densities required for geometry optimization.
1.4.2 Docking methods to identify binding interactions modes of the ligand

Virtual screening has been proved to be a very efficient approach for finding potential interactions of ligand with protein target. Therefore, it facilitates lead optimization in structure-based drug discovery projects. Most of the docking software considers active site as rigid and ligand as flexible. With the availability computing resources, docking process facilitates to screen chemical molecule databases (ZINC) and lead like molecule databases against the target protein with an objective to identify potential molecule for experimental validation. After identification of lead molecules, this software can also be used for design of more potent lead molecules through analyzing protein-ligand interactions. Currently, most of the drug design & development labs combine these methods as a regular protocol to identify new lead molecules.

Schrodinger software (Maestro 10.5) Glide module is used for the docking study of the compounds. The Glide module consists of high throughput virtual screening (HTVS), standard precision (SP) and, Xtra Precision (XP) docking methodologies. Glide HTVS and SP implement a series of hierarchical filters to predict for possible best interactions mode of the ligand in the binding-site region of a receptor. The shape and properties of the receptor binding site are represented on a grid value by different sets of fields that provide more accurate scoring of the ligand pose in a faster manner. A collection of ligand conformations that are created and examined during the docking process are evaluated by exhaustive enumeration of ligand torsions. With different ligand conformations, preliminary screens are performed over the entire phase space to locate promising ligand poses. From poses selected by initial screening, the ligand is refined in torsional space using the force field OPLS3 (Glide SP & XP) with a distance-dependent dielectric model. This force-field (OPLS3) employs more reference data and allied parameter types in comparison to other commonly used force fields (e.g. MMFF and OPLS_2005). Therefore, OPLS3 provides a more accurate docking score. Finally, a small number of significant poses are minimized within the active site of the receptor with full ligand flexibility.

The adverse effect of the drugs on patients along with other limitations like low brain penetration effect, lower solubility etc. assures that there is a requirement for novel compounds that can be developed into better drug for the cure of AD. This prompted us to take this work and
synthesize new series of heterocyclic compounds which on *in silico* and *in vitro* study can give lead molecules. Therefore, the following three are objectives of my thesis:

### 1.5 Objectives

**Scheme 1:** Design, synthesis and evaluation of 3-[2-(4-phenylthiazol-2-y lamino)-acetyl]-chromen-2-one derivatives as cholinesterase inhibitors

**Scheme 2:** Design, synthesis and evaluation of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-y lamino)-acetamide derivatives as cholinesterase inhibitors

**Scheme 3:** Design, synthesis and evaluation of N-(3-mercapto-5-phenyl-4H-1,2,4-triazole-4-yl)2-oxo-2H-chromene-3-carboxamide derivatives as cholinesterase inhibitors

The thesis will be organized into five Chapters. The Chapter 1 discusses the overview of Alzheimer Disease (AD). Chapters 2, 3 and 4 describe the synthesis of novel heterocyclic molecules along with their evaluations against the AChE and BuChE. Chapter 5 discusses conclusion and future perspectives.

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