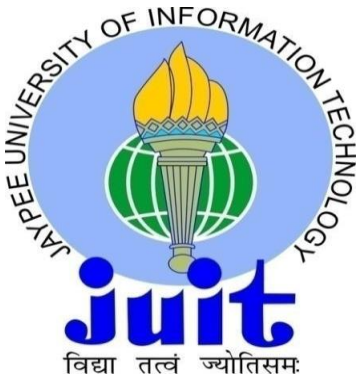
SYNTHESIS OF HETEROCYCLIC MOLECULES AS POTENTIAL ANTI-ALZHEIMERIC AGENTS

Thesis Submitted in fulfillment for the requirement of the Degree of

DOCTOR OF PHILOSOPHY

By

DEEPAK MISHRA



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WAKNAGHAT, DISTRICT SOLAN, H.P., INDIA
May 2018

Dedicated

To

My family

DECLARATION

I hereby declare that the work reported in the Ph.D. thesis entitled "**SYNTHESIS OF HETEROCYCLIC MOLECULES AS POTENTIAL ANTI-ALZHEIMERIC AGENTS**" submitted at **Jaypee University of Information Technology, Waknaghat, India,** is an authentic record of my work carried out under the joint guidance and supervision of **Dr. Chittaranjan Rout**, Associate professor, Department of Biotechnology & Bioinformatics, Jaypee University of Information Technology, Waknaghat, Himachal Pradesh and **Dr. Ram Singh**, Assistant Professor, Department of Applied Chemistry, Delhi Technological University (DTU), Delhi, India. This thesis is a presentation of my original research work. Wherever contributions of others are involved, every effort has been made to indicate this clearly.

I have not submitted this work elsewhere for any other degree or diploma. I am fully responsible for the contents of my Ph.D. Thesis.

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Date

SUPERVISOR'S CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled "**SYNTHESIS OF HETEROCYCLIC MOLECULES AS POTENTIAL ANTI-ALZHEIMERIC AGENTS**", submitted by **Deepak Mishra** at **Jaypee University of Information Technology, Waknaghat, India**, is a bonafide record of his original work carried out under our supervision. This work has not been submitted elsewhere for any other degree or diploma.

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Deepak Mishra

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LIST OF ABBREVIATIONS

ABBREVIATIONS	Full form
AD	Alzheimer's Disease
ARDSI	Alzheimer's and related disorders society of India
ACh	Acetylcholine
AChE	Acetylcholinesterase
AMP	Ammonium-molybdophosphate
APP	Amyloid precursor protein
BuChE	Butyrylcholinesterase
CT	Computed tomography
DBU	1,8-Diazabicyclo-[5.4.0]undec-7-ene
DABCO	1,4-Diazobicyclo(2,2,2)-octane
DCC	N,N′-Dicyclohexylcarbodiimide
DCM	Dicholromethane
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DTNB	5,5'-Dithio-bis-[2-nitrobenzoic acid]
EDC	1-Ethyl-3-(3′-dimethylaminopropyl)-carbodiimidehydrochloride
FDA	Food and Drugs Administration (U.S)
FTIR	Fourier-transform infrared spectroscopy
HOBt	Hydroxybezotriazole
HTVS	High throughput virtual screening
MRI	Magnetic resonance imaging
NBS	N-Bromosuccinimide
NMR	Nuclear magnetic resonance spectroscopy
PBS	Phosphate-buffered saline
PET	Positron emission tomography
TEA	Triethylamine
TMS	Tetramethylsilane
TLC	Thin-layer chromatography
THF	Tetrahydrofuran

ABSTRACT

Alzheimer's disease (AD) has a destructive impact on society, healthcare and cost. It is the most common form of dementia, which mainly occurs in elderly aged people. The disease affects cognitive function of patient. An approximately 9.4 million Americans are affected by this disease and this number is increased up to 30 million in 2050. While in Asia 23 million people suffered from this disease and this number is increase up to 38 million in 2030 and 67 million in 2050. The human cost is incalculable, the financial burden of caring for these patients is now $150 billion a year in America and the crisis is spreading. From Indian perspective, in the late nineties, of about 820 million people in the country, about 8.5% (~ 70 million people) were over 60 years of age. Today, this population increased to 10% and by the year 2021, this is expected that every seventh Indian will be a senior citizen. In 2010, there are 3.7 million Indians with dementia and the total societal costs is about 14,700 crore. While the numbers are expected to double by 2030, costs would increase three times. It is the sixth leading cause of death in USA and 5 leading cause of death for those people who aged above 60. The cause and progression of AD is not well understood till now. Based on the ongoing research several hypothesis are given for the treatment of AD at early stage but, till now no drugs are available which permanently cure this disease. There are only few drugs are available which is mainly based on the cholinergic hypothesis and was approved by FDA, used for the treatment of AD at early stage. These known drugs not cure the disease permanently, they only slow down the progression of this disease. Some of these drugs have side effect also which a major problematic issue for the elderly aged people. Based ongoing research and hypothesis available for the treatment of AD, herein we have design, synthesize and evaluated some novel heterocyclic molecule based on coumarin, thiazole and trialzole moieties to obtain a lead novel molecule which can act as cholinesterase inhibitor.

Chapter 2 deals with the synthesis and evaluation of coumarin-thiazole based cholinesterase inhibitors. We have design our scheme on the basis of preliminary *in-silico* studies and synthesize the novel molecule *via* multistep synthesis and characterized by spectroscopic techniques. The synthesized molecules were evaluated towards AChE and BuChE enzyme. In chapters 3 and 4, we have synthesized benzothiazole-triazole and coumarin-triazole based novel molecule respectively by following the same protocol as in chapter 2 and was evaluated against AChE and BuChE enzymes.

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.[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.[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.[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.[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 6th leading cause of death in USA, and 5th leading cause of death for those who aged above 70.[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.[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.[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).[5] The larger percentage of older women than men who is suffering from dementia is because the women live longer in India.[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),[5] and 90% cases of dementia in India is due to AD and this number is expected to double by 2030 (Figure 1.2).[5]

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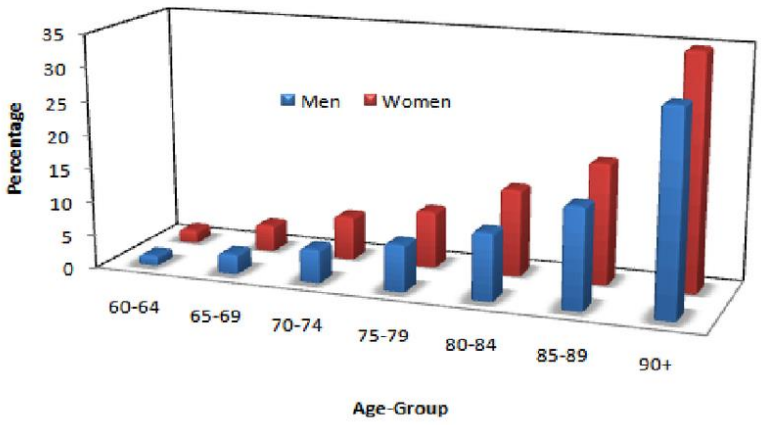


Figure 1.1: Prevalence of individual with dementia by age and gender in India[5]

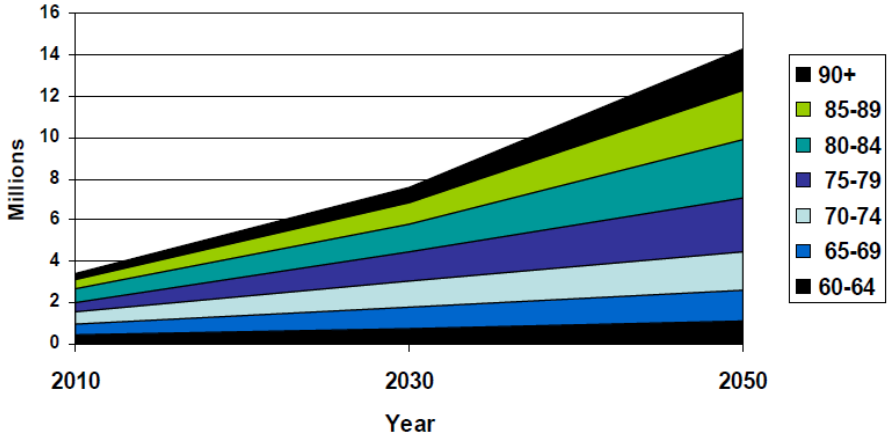
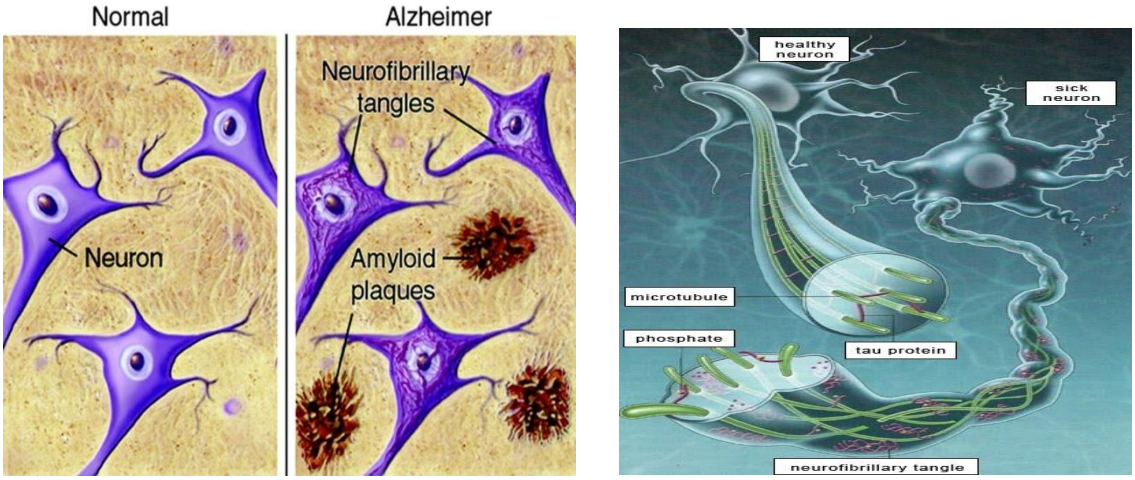


Figure 1.2: Number of individual with dementia by age in India, 2010-2050[5]

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.[1] 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.[1] 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.[6] 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.



(a) (b)

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

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 sever 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).[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.[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).[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.[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). [7,8]

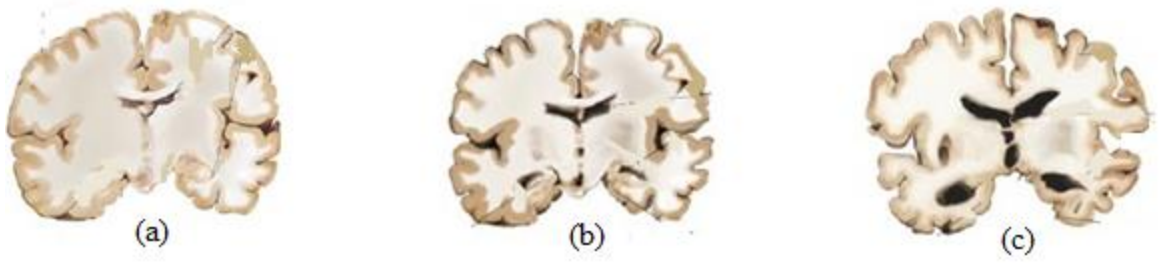


Figure 1.4: Structure of brain at the different stages of Alzheimers disease[1]

(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.[9] Besides above, brain

scans such as magnetic resonance imaging (MRI),[10] positron emission tomography (PET)[11] and computed tomography (CT)[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.[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 cholinerginc 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.[13-15] According to Francis et al[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[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.[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.[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.[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.[25] Thus, by inhibiting these two enzymes the amount of free acetylcholine which interacts with neuronal receptors for signaling can be increased. [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 super-family belong to a broad group of proteins which are involved in several important physiological processes like blood coagulation,[27] digestion,[28] as well as in neurotransmission.[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.[30] However, there are many serine hydrolases available that need to be characterized as their function and substrate specificity are still unknown.[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[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,[34] brain cholinergic synapses,[35] autonomic ganglia[36] and red blood cell membranes.[37] This enzyme is also known as a modulator of neurotransmission which hydrolyses neurotransmitter acetylcholine (ACh) that is synthesized from acetyl coenzyme A

(AcCoA, which is synthesized from glucose). Acetylcholine is synthesized from choline by the catalytic action of choline acetyltransferase enzyme and stored into synaptic vesicles.[15] From this vesicles, the ACh is released to presynaptic nicotinic (N) and muscarine type 2 (M_2) receptors which further release this ACh to postsynaptic M_1 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[15]. Several AChE functions have also been reported such as cellular differentiation and tumorigenesis,[38] apoptosis.[39,40] etc. Unattended release of ACh results in the continuous stimulation of receptors which causes symptoms like confusion, vomiting, convulsion and respiratory failure.[41] On the contrary, lack of ACh lowers receptor stimulation leading to cognitive impairment (significant symptom of AD).[42] Therefore, it is essential to keep a balance of ACh activity.

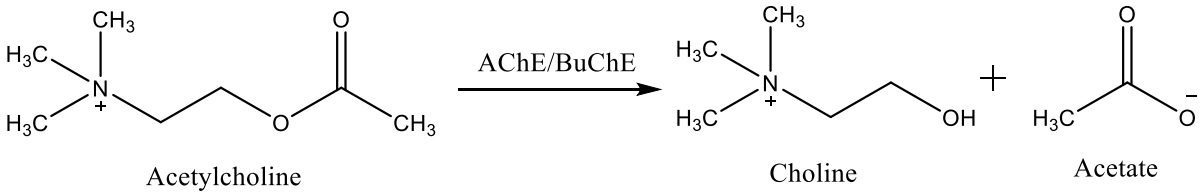


Figure 1.5: Acetylcholinesterase/ Butyrylcholinesterase hydrolyzing acetylcholine

Butyrylcholinesterase

Butyrylcholinesterase (BuChE), is a serine hydrolase and also called as pseudocholinesterase, which catalyzes the hydrolysis of choline ester including butyrylcholine, succinylcholine and acetylcholin.[43] 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[16] and mostly found in the serum and glial cells.[44,45] AChE exhibits specificity towards the neurotransmitter acetylcholine,[46] whereas BuChE catalyzes the hydrolysis of a wide variety of choline and non-choline esters[47] such as ACh,[48] succinylcholine,[49] cocaine[50] and aspirin.[51] 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[52] and myasthenia gravis.[53] These drugs are also beneficial for the management of several other disease like chronic pain[54,55] and type 2 diabetes.[56] Most of the early drug development efforts against AD targeted cholinesterases.

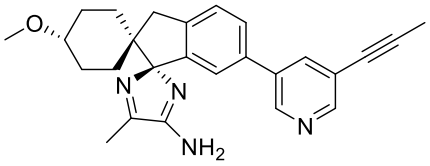
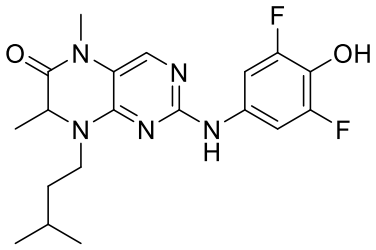
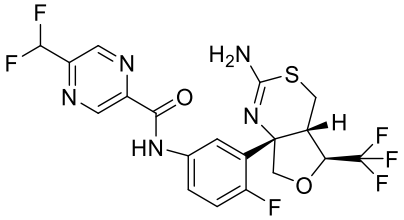
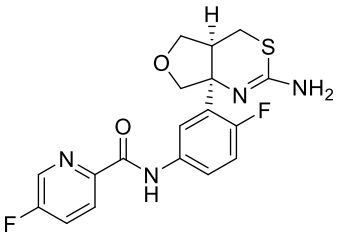
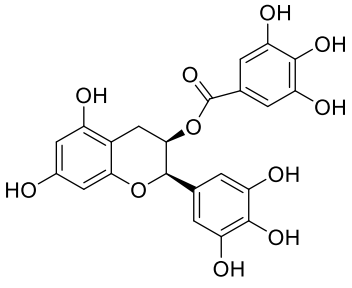
1.2.4 Amyloid hypothesis for Alzheimer's Disease (AD)

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.[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.[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.[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.[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.[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).[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.[65] It mainly catalyzes the oxidative deamination of amines particularly, primary amines to produce aldehyde, ammonia and hydrogen peroxide (Figure 1.6).[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).[66,67]

Table 1.1 Potential drug molecules in clinical trial as BACE inhibitors

Drugs	Clinical Trial Phase and Current Status
 (AZD3293)	Phase III Study start date Sep. 2014 Study end date Aug 2019 adopted from https://www.nia.nih.gov/alzheimers/clinical-trials/azd3293-early-alzheimers-disease-amaranth (accessed on 11 Oct 2017)
 BI 1181181	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	Phase III started on 27 April 2017 https://www.biocentury.com/bc-week-review/clinical-news/clinical-status/2017-04-26/elenbecestat-ph-iii-missionad2-started?Kwh=%22elenbecestat%22+%22E2609%22 (accessed on 11 Oct. 2107)
 LY2886721	Phase II discontinued due to liver biochemistry in 2013 http://www.alzforum.org/therapeutics/ly2886721 (accessed on 11 Oct 2017)
 PF05297909	Studied of Phase I was completed in 2012 and goes for further studies. https://clinicaltrials.gov/ct2/show/NCT01462851 accessed on 11 Oct 2017.

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,[68,69] AD[70,71] and other types of dementia.[72] During oxidative deamination of amine by MAO, the formation of H_2O_2 takes place resulting in oxidative stress which plays a central role in neurodegeneration.[65] Literature also shows that neurotransmitter containing monoamine systems play a important role in cognitive function, like memory, attention, thinking, behavior and emotion.[65] MAO disturb the balance of neurotransmitters by oxidative deaminaton, which includes glutamatergic action, ChE, serotonin and norepinephrine and these may result in cognitive impairment.[65] 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.[73-75] On the other hand, MAO-B catabolizes 2-phenylethylamine and benzylamine.[76,77] 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.[65] 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.[65]

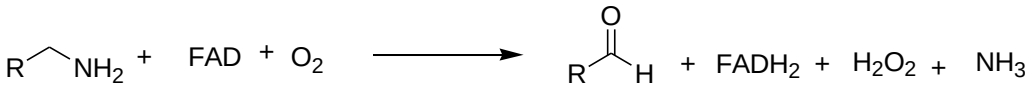


Figure 1.6: Oxidative deamination of amines by MAO proteins

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).[78-80] 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[81] was the first drug, approved by

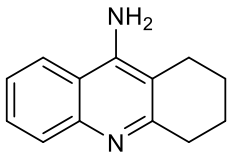
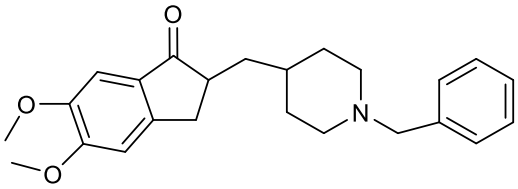
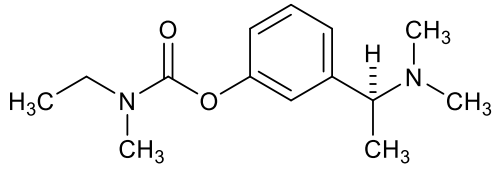
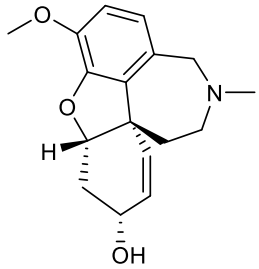
FDA in 1993, entered the market for the treatment of AD. It is a non-competitive AChE inhibitor which also inhibits BuChE .[82] Due to toxicitity of this drug,[83,84] it is not commonly in use. However, many medicinal chemists used its scaffold to synthesize many cholinesterase inhibitors (Section 1.3.1-1.3.2).[85-87] Later on few drugs like donepezil (1996), rivastigmine (2000) and galantamine (2001)[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.[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.[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.[90,91] This glutamate binds to N-methyl-D-aspartate (NMDA) receptors and opens the calcium ion channel leading to hyperpolarization ofneurons results in cellular appotosis.[91] Memantine mainly blocks the NMDA receptors; therefore, prevents the nerves from excessive glutamate stimulation.[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

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

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.[30,93] 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).[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.[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.[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.[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.[96] This molecule is also free from their adverse side effects which were observed in tacrine.[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,[97] benzo[b]pyrazolo[4,3-g][1,8] napthyridine,[98] Tacrine-benzofuran Hybrids,[99] and benzochromene.[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.[102,103]

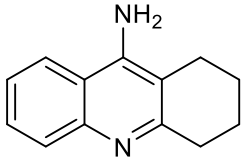


Figure 1.7: Tacrine molecule (**I**)

1.3.2 Tacrin-phenylthiazole hybrids

Thiazole is a five-membered heterocyclic molecule having molecular formula C_3H_3NS. 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.[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.[104] 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).[105]

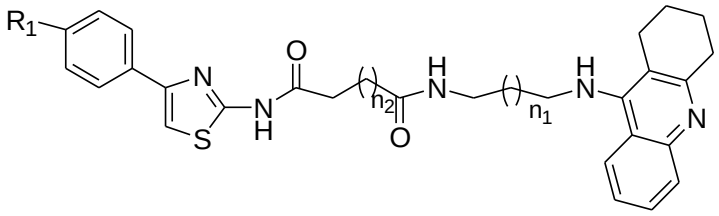


Figure 1.8: Phenylthiazole-tacrine conjugates (**II**)

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).

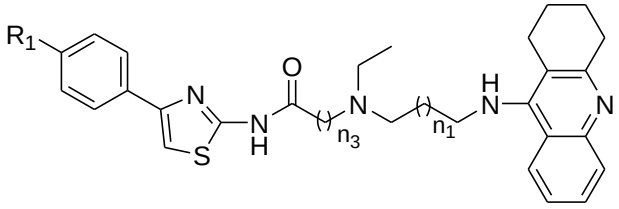


Figure 1.9: Phenylthiazole-tacrine conjugates (**III**)

1.3.3 Coumarins derivatives

Ensaculin[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_{50} value 0.36 µM against AChE.

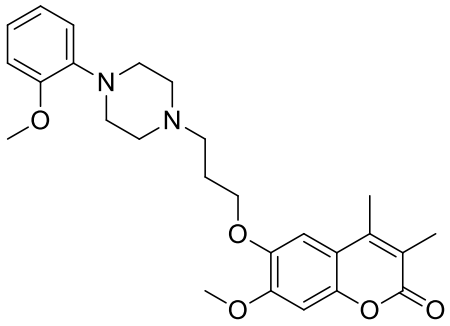
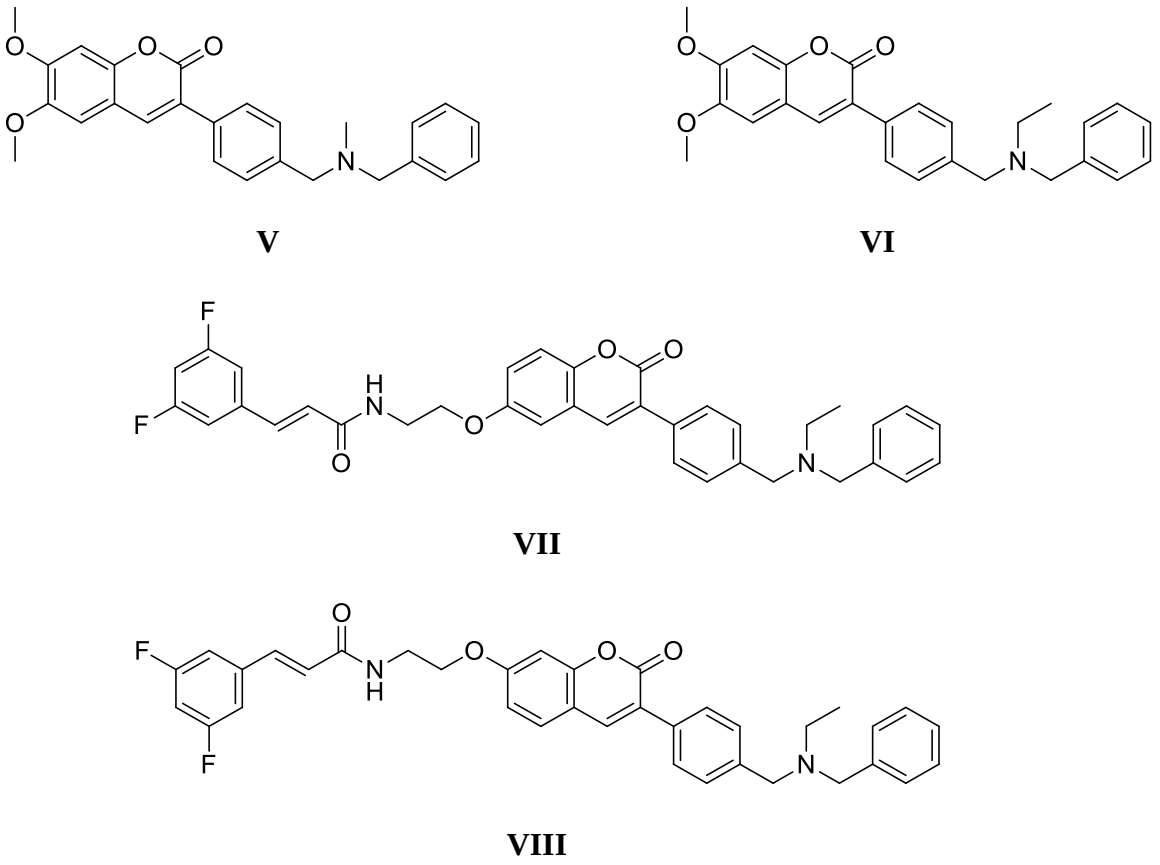


Figure 1.10: Ensaculin, KA-672 (**IV**)

Piazzi et al[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_{50} value 44.5 nM (Figure 1.11). This molecule is highly selective towards AChE in comparison to BuChE with IC_{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 Aβ 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_{50} = 18.3 nM) which was due to its increase in lipophilicity.[108] When $–OCH_3$ group at 6^{th} or 7^{th} position was replaced by bulkier halogenated phenyl group, the AChE inhibitory activity was reduced (Compound **VII** IC_{50}= 7.16 µM and **VIII**, IC_{50}= 4.57µM). This decrease in activity suggests that molecule with bulky groups at 6^{th} and 7^{th} position are not allowed to penetrate into the active site of AChE.



V **VI**

VII

VIII

Figure 1.11: AP2238 and its derivatives

Shen et al[109] reported franocoumarin derivatives and provided substitution at 4th position of the coumarin with substituted aryl amino group with one atom as spacer. These derivatives displayed moderate AChE inhibitory activity having IC_{50} value in µM range. The compounds having electron donating groups such as $–OCH_3$, $–NH_2$ and –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 $–CH_3$. The most potent compound of this series was compound **IX** (IC_{50} = 0.19µM) having $-OCH_3$ group at the second position of phenyl ring of anilino moiety (Figure 1.12).

Bruhlmann et al[110] reported 7-benzyloxycoumarin 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 orthro-, meta- and para-positions by any electron donating groups like -CH_3, -OH, -OCH_3 or other electron withdrawing groups. The 3-chlorobenzyloxycoumarin (Compound **X**, Figure 1.12) showed exceptional behavior towards the inhibition of AChE and MAO.

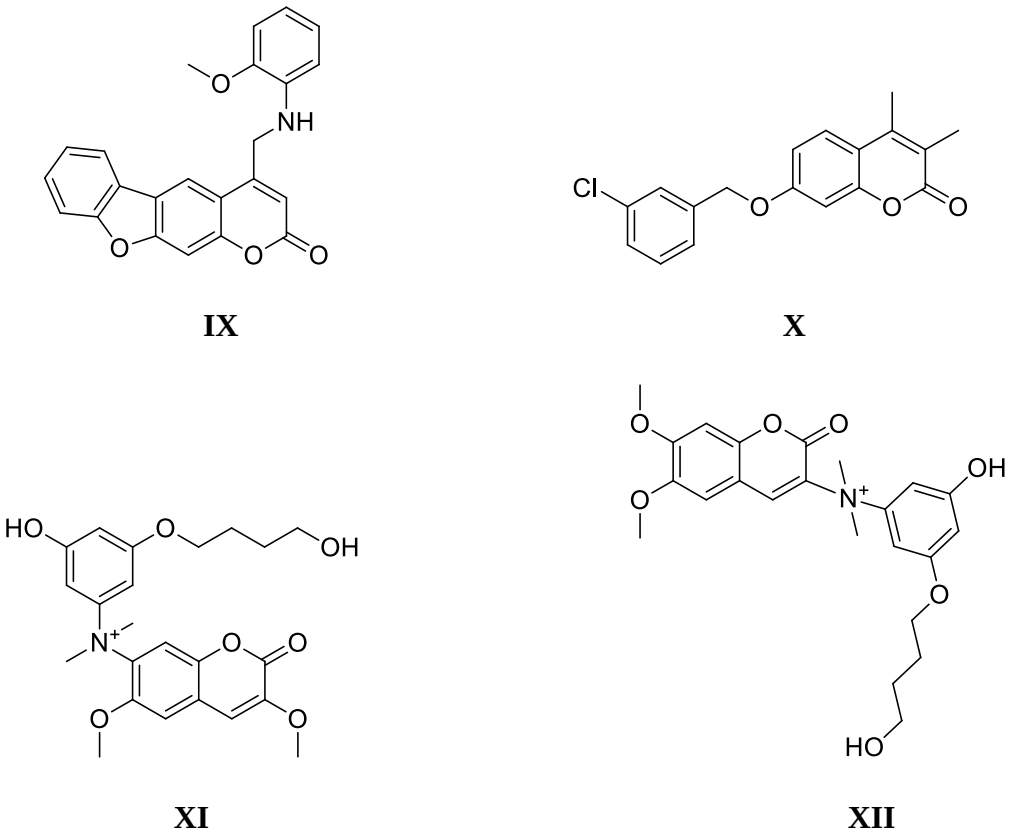


Figure 1.12: Coumarin derivatives

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 (**XI**, IC_{50} = 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 coumrin 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-dimethyanilino group at 4^{th}, 5^{th} , 6^{th} ,7^{th} and 8^{th} positions respectively. They were reported compound (**XII,** IC_{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.

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.[112] The quantum chemistry method predicts/models partial atomics charges more effectively resulting in more accurate polar interaction energy calculation.

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 ($H\Psi=E\Psi$) 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, ρ 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.[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.[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.[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.[115] 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.[116] 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-ylamino)-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-ylamino)-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.

*****

CHAPTER 2

Design, Synthesis and Evaluation of 3-[2-(4-phenyl thiazol-2-ylamino)acetyl]chromen-2-one Derivatives as Cholinesterase Inhibitors

2.1 Introduction

Coumarin (2H-chromen-2-one or 1-benzopyran-2-one, Figure 2.1) is benzopyran derivative having oxygen hetero-atom in the six membered ring called pyran which is fused with the benzene ring.[117] This molecule was first time isolated by August Vogel from plant Tonka bean, *coumarou* in 1820.[118,119] Coumarins are structurally constructed by the fusion of lactone and benzene ring. In general, the structure formed by benzene and lactones are called benzopyranone. They are classified on the basis of their fusion position commonly known as coumarins and chromones (Figure 2.1). Both differ only in the position of the carbonyl group in the heterocyclic ring.[120] These are naturally occurring phytochemicals which are found in many plant species like woodruff, lavender, licorice, strawberries, apricots, cherries, cinnamon, sweet clover, bison grass, etc.[121,122] It has a broad range of biological activities such as anti-inflammatory,[123] anti-tumor,[124] hepatoprotective, anti-allergic, anti-HIV-1, antiviral, antifungal, antimicrobial, antiasthmatic,[125] antioxidant,[126] antinociceptive,[127] anti-diabetic, antidepressant effects, etc. (Figure 2.2).[128]

The coumarins are further classified as simple coumarin, furanocoumarin and pyranocumarin (Figure 2.3).[129]

- Simple coumarins – these molecules are either unsubstituted, or hydroxylated, alkoxylated and alkylated derivatives of coumarin, along with their glycosides
- Furanocoumarins – they are the compounds comprise of a five-membered furan ring attached to the coumarin nucleus.
- Pyranocoumarins – they are the members of furanocoumarins, having a six-membered ring.

In recent years, coumarin derivatives have attracted attention due to their medicinal applications in neurological disorders. The molecules based on coumarins are widely studied as potential anti-alzheimeric agents.[130] Furthermore, derivatization of the aromatic center of coumarins has led to the development of novel analogs that are capable of inhibiting Aβ aggregation.[108] The studies have also shown the anti-amnestic and memory restorative functions of the coumarin derivatives in many different experimental models of amnesia.[131]

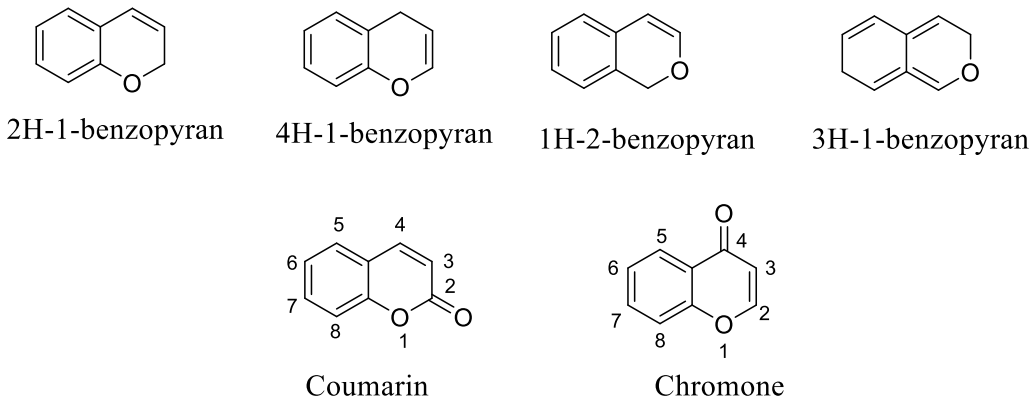


Figure 2.1: Structure of benzopyran derivatives

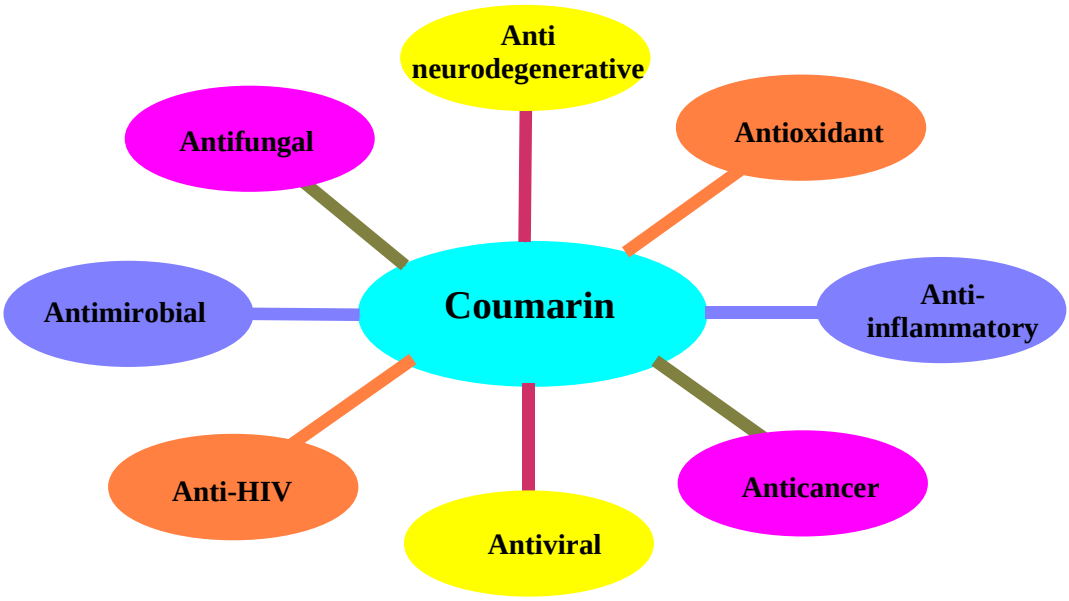


Figure 2.2: Potential medicinal applications of coumarins

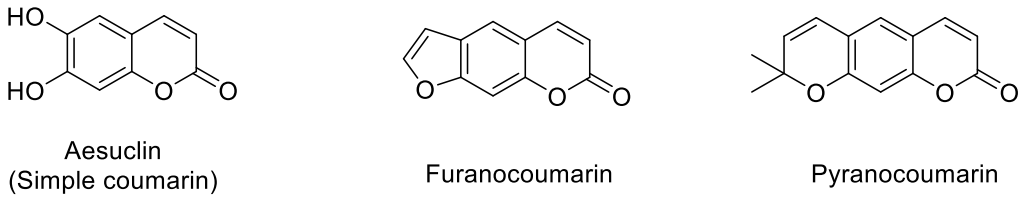


Figure 2.3: Classification of coumarins

Protection to neurons against Aβ-induced oxidative stress and free radicals is also provided by the coumarin derivatives.[132] Studies on coumarin analogues have also proven that naturally occurring as well as the chemically synthesized coumarin analogs exhibit potent acetyl cholinesterase (AChE) inhibitory activity.[127] The length of linker, linking coumarin heterocycle and catalytic site interacting moiety, is an important parameter for influencing the AChE inhibitory activity.[133] Most of the studies have reported that compounds with tetramethylene or phenyl linker are more potent AChE inhibitors.[133] Studies also reported that the 3rd or 4th position of coumarin moiety (Figure 2.1) is the most favorable position for linking to the catalytic site interacting moiety for obtaining potent dual site AChE inhibitors.[133] The substitution at 6th or 7th position generally does not increase the potency of compounds. When bulkier substituents are present at 6th and 7th positions of the coumarin, the inhibitor activity towards AChE has found to be lower.[133]

Hypothesis of proposing coumarin-thiazole conjugate as cholinesterase inhibitor

Among six drugs that have been approved by FDA for the treatment AD, five of them are AChE inhibitors (discussed in Chapter 1). However, these drugs only slow down the progression of this disease on mild to moderate stage of AD. Coumarins and thiazoles were extensively studied as they exhibited antioxidative and enzymatic inhibition properties. Numerous derivatization have been presented on coumarins and thiazole moiety alone, which act as potent MAO and/or AChE as well as BuChE inhibitors, and some of them have been proposed for AD treatment.[110] Moreover, coumarin and thiazole derivatives are usually easy to synthesize and also they possess good solubility, low cytotoxicity, and excellent cell permeability. Therefore, it is expected that hybrid of coumarin-thiazole would improve the potency as compared to coumarin or thiazole alone. Herein, a series of hybrids of coumarin-thaizole (**8a-l**) using appropriate linker have been designed on the basis of preliminary *in-silico* studies. These compounds were synthesized and then their activity was evaluated through *in-silico* and *in-vitro* studies. The substitution was introduced at the 3rd position of coumarin with the appropriate linker as shown in figure 2.4.

Studies have reported that coumarin and thiazole fragments showed activity against ACHE. Preliminary docking studies of these fragments had indicated that coumarin and thiazole interact with the active site of AChE and BuChE. In view of these findings, this chapter deals

with the design, synthesis and evaluation of coumarin-thiazole conjugates as cholinesterase inhibitors (Figure 2.5).

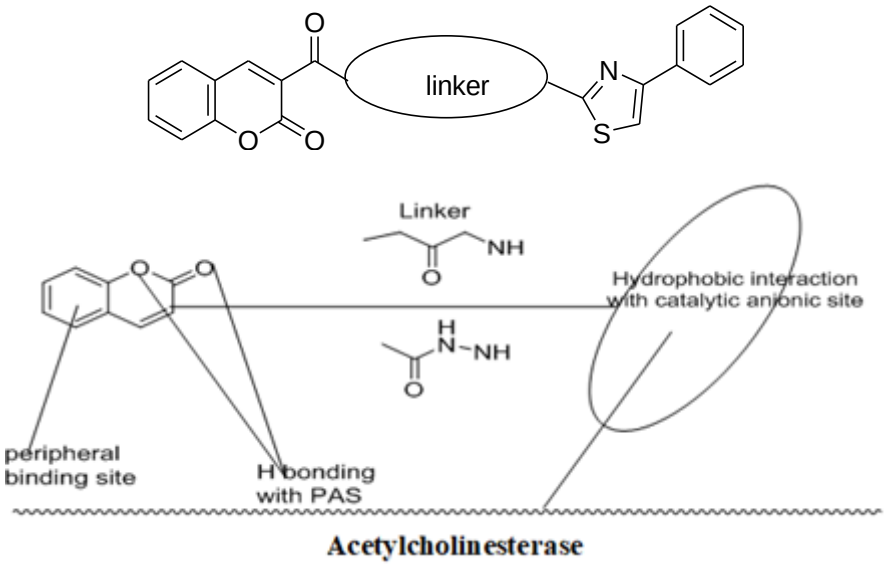


Figure 2.4: Potential interactions between AChE with coumarin-thiazole conjugate

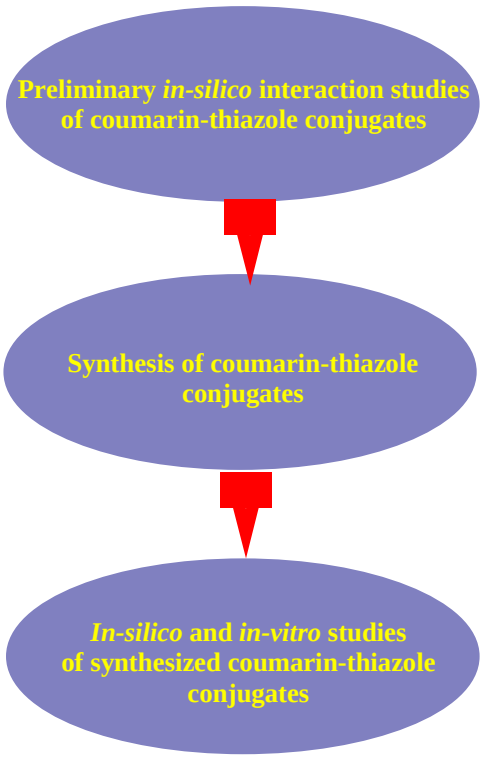


Figure 2.5: Flow chart indicating design, synthesis and evaluation of coumarin-thiazole conjugates

2.2 Experimental

2.2.1 Preliminary *in-silico* interaction studies of coumarin-thiazole conjugates

Preliminary docking studies of the coumarin and thiazole fragments with cholinesterase enzymes such as AChE (1EVE) and BuChE (4TPK) was performed using Glide module of Schrodinger. Favourable interactions of these fragments with these enzymes (AChE and BuChE) were identified. These fragments were interacted in different parts of the active site. On the basis of these results, we designed and synthesized 3-[2-(4-phenylthiazol-2-ylamino)acetyl]chromen-2-one derivatives. These synthesized novel compounds were validated by carrying out *in-silico* and *in-vitro* studies.

2.2.2 Synthesis of 3-[2-(4-phenylthiazol-2-ylamino)acetyl]chromen-2-one derivatives (8a-l)

All commercially available solvents and reagents were purchased from reputed company, and were used without further purifications. Melting points were determined on a laboratory capillary melting apparatus and were uncorrected. FTIR spectra were recorded on a Perkin Elmer Spectrum Version 10.5.3 FTIR spectrophotometer. The ν_{max} are expressed in cm^{-1}. ^{1}H and ^{13}C NMR were recorded on a Bruker spectrophotometer and Jeol spectrophotometer (400/100 MHz) using TMS as internal standard. The chemical shifts are expressed in ppm. The abbreviation s, d, t, q, m and bs stand for singlet, doublet, triplet, quartet, multiplet and broad singlet respectively. The elemental analysis was measured by PerkinElmer 2400. Thin-layer chromatography was performed on aluminium-coated silica plates purchased from Merck. The synthesis of compound **8a-l** has been achieved by following the Scheme 2.1 (Section 2.3.1).

Synthesis of 3-acetyl-2H-chromen-2-one (3)

A solution of ethylacetoacetate (**2**, 3.0 mL, 23.5 mmol) in ethanol (15 mL) was taken in a round bottom flask (250 mL). The solution was kept at 0^{o}C and then piperidine (0.2 mL, 2.0 mmol) was added. The resulting reaction mixture was stirred at 0^{o}C for 5 min followed by addition of salicylaldehyde (**1**, 2.5 mL, 23.5 mmol). The reaction mixture was allowed to come at room temperature and stirred for 3 hours. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 7:3, v/v). The reaction mixture was filtered and product was washed with

ice cold water-ethanol (7:3, v/v) mixture. The yellow solid product was further recrystallized by using ethanol to get the pure product **3**.

Yield: 94%; Mp: 120-121^{o}C (Lit.[134] Mp.: 120^{o}C); FTIR (KBr): 3031, 1937, 1739, 1675, 1555, 1453, 1209, 1159, 920, 872, 575 cm^{-1}; ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) δ: 2.49 (s, 3H, CH_3), 7.40-7.96 (m, 4H, ArH), 8.64 (s, 1H, H of pyran ring); ^{13}C NMR (DMSO-*d*$_6$, 100 MHz) δ: 30, 116, 118, 124, 125, 131, 134, 147, 155, 158, 195.

Synthesis of 3-(2-bromoacetyl)chromen-2-one (4)

A solution of 3-acetyl-2H-chromen-2-one (**3**, 28.2 g, 150 mmol) in 150 mL of alcohol free chloroform was taken in a round bottom flask (250 mL). The solution was kept at 0^{o}C and then Br_2 (7.6 mL in 20 mL $CHCl_3$, 150 mmol) was added with the help of dropping funnel. After addition of all bromine, the reaction mixture was allowed to come at room temperature and stirred vigorously for 6 hours. The progress of reaction was monitored by TLC (CH_2Cl_2). After completion of reaction, the reaction mixture was heated on water bath for 20 min and further cooled to room temperature. The solid product was separated out which was further separated by column chromatography into **4a** and 3-(2,2-dibromoacetyl)-2-chromen-2-one (**4b**) (Scheme 2.1). The individual compounds were re-crystallized using glacial acetic acid to get colorless needle shaped crystal of **4**.

3-(2-Bromoacetyl)chromen-2-one (4a)

Yield: 85.5%; Mp.: 162-163^{o}C (Lit.[135] Mp.: 162^{o}C); FTIR (KBr): 3025, 2959, 2030, 1727, 1685, 1612, 1552, 1450, 1366, 1248, 1056, 978, 754, 668, 571 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.75 (s, 2H, CH_2), 7.36-7.72 (m, 4H, ArH), 8.64 (s, 1H, H of pyran ring).

3-(2,2-Dibromoacetyl)-2H-chromen-2-one (4b)

Yield: 12.5% Mp.: 100-102^{o}C (Lit.[136] Mp.: 102^{o}C); FTIR (KBr): 3024, 2968, 2029, 1729, 1682, 1548, 1446, 1372, 1257, 1069, 962, 768, 657, 573 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.57 (s, 1H, CH), 7.28-7.77 (m, 4H, ArH), 8.78 (s, 1H, H of pyran ring).

General procedure for the synthesis of 2-aminophenylthiazole derivatives (7a-l)

A solution of substituted phenacyl bromide (**5**, 4.00 g, 20.0 mmol) in 6 mL THF was taken in round bottom flask (100 mL). The solution was kept at room temperature and added thiourea (**6**,

1.83 g, 24.0 mmol). The reaction mixture was stirred at room temperature for 30 min. The progress of reaction mixture was monitored by TLC (hexane:ethyl acetate, 7:3, v/v). After completion of reaction, the solid products was filtered and washed with water. The crude products were further re-crystallized by using ethanol to get the pure compounds **7a-l** (Scheme 2.1).

4-Phenyl-1,3-thiazol-2-amine (7a)

Yield: 95%; Mp.: 147-148^{o}C (Lit.[137] Mp.: 150-151^{o}C); FTIR (KBr): 3435, 3252, 3154, 2920, 2852, 1600, 1520, 1481, 1440 1333, 1215, 1072, 912, 846, 775 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.12 (bs, 2H, NH_2), 6.75 (s, 1H, H of thiazole), 7.33 (d, 1H, J = 7.6 Hz, ArH), 7.42-7.38 (m, 2H, ArH), 7.80 (d, 2H, J = 7.2 Hz, ArH).

4-(4-Fluorophenyl)-1,3-thiazol-2-amine (7b)

Yield: 85%; Mp.: 105-108^{o}C (Lit.[138] Mp.: 102-103^{o}C); FTIR (KBr): 3434, 3243, 3150, 2928, 2857, 1590, 1520, 1482, 1440, 1333, 1216, 1073, 911, 846, 773 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.10 (bs, 2H, NH_2), 6.67 (s, 1H, H of thiazole), 7.11 (d, 2H, J = 8.1 Hz, ArH), 7.78 (d, 2H, J = 8.1 Hz, ArH).

4-(4-Chlorophenyl)-1,3-thiazol-2-amine (7c)

Yield: 83%; Mp.: 162-164^{o}C (Lit.[139] Mp.: 167-168^{o}C); FTIR (KBr): 3436, 3243, 3145, 2920, 2850, 1599, 1522, 1482, 1440, 1336, 1216, 1071, 912, 846, 775 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.04 (bs, 2H, NH_2), 6.74 (s, 1H, H of thiazole), 7.35 (d, 2H, J = 8.4 Hz, ArH), 7.72 (d, 2H, J = 8.4 Hz, ArH).

4-(4-Bromophenyl)-1,3-thiazol-2-amine 7(d)

Yield: 92%; Mp.:180-181^{o}C (Lit.[140] Mp.: 176-177^{o}C); FTIR (KBr): 3427, 3282, 3106, 2924, 1530, 1466, 1390, 1334, 1065, 1032, 1001, 820, 727, 666 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.02 (bs, 2H, NH_2), 6.75 (s, 1H, H of thiazole), 7.50 (d, 2H, J = 8.4 Hz, ArH), 7.66 (d, 2H, J = 8.8 Hz, ArH).

4-(4-Methylphenyl)-1,3-thiazol-2-amine (7e)

Yield: 87%; Mp.: 134-136^{o}C (Lit.[141] Mp.: 135-136^{o}C); FTIR (KBr): 3427, 3216, 3128, 2945, 2821, 1588, 1509, 1451, 1341, 1221, 1078, 918, 836, 763 $cm.^{-1}$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ:

2.38 (s, 3H, CH_3), 5.09 (bs, 2H, NH_2), 6.68 (s, 1H, H of thiazole), 7.19 (d, 2H, J = 7.6 Hz, ArH), 7.67 (d, 2H, J = 8.0 Hz, ArH).

4-(4-Methoxyphenyl)-1,3-thiazol-2-amine (7f)

Yield: 92%; Mp.: 204-206ºC (Lit.[139] Mp.: 208-209ºC); FTIR (KBr): 3434, 3253, 2948, 1524, 1482, 1336, 1216 , 1071, 910, 846, 773 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 3.80 (s, 3H, OCH_3) 5.01 (bs, 2H, NH_2), 6.58 (s, 1H, H of thiazole), 6.81 (dd, 2H, J = 8.2 Hz, J = 1.8 Hz, ArH) 7.72 (dd, 2H, J = 8.4 Hz, J = 1.6 Hz, ArH).

4-(4-Nitrophenyl)-1,3-thiazol-2-amine (7g)

Yield: 94%; Mp.: 280-284ºC (Lit.[141] Mp.: 284-286ºC); FTIR (KBr): 3430, 3243, 3091, 2933, 1572, 1514, 1377, 1360, 1310, 1216, 1071, 915, 846, 766 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 7.23 (bs, 2H, NH_2), 7.41 (s, 1H, H of thiazole), 8.03 (d, 2H, J = 8.0 Hz, ArH), 8.25 (d, 2H, J = 8.8 Hz, ArH).

4-(4-Cyanophenyl)-1,3-thiazol-2-amine (7h)

Yield: 93%; Mp.: 259-265ºC (Lit.[142] Mp.: 257-268ºC); FTIR (KBr): 3420, 3227, 3167, 2812, 2237, 1599, 1472, 1440, 1339, 1211, 1042, 915, 856, 783 $cm.^{-1}$; ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.10 (bs, 2H, NH_2), 6.78 (s, 1H, H of thiazole), 8.03 (d, 2H, J = 8.0 Hz, ArH), 8.25 (d, 2H, J = 8.8 Hz, ArH).

4-(3-Bromophenyl)-1,3-thiazol-2-amine (7i)

Yield: 89%; Mp.: 132-135ºC (Lit.[142] Mp.: 132-135ºC); FTIR (KBr): 3440, 3281, 3126, 2924, 1530, 1466, 1350, 1334, 1065, 1033, 1021, 822, 729, 667 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.02 (bs, 2H, NH_2), 7.15 (s, 1H, H of thiazole), 7.23-7.35 (m, 3H, ArH), 7.65 (d, 1H, J = 1.8Hz, ArH).

4-(3-Nitrophenyl)-1,3-thiazol-2-amine (7j)

Yield: 90%; Mp.: 190-192ºC (Lit.[138] Mp.: 189-190ºC); FTIR (KBr): 3429, 3240, 3071, 2933, 1570, 1514, 1373, 1360, 1312, 1213, 1074, 915, 846, 767 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 7.11 (bs, 2H, NH_2), 7.67 (s, 1H, H of thiazole), 7.73-7.78 (m, 3H, ArH), 7.88 (d, 1H, J = 1.8 Hz, ArH).

4-(3-Methoxphenyl)-1,3-thiazol-2-amine (7k)

Yield: 89%; Mp.: 99-100^{o}C (Lit.[143] Mp.: 98-100^{o}C); FTIR (KBr): 3453, 3278, 2918, 2855, 1599, 1440, 1240, 1171, 908, 856, 780 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.85 (s, 3H, OCH_3), 5.18 (bs, 2H, NH_2), 6.71 (s, 1H, H of thiazole), 6.86 (d, 1H, J = 1.8 Hz, ArH), 7.34-7.25 (m, 3H, ArH).

4-(3,4-Dichlorophenyl)-1,3-thiazol-2-amine (7l)

Yield: 92%; Mp.: 190-195^{o}C (Lit.[142] Mp.: 190-195^{o}C); FTIR (KBr): 3425, 3235, 3144, 2919, 2864, 1517, 1486, 1423, 1316, 1096, 1062, 907, 834, 753 cm.$^{-1}$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.02 (bs, 2H, NH_2), 6.75 (s, 1H, H of thiazole), 7.47 (d, 1H, J = 8.1 Hz, ArH), 7.59 (dd, 1H, J = 8.4 Hz, J = 2.1 Hz, ArH), 7.89 (d, 1H, J = 2.1 Hz, ArH).

General procedure for the synthesis of 3-[2-(4-phenylthiazol-2-ylamino)acetyl]chromen-2-one derivatives (8a-l)

A solution of substituted phenylthiazole-2-amines (**7**, 2.84 mmol) in 3 mL DMF were taken in a round bottom flask (50 mL). To this solution, 3-(2-bromoacetyl)chromen-2-one (**4a**, 2.84 mmol) and K_2CO_3 (0.72 mmol) were added. The reaction mixture was stirred at 60^{o}C for 3 hours and the progress of reaction was monitored by TLC (hexane:ethyl acetate, 7:3, v/v). After completion of reaction, the reaction mixture was quenched by water and was extracted with ethyl acetate (3×50 mL). The organic layer was washed with water (3×50 mL) and dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure. The residue was purified by column chromatography using petroleum ether:ethyl acetate, (4:1, v/v) as mobile phase and silica gel (60-120 mesh) as stationary phase to get the pure products **8**.

3-[2-(4-Phenylthiazol-2-ylamino)acetyl]chromen-2-one (8a)

Yield: 67%; Mp.: 190-195^{o}C; FTIR (KBr): 3436, 1705, 1604, 1532, 1518, 1483, 1442, 1332, 1148, 1039, 845, 714 cm.$^{-1}$; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.21 (s, 2H, CH_2), 7.07 (s, 1H, H of thiazole), 7.11-7.91 (m, 8H, ArH), 8.53 (s, 1H, H of pyran); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 51, 102, 111, 113, 118, 124, 125, 129, 131, 136, 147, 150, 155, 159, 168, 195; Anal. calc. for $C_{20}H_{14}N_2O_3S$: C, 66.28; H, 3.89; N, 7.73; S, 8.85; found C, 66.20; H, 3.81; N, 7.68; S, 8.80.

3-{2-[4-(4-Fluorophenyl)thiazole-2-ylamino]acetyl}chromen-2-one (8b)

Yield: 64%; Mp.: 125-130^{o}C; FTIR (KBr): 3441, 2923, 2853, 1701, 1625, 1538, 1488, 1384, 1261, 1095, 801, 730 cm^{-1}; ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) δ: 4.52 (s, 2H, CH_2), 6.98 (s, 1H, H of thiazole), 7.20 (d, J = 8.8 Hz, 2H, ArH), 7.45-7.62 (m, 4H, ArH), 7.90 (d, 2H, J = 8.8 Hz, ArH), 8.68 (s, 1H, H of pyran); ^{13}C NMR (DMSO-*d*$_6$, 100 MHz) δ: 51, 104, 116, 126, 127, 129, 131, 135, 147, 158, 162, 168, 198; Anal. calc. for $C_{20}H_{13}FN_2O_3S$: C, 63.15; H, 3.44; N, 7.36; S, 8.43; found C, 63.09; H, 3.40; N, 7.28; S, 8.39.

3-{2-[4-(4-Chlorophenyl)thiazole-2-ylamino]acetyl}chromen-2-one (8c)

Yield: 67%; Mp.: 182-186^{o}C; FTIR (KBr): 3438, 3283, 3111, 2962, 1708, 1633, 1535, 1477, 1401, 1261, 1088, 820, 730 cm^{-1}; ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) δ: 4.75 (s, 2H, CH_2), 6.73 (s, 1H, H of thiazole), 7.16-7.21 (m, 2H, ArH), 7.37 (d, 2H, J = 8.4 Hz, ArH), 7.67-7.70 (m, 2H, ArH), 8.17 (d, 2H, J = 8.4 Hz, ArH); 8.73 (s, 1H, H of pyran); ^{13}C NMR (DMSO-*d*$_6$, 100 MHz) δ: 48, 103, 114, 124, 128, 130, 133, 136, 145, 155, 161, 167, 195; Anal. calc. for $C_{20}H_{13}ClN_2O_3S$: C, 60.53; H, 3.30; N, 7.06; S, 8.08; found C, 60.49; H, 3.26; N, 6.98; S, 8.01.

3-{2-[4-(4-Bromophenyl)thiazol-2-ylamino]acetyl}chromen-2-one (8d)

Yield: 61%; Mp.: 210-215^{o}C; FTIR (KBr): 3425, 2926, 1720, 1611, 1532, 1458, 1342, 1225, 1075, 1045, 990, 895 cm^{-1}; ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) δ: 4.72 (s, 2H, CH_2), 7.11 (s, 1H, H of thiazole), 7.52-7.62 (m, 2H, ArH), 7.72 (d, 2H, J = 6.8 Hz, ArH), 7.78-7.90 (m, 2H, ArH), 8.07 (d, 2H, J = 8.4 Hz), 8.67 (s, 1H, H of pyran); ^{13}C NMR (DMSO-*d*$_6$, 100 MHz) δ: 50, 106, 112, 123, 129, 131, 133, 136, 149, 153, 160, 166, 192; Anal. calc. for $C_{20}H_{13}BrN_2O_3S$: C, 54.43; H, 2.97; N, 6.35; S, 7.27; found C, 54.40; H, 2.92; N, 6.31; S, 7.24.

3-{2-[4-(4-Methylphenyl)thiazol-2-ylamino]acetyl}chromen-2-one (8e)

Yield: 63%; Mp.: 156-160^{o}C; FTIR (KBr): 3450, 3295, 2921, 2853, 1715, 1633, 1530, 1487, 1359, 1331, 1222, 1033, 820, 728 cm^{-1}; ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) δ: 2.29 (s, 3H, CH_3), 4.40 (s, 2H, CH_2), 6.91 (s, 1H, thiazole), 7.04-7.17 (m, 2H, ArH), 7.23 (d, 2H, J = 8.0 Hz, ArH), 7.68 (d, 2H, J = 8.0 Hz, ArH), 7.78-7.88 (m, 2H, ArH), 8.52 (s, 1H, H of pyran); ^{13}C NMR (DMSO-*d*$_6$, 100 MHz) δ: 23, 54, 109, 114, 120, 128, 132, 135, 138, 147, 156, 162, 167, 194; Anal. calc. for $C_{21}H_{16}N_2O_3S$: C, 67.00; H, 4.28; N, 7.44; S, 8.52 found C, 66.96; H, 4.20; N, 7.42; S, 8.48.

3-{2-[4-(4-Methoxyphenyl)thiazol-2-ylamino]acetyl}chromen-2-one (8f)

Yield: 65%; Mp.: 224-228^{o}C; FTIR (KBr): 3411, 3120, 2940, 1712, 1698, 1594, 1528, 1486, 1284, 1263, 1188, 1045, 862, 785 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.87 (s, 3H, OCH_3), 4.67 (s, 2H, CH_2), 6.58 (s, 1H, H of thiazole), 6.92-6.98 (m, 4H, ArH), 7.34 (d, 2H, J = 8.4 Hz, ArH), 7.71 (d, 2H, J = 8.4 Hz, ArH), 8.23 (s, 1H, H of pyran); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 52, 55, 107, 115, 118, 125, 129, 134, 140, 148, 159, 164, 169, 196 ; Anal. calc. for $C_{21}H_{16}N_2O_4S$: C, 64.27; H, 4.11; N, 7.14; S, 8.17; found C, 64.23; H, 4.08; N, 7.10; S, 8.11.

3-{2-[4-(4-Nitrophenyl)thiazol-2-ylamino]acetyl}chromen-2-one (8g)

Yield: 54%; Mp.: 302-306^{o}C; FTIR (KBr): 3399, 3306, 3146, 1708, 1642, 1593, 1537, 1502, 1324, 1108, 1039, 853, 719 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.78 (s, 2H, CH_2), 7.22 (s, 1H, H of thiazole), 8.03-8.31 (m, 8H, ArH), 8.56 (s, 1H, H of pyran) ; ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 56, 105, 111, 120, 124, 130, 136, 142, 149, 158, 161, 170, 198; Anal. calc. for $C_{20}H_{13}N_3O_5S$: C, 58.96; H, 3.22; N, 10.31; S, 7.87 found C, 58.94; H, 3.19; N, 10.27; S, 7.83.

3-{2-[4-(4-Cyanophenyl)thiazol-2-ylamino]acetyl}chromen-2-one (8h)

Yield: 58%; Mp.: 286-290^{o}C; FTIR (KBr): 3375, 3114, 2227, 1716, 1642, 1603, 1540, 1453, 1341, 1230, 1173, 1042, 837, 752 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.54 (s, 2H, CH_2), 7.12 (s, 1H, H of thiazole), 7.54-7.56 (m, 2H, ArH), 7.63 (d, 2H, J = 8.4 Hz, ArH), 7.74-7.81 (m, 2H, ArH), 7.86 (d, 2H, J = 8.4 Hz, ArH), 8.54 (s, 1H, H of pyran); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 52, 102, 113, 117, 119, 125, 131, 138, 144, 151, 159, 163, 168, 199; Anal. calc. for $C_{21}H_{13}N_3O_3S$: C, 65.11; H, 3.38; N, 10.85; S, 8.28; found C, 65.07; H, 3.35; N, 10.83; S, 8.21.

3-{2-[4-(3-Bromophenyl)thiazol-2-ylamino]acetyl}chromen-2-one (8i)

Yield: 61%; Mp: 156-158^{o}C; FTIR (KBr): 3435, 2998, 2830, 1709, 1609, 1528, 1455, 1339, 1227, 1070, 1047, 897, 756 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.68 (s, 2H, CH_2), 7.19 (s, 1H, H of thiazole), 7.30-7.34 (m, 4H, ArH), 7.62-7.65 (m, 4H, ArH), 8.57 (s, 1H, H of pyran); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 51, 107, 112, 122, 129, 132, 135, 139, 148, 154, 159, 168, 193; Anal. calc. for $C_{20}H_{13}BrN_2O_3S$: C, 54.43; H, 2.97; N, 6.35; S, 7.27; found C, 54.38; H, 2.94; N, 6.32; S, 7.19.

3-{2-[4-(3-Nitrophenyl)thiazol-2-ylamino]acetyl}chromen-2-one (8j)

Yield: 51%; Mp.: 215-220^{o}C; FTIR (KBr): 3447, 3240, 3114, 1714, 1635, 1579, 1537, 1513, 1342, 1204, 1052, 869, 714 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.76 (s, 2H, CH_2), 7.24 (s, 1H, H of thiazole), 7.68-7.98 (m, 4H, ArH), 8.02-8.28 (m, 4H, ArH), 8.67 (s, 1H, H of pyran); ^{13}C NMR (DMSO-d_6, 100 MHz): 54, 106, 113, 120, 124, 131, 136, 143, 148, 157, 160, 171, 199; Anal. calc. for $C_{20}H_{13}N_3O_5S$: C, 58.96; H, 3.22; N, 10.31; S, 7.87; found C, 58.92; H, 3.18; N, 10.29; S, 7.82.

3-{2-[4-(3-Methoxyphenyl)thiazol-2-ylamino]acetyl}chromen-2-one (8k)

Yield: 66%; Mp.: 120-125^{o}C; FTIR (KBr): 3400, 2942, 1715, 1598, 1537, 1523, 1488, 1464, 1281, 1048, 863, 783 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.85 (s, 3H, OCH_3), 4.68 (s, 2H, CH_2), 6.78 (s, 1H, H of thiazole), 6.93-7.12 (m, 4H, ArH), 7.42-7.45 (m, 4H, ArH), 8.46 (s, 1H, H of pyran); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 53, 56, 109, 116, 120, 126, 130, 136, 141, 149, 161, 166, 170, 196; Anal. calc. for $C_{21}H_{16}N_2O_4S$: C, 64.27; H, 4.11; N, 7.14; S, 8.17; found C, 64.23; H, 4.07; N, 7.12; S, 8.12.

3-{2-[4-(3,4-Dichlorophenyl)thiazol-2-ylamino]acetyl}chromen-2-one (8l)

Yield: 63%; Mp.: 210-212^{o}C; FTIR (KBr): 3432, 3316, 3142, 2921, 1716, 1608, 1560, 1523, 1463, 1376, 1229, 1050, 893, 755, 722 cm^{-1} ; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.42 (s, 2H, CH_2), 7.20 (s, 1H, H of thiazole), 7.52-7.94 (m, 7H, ArH), 8.32 (s, 1H, H of Pyran); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 52, 58, 110, 114, 123, 128, 132, 138, 143, 151, 164, 168, 172, 194; Anal. calc. for $C_{20}H_{12}Cl_2N_2O_3S$: C, 55.70; H, 2.80; N, 6.50; S, 7.43 found C, 55.68; H, 2.77; N, 6.47; S, 7.39.

2.2.3 *In- silico* (Docking) studies

Geometries of the compounds **3**, **4, 7a-l** and **8a-l** were optimized at the level B3LYP/6-31G* using Gaussian 09 quantum chemistry software.[144] The global minima of the structures were verified using vibrational frequencies. Crystal structure of the protein AChE (PDB Id: 1EVE) was downloaded from protein data bank (PDB: www.rcsb.org). Though many structures of AChE are available, but the above protein structure from *Tetronarce californica* organism was

opted as assay used for *in vitro* experiment was also carried on enzyme from the same organism. Similarly for BuChE structure PDB Id (4TPK) was used.

Before docking the ligand molecules and enzymes were prepared by Glide 'ligprep' and 'Protein preparation' modules respectively. The ligand was refined in torsional space using the force field OPLS3 (Glide XP) with a distance-dependent dielectric model. Finally, a small number of poses are minimized within the field of the receptor with full ligand flexibility. The Glide module of Schrodinger uses high throughput virtual screening (HTVS), standard Precision (SP) and Xtra precision (XP) docking methodologies. As the last one provided more appropriate results, the current study provided XP docking score for all the ligands (Table 2.3).

2.2.4 *In-vitro* studies

Inhibition of acetylcholinesterase (AChE) and butrylcholinesterase (BuChE) activity assay

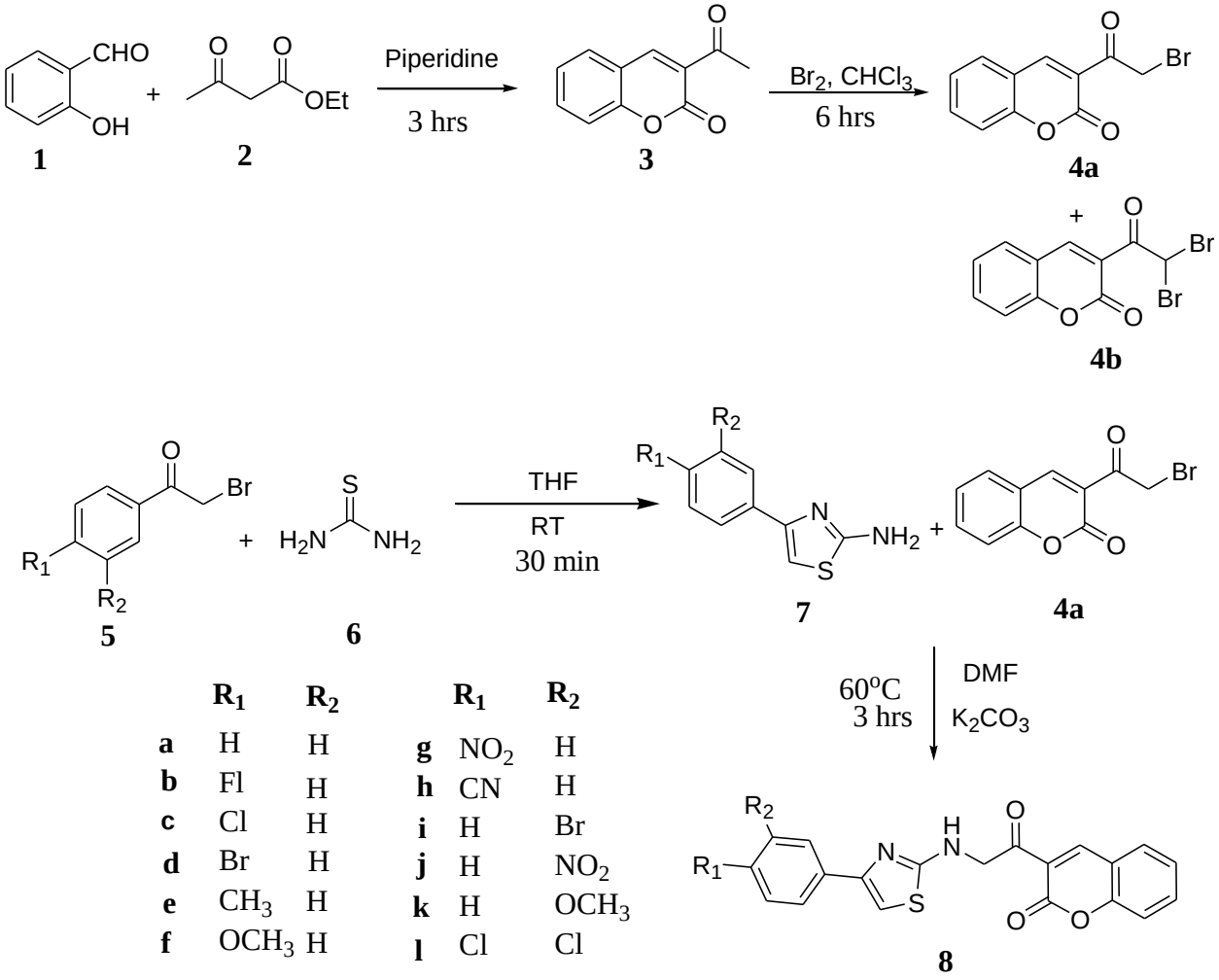
The synthesized molecules were tested for AChE and BuChE inhibitory activities according to the method described by Najafi et al, 2017.[145] Enzyme inhibition assay was performed in a 96-well plate by using Ellman's reagent 5,5'-dithio-bis-[2-nitrobenzoic acid] (DTNB) method. Briefly, 25 µL AChE/BuChE (25 mU in 100 µM PBS) was incubated with 75 µL DTNB (100 µM PBS containing 600 µM $NaHCO_3$) for 5 min at room temperature. To this, 25 µL of test compounds (1 – 1000 µM), and 50 µL PBS (pH 7.4) were added. The reaction mixture was then incubated for 15 min at room temperature. Reaction was initiated by adding 25 µL of acetylthiocholine iodide and butylthiocholine (75 mM) in phosphate-buffered saline (PBS) for AChE and BuChE inhibitory assay respectively. Change in absorbance was recorded spectrophotometrically during the experimental duration of 4 min at 412 nm by using UV-spectrophotometer. A blank reaction was run simultaneously, which was having 25 µL solvent (1% DMSO) in place of drugs. Percent inhibition of AChE activity was calculated by using following equation. Similar method was also used to determine the inhibition of BuChE activity.

$$\%\text{AChE/BuChE inhibition} = \frac{(Absorbance\ of\ control - Absorbance\ of\ test) \times 100}{Absorbance\ of\ control}$$

2.3 Results and discussion

2.3.1 Synthesis and characterization of 3-[2-(4-phenylthiazol-2-ylamino)acetyl]chromen-2-one derivatives (8a-l)

As shown in scheme 2.1, synthesis of 3-[2-(4-phenylthiazol-2-ylamino)acetyl]chromen-2-one derivatives (**8a-l**) was achieved in four steps. First step involved the synthesis of 3-acetyl-2H-chromen-2-one (**3**). In second step, compound **3** was brominated to produce the compound 3-(2-bromoacetyl)chromen-2-one (**4a**). The other compound, substituted 2-aminothiazoles (**7a-l**) were prepared in third step by using substituted phenacyl bromide with thiourea. In final step, compounds **7a-l** and **4a** were dissolved in DMF and heated at 60^{o}C for 3 hours in presence of K_2CO_3 to generate the final derivatives **8a-l**).



	R_1	R_2		R_1	R_2
a	H	H	**g**	NO_2	H
b	Fl	H	**h**	CN	H
c	Cl	H	**i**	H	Br
d	Br	H	**j**	H	NO_2
e	CH_3	H	**k**	H	OCH_3
f	OCH_3	H	**l**	Cl	Cl

Scheme 2.1: Synthesis of 3-[2-(4-phenylthiazol-2-ylamino)acetyl]chromen-2-one (**8a-l**)

Synthesis of 3-acetyl-2H-chromen-2-one (3)

Several methodologies have been reported for the synthesis of coumarin derivatives such as the Pechmann,[146] Perkin,[147] Knoevenagel,[148] Wittig,[149,150] and Reformastsky reactions.[151] Among these, the Knoevenagel and Pechmann reactions are the most widely used method, due to their cheap starting materials and good yield of coumarins.[152,153] We have synthesized 3-acetyl-2H-chromen-2-one (**3**) from salicylaldehyde (**1**) and ethyl acetoacetate (**2**) in the presence of piperidine, which undergo Knoevenagel condensation reaction to obtain the product with 94% yield. The product was recrystallized with absolute ethanol and were characterized by FTIR, ^{1}H NMR and ^{13}C NMR. The absorption at 3031, 1739 and 1675 cm^{-1} in the FTIR spectrum of **3** have been assigned to C-H stretching, C=O stretching of coumarin and C=O stretching of acetyl respectively. In the ^{1}H NMR spectrum, a singlet at 2.49 ppm for three CH_3 protons adjacent to carbonyl group and singlet at 8.64 ppm for one H proton of pyran ring has been observed. A multiplet in the region 7.40-7.96 ppm is due to four proton of aromatic ring (Figure 2.6). The appearance of peaks at 30, 158, 195 ppm for CH_3 and C=O in ^{13}C NMR spectrum further confirms the formation of product (Figure 2.7).

Synthesis of 3-(2-bromoacetyl)chromen-2-one (4a)

Synthesis of 3-(2-bromoacetyl)chromen-2-one (**4a**) was achieved by α-bromination of 3-acetyl-2H-chromen-2-one (**3**). We have tried several methodologies for the synthesis of **4a** as shown in table 2.1. The best result was obtained when the bromination was carried out using bromine with alcohol free chloroform. The other brominating reagents such as $CuBr_2$, Br_2 in glacial acetic acid and NBS were used in different solvents. The yield and reagent information have been summarized in table 2.1. Dibromination (**4b**) was observed with most of the other reagents leading to low yield of the monobromo compound **4a** (Table 2.1). The formation of dibromo derivative (**4b**) was confirmed by ^{1}H NMR spectrum (Figure 2.9). The appearance of a singlet at down field of 7.57 ppm due to presence of two bromine atom confirmed the dibromination. The monobrominated product **4a** was obtained with alcohol free chloroform and Br_2. The reaction mixture was stirred at room temperature after addition of all bromine for 6 hours. After the completion of reaction, the reaction mixture was heated for 20 min to expel the HBr from reaction mixture.

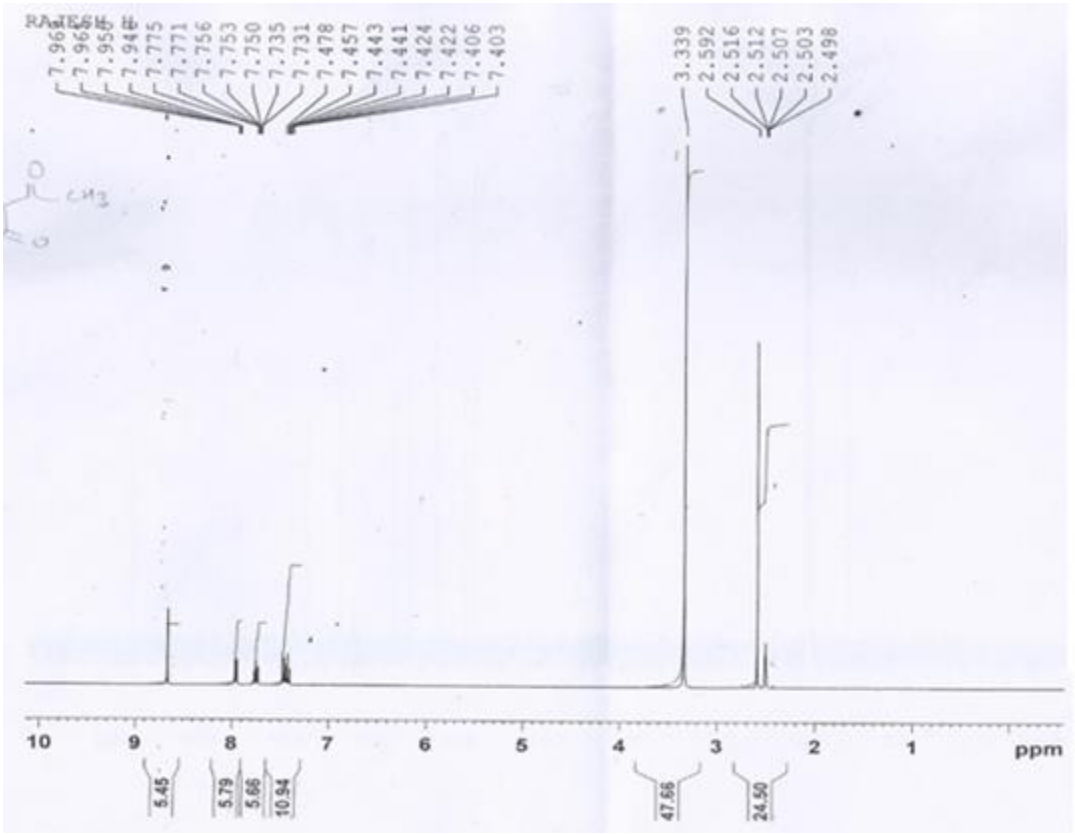


Figure 2.6: ^{1}H NMR spectrum of 3-acetyl-2H-chromen-2-one (**3**)

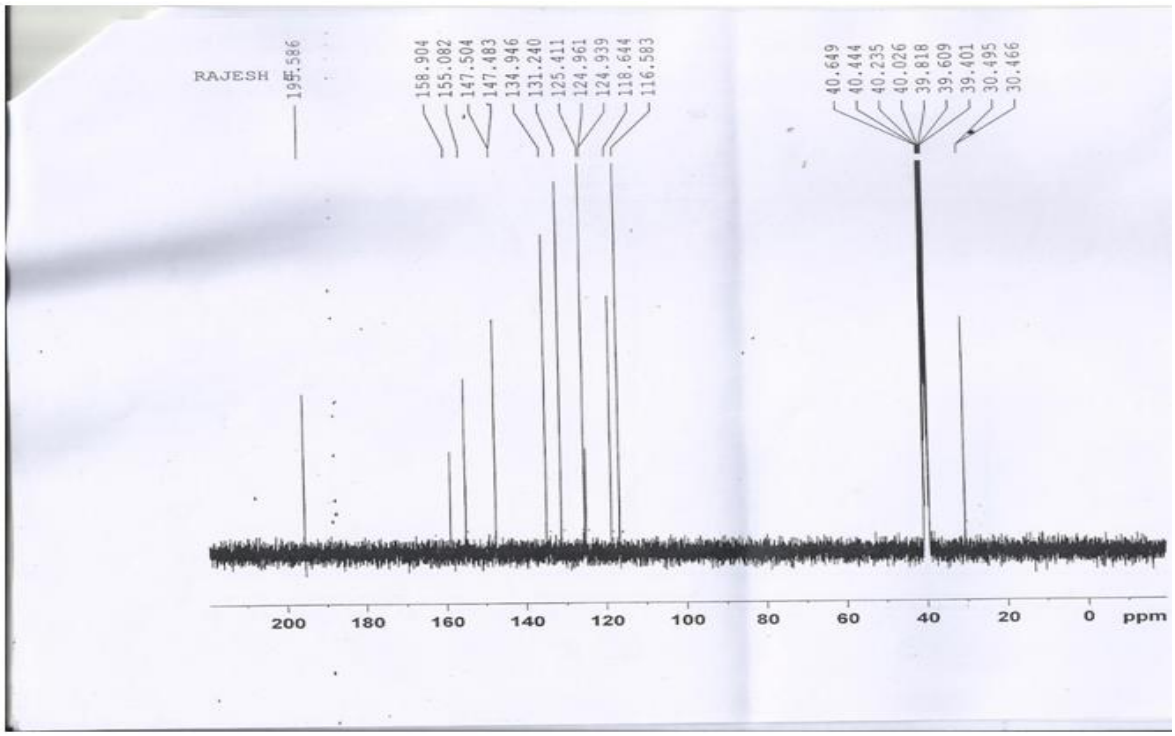


Figure 2.7: ^{13}C NMR spectrum of 3-acetyl-2H-chromen-2-one (**3**)

The HBr was further quenched by using saturated solution of sodium thiosulpahate. For this, the reaction mixture was washed (3×100 mL) with saturated solution of sodium thiosulphate. The organic layer was separated out and dried over anhydrous sodium sulphate, and then evaporated under reduced pressure. The solid was separated and then was purified by column chromatography and re-crystallized using glacial acetic acid to get colorless needle shaped crystal which was characterized by usual spectroscopic data. In the ^{1}H NMR spectrum, a singlet at 4.75 ppm for two CH_2 protons confirms the formation of (**4a**). The other peaks in the aromatic region are in agreement with the structure (Figure 2.8).

Synthesis of 2-aminophenylthiazole and its derivatives (7a-l)

Synthesis of 2-aminophenylthiazole derivatives (**7a-l)** was achieved by the reaction between phenacyl bromide (**5**) and thiourea (**6**) in THF. The literature revealed that a variety of solvents were used for the reaction of haloketones with thioamide.[139,154-157] The methods employed for the synthesis of phenylthiazole derivatives include the use of β-cyclodextrin,[157] ammonium-molybdophosphate (AMP),[158] iodine,[159] silica-chloride,[160] ionic liquids[161] and microwave irradiation.[162] However, in spite of their potential utility, many of these reported methods suffered from drawbacks such as harsh reaction conditions, long reaction times, unsatisfactory yields, tedious product isolation procedures and use of expensive catalysts. So development of an improved protocol is of considerable interest.

Table 2.1: Optimization of 3-(2-bromoacetyl)chromen-2-one (**4a**) yields

S. No.	Reagents and reaction conditions	%Yield	
		(**4a**)	(**4b**)
1	Glacial acetic acid, Br_2, 24 hr, rt	54	36
2	Chloroform-ehthlyacetate $CuBr_2$, 24 hr, 80^{o}C	42	40
3	Ethanol, NBS, 12 hr, rt	36	48
4	Chloroform, NBS, 12 hr, rt	36	46
5	Chloroform, Br_2, 6 hr, rt	85.5	12.5

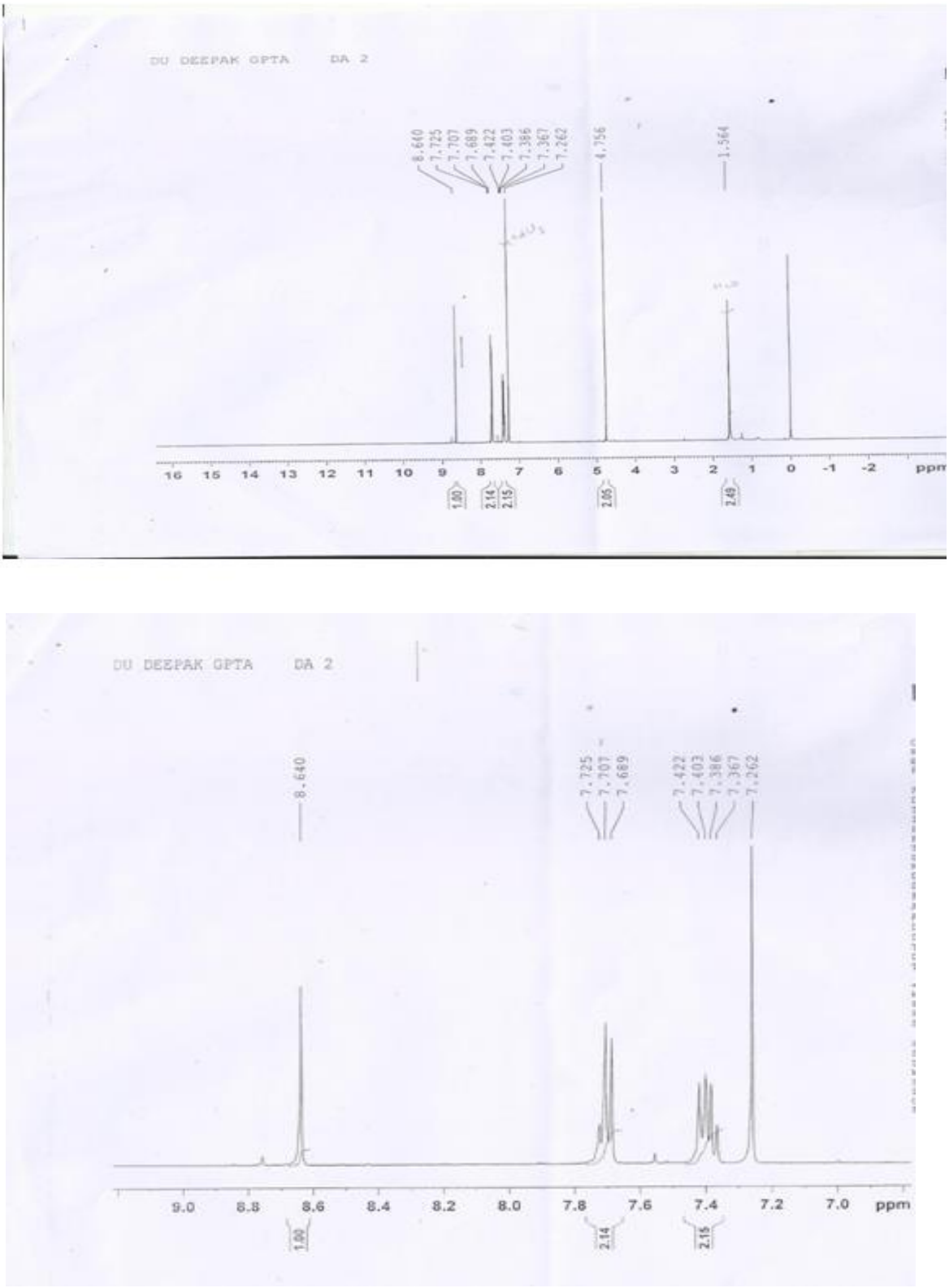


Figure 2.8: ^{1}H NMR spectrum of 3-(2-bromoacetyl)chromen-2-one (**4a**)

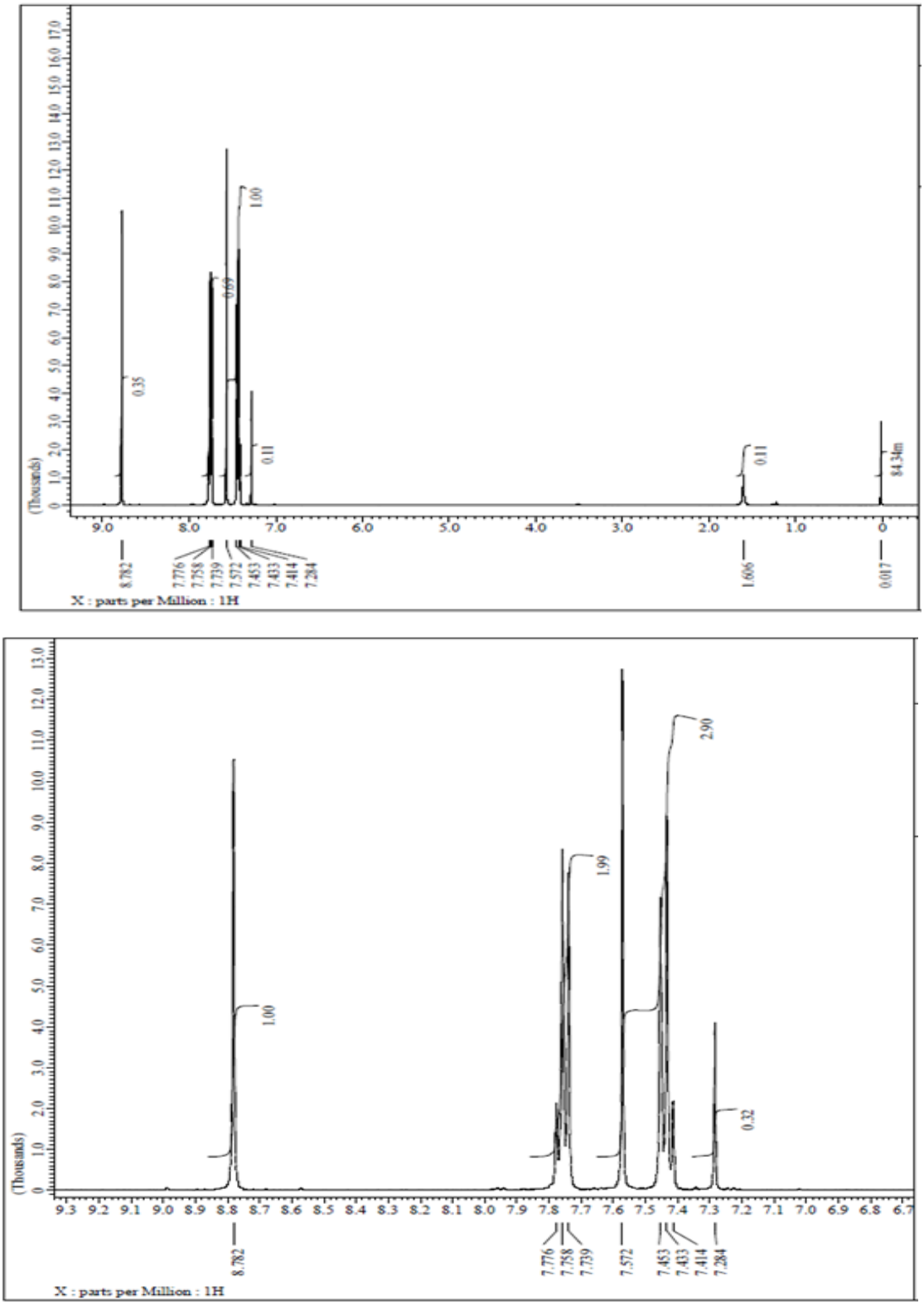


Figure 2.9: ^{1}H NMR spectrum of 3-(2,2-dibromoacetyl)chromen-2-one (**4b**)

As a part of our ongoing effort towards the synthesis of biologically active compounds,[163-165] We tried to develop a synthetic protocol which could give high yield with easy work up. We had tried the reaction in various solvent systems (Table 2.2). Tetrahydrofuran (THF) gave the maximum yield in minimum time with easy workup procedure. The phenacyl bromides carrying different functional groups were reacted with thiourea in THF at room temperature for 30 min to get 83-95% yield of 2-aminophenylthiazole derivatives (**7**). We had also explored the possibility of using other solvent systems as the reaction media but the yield of the products was not appreciable even after refluxing for about 3-6 hours (Table 2.2).

To assess the feasibility of the methodology on higher scale under identical reaction conditions, we carried out the reaction on a 20 g scale twice for compound **7a.** It was observed that the reaction proceeded smoothly and the desired product was isolated in 94% and 93% yields respectively. The structures of all of the compounds were identified by their spectral data. The absorption at 3435 cm^{-1} in the FTIR spectrum of **7a** has been assigned for NH_2 stretching. In the ^1H NMR spectrum, a broad singlet at 5.12 ppm is for 2H of NH_2, and singlet at 6.75 ppm is for one H proton of thiazole ring. The five protons of benzene ring lie in between 7.33-7.80 ppm (Figure 2.10, 2.11). Above data further confirmed the formation of **7a**.

Table 2.2: Reaction of **5** and **6** in different reaction conditions

S. No	Reagents/Reaction condition	%Yield of **7a**
1	H_2O:DMF, 4 h (Reflux)	60
2	Benzene, 6 h (Reflux)	62
3	Dioxane, 3 h (Reflux)	58
4	H_2O:Dioxane, 3 h (Reflux)	63
5	H_2O:Toluene, 4 h (Reflux)	65
6	Tetrahydrofuran (THF), 30 min (Room Temp.)	95

Synthesis of 3-[2-(4-phenylthiazol-2-ylamino)acetyl]chromen-2-one derivatives (8a-l)

The synthesis of twelve novel coumarin-phenylthiazole conjugates (**8a-l**) was achieved (Scheme 2.1). For the synthesis of **8a**, the compound **7a** and **4a** were dissolve in DMF in

presence of K_2CO_3 and heated at 60°C with stirring for 3 hours. The formation of product takes place *via* nucleophilic attack of NH_2 to **4a**. The formed product was characterized by spectroscopic techniques and elemental analysis. In the FTIR spectrum of **8a** the absorption peak at 3436, 1705 and 1604 cm^{-1} in the FTIR spectrum of **8a** have been assigned for NH and C=O stretching respectively (Figure 2.12). In the ^1H NMR spectrum of **8a**, singlet at 4.21 ppm is for two CH_2 protons adjacent to carbonyl group. The singlet at 7.07 ppm is for one H of thiazole ring, the multiplet of eight protons of benzene ring appears at 7.11-7.91 ppm. A singlet at 8.53 ppm is for one H of pyran ring (Figure 2.13). The peaks at 51, 159, 168, 192 ppm for CH_2, aromatic carbon and carbonyl carbon respectively, in ^{13}C NMR spectrum (Figure 2.14) further confirmed the formation of product **8a**. Similarly, the other compounds (**8b-l**) were synthesized and characterized.

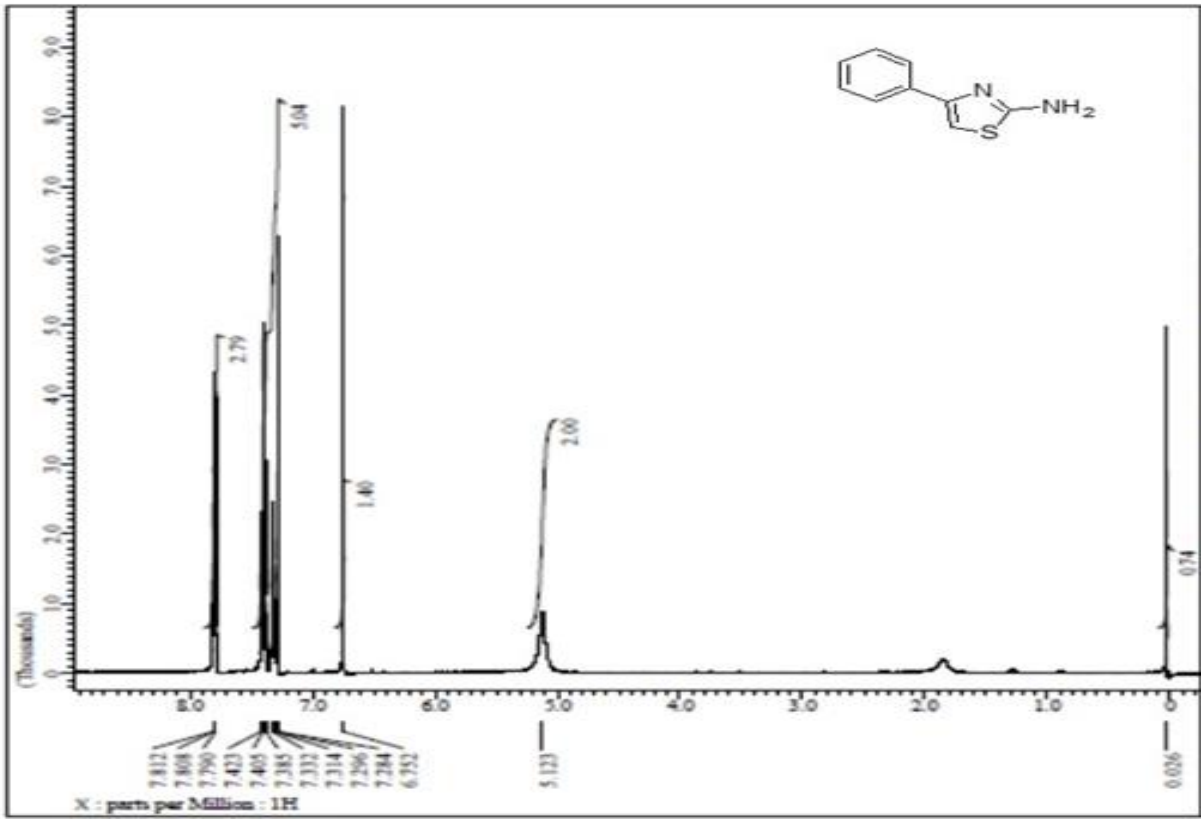


Figure 2.10: ^1H NMR spectrum of 4-phenyl-1,3-thiazol-2-amine (**7a**)

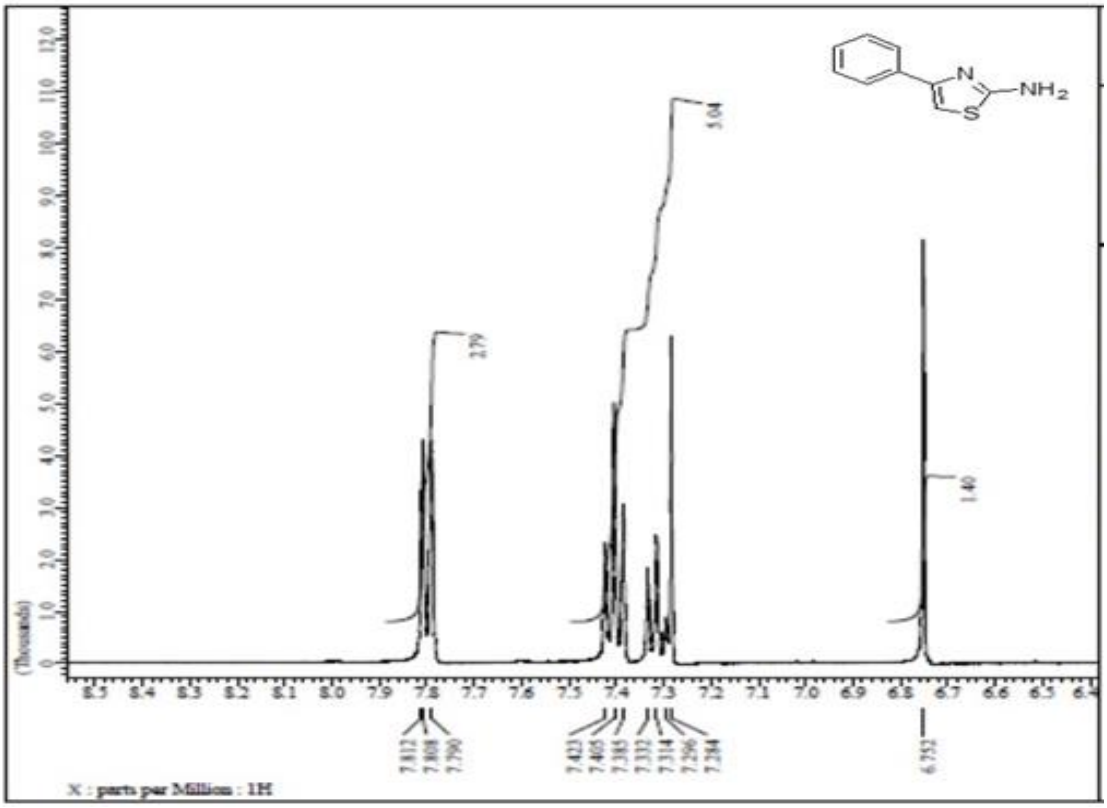


Figure 2.11: Expansion ^{1}H NMR spectrum of 4-phenyl-1,3-thiazol-2-amine (**7a**)

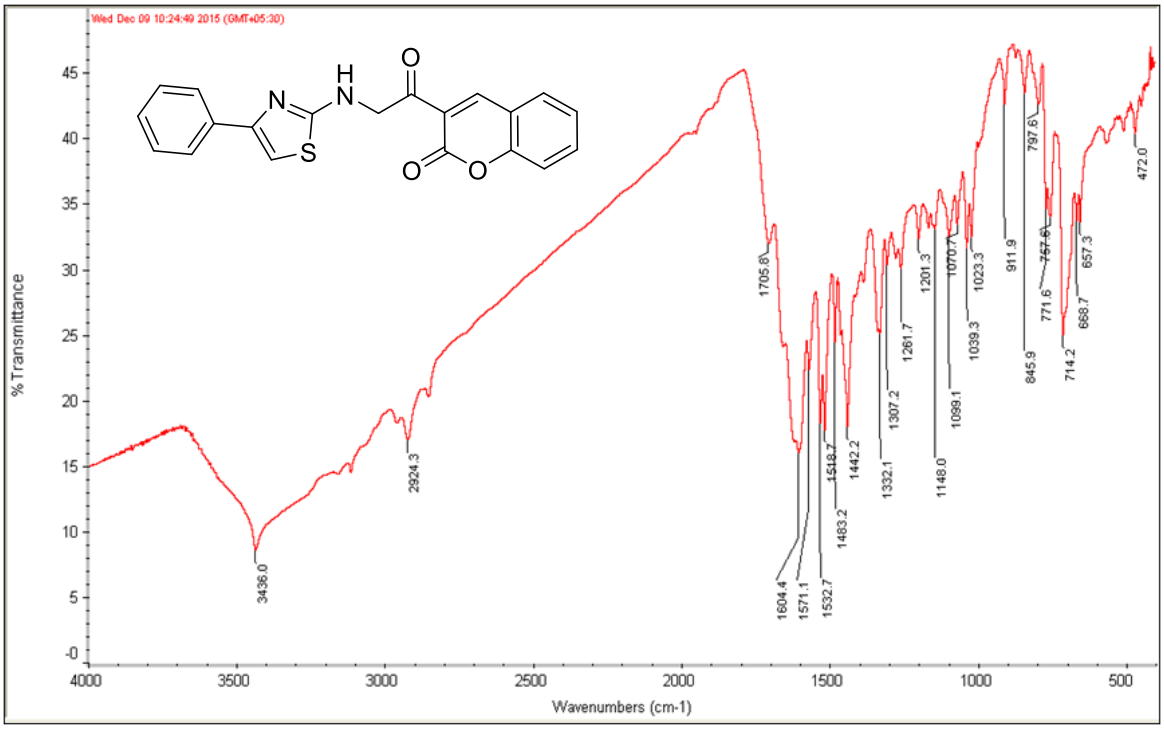


Figure 2.12: FTIR spectrum of 3-[2-(4-phenylthiazol-2-ylamino)acetyl]chromen-2-one (**8a**)

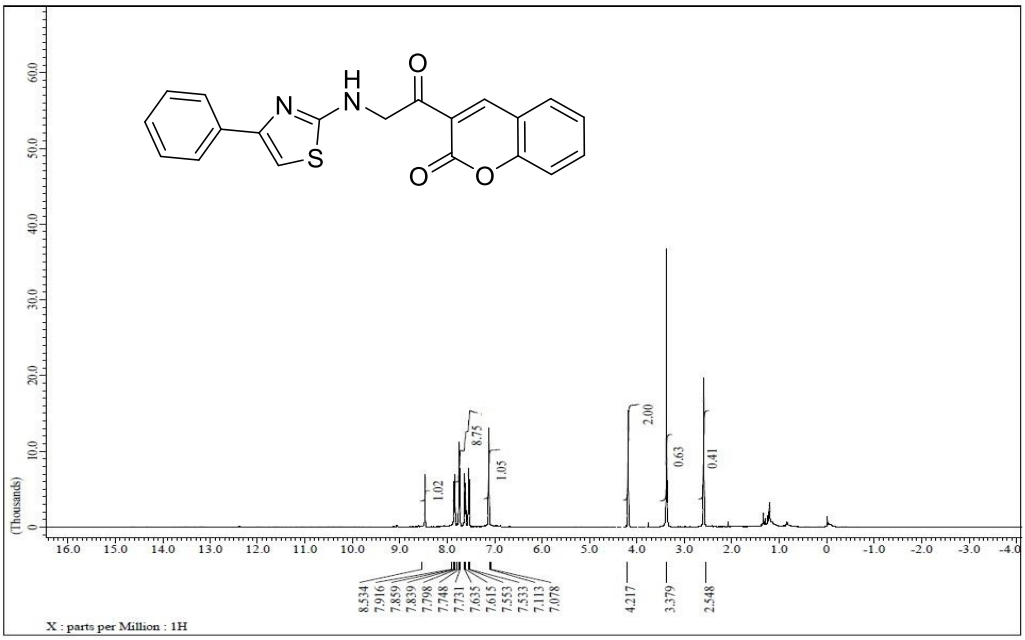


Figure 2.13: [1]H NMR spectrum of 3-[2-(4-phenylthiazol-2-ylamino)acetyl]chromen-2-one (**8a**)

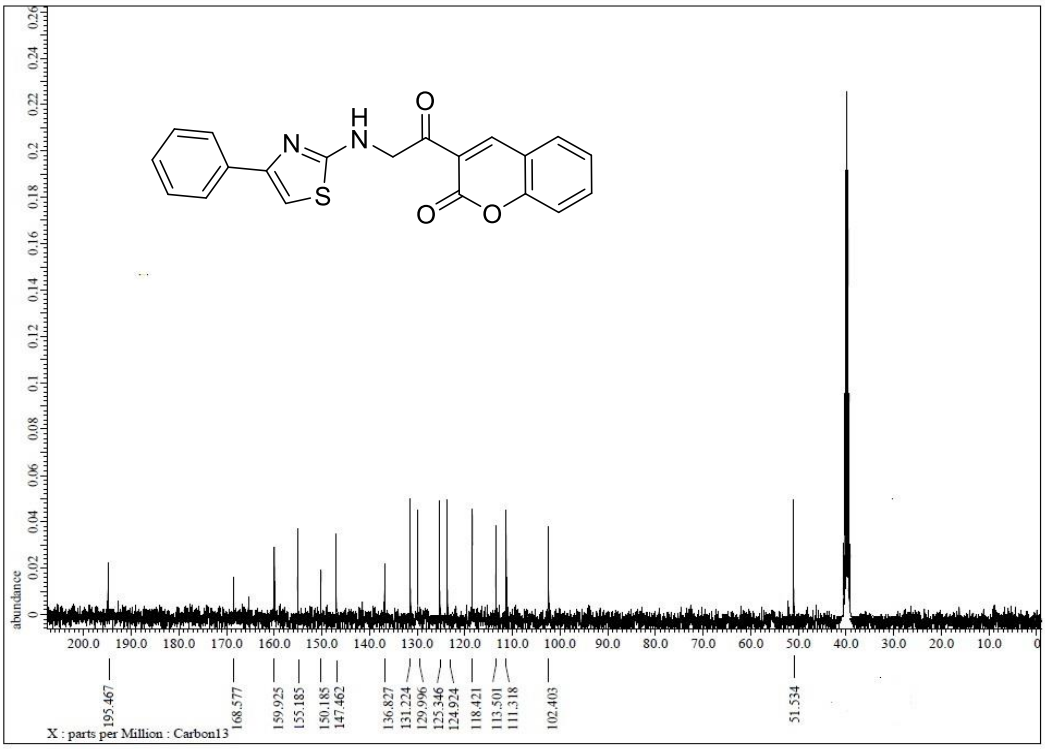


Figure 2.14: [13]C NMR spectrum of 3-[2-(4-phenylthiazol-2-ylamino)acetyl]chromen-2-one (**8a**)

2.3.2 *In-silico* interaction analysis

Potential binding affinity of the novel synthesized compounds **(3, 4, 7a-l** and **8a-l)** with AChE and BuChE enzymes have been studied by performing docking studies. In spite of diverse series of compounds, the interactions of these molecules are quite high for almost all molecules. This may be an indication of inaccurate score calculation. *In-vitro* results have also indicated that none of the molecule is active against AChE (Table 2.3). The compound series shows less interaction with AChE. However, a range of docking scores indicating favorable to unfavorable interactions are obtained for docking of diverse compounds against BuChE. Further, the docking results of BuChE are correlating well with the *in-vitro* experimental studies. Analyses of the docked structures revealed that in the active site of BuChE, His 438 and Phe 329 makes π- π interaction with the thiazole moiety of **8j** whereas Trp 231 and Phe 329 makes π- π interaction with 3-nitro phenyl ring of **8j** (Scheme 2.1 and Figure 2.15). Ser 198 makes a hydrogen bond with the N atom of thiazole ring and Tyr 128 makes a strong H bond with the lactone of coumarin. In many compounds, we also observed hydrophobic and aromatic interactions among the compounds and enzyme indicating compounds good binding affinity with the BuChE.

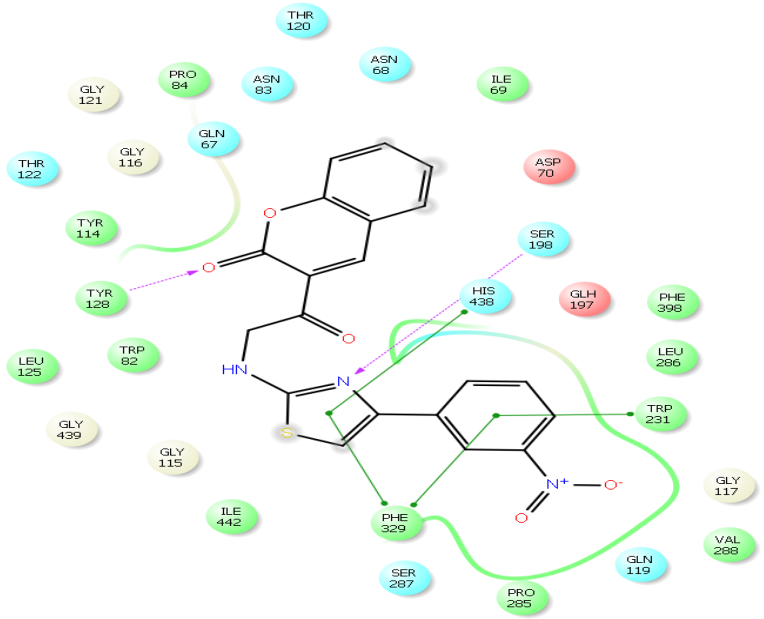


Figure 2.15: Interaction of 3-{2-[4-(3-Nitrophenyl)thiazol-2-ylamino]acetyl}chromen-2-one (**8j**) with active site of BuChE

2.3.3 *In-vitro* inhibition studies of AChE and BuChE

The inhibition activity of 3-[2-(4-phenylthiazol-2-ylamino)acetyl]chromen-2-one against AChE & BuChE are given in table 2.3. Based on the IC_{50} value, the synthesized compounds (**3**, **4**, **7a-7l** and **8a-8l**) showed low to poor activity towards AChE, but a remarkably high activity towards BuChE. The best results are being displayed by **8j**, **8i** and **8b** with IC_{50} value of 46.47, 61.64 and 76.41 μM respectively. These synthesized compounds are composed of two fragments bromocoumarine and substituted phenylthiazole joined *via* carboxamide linkers. The IC_{50} values of bromocoumarine phenylthiazole are found to be 346.68 and 385.69 μM, respectively. On coupling both the moieties, the activity increases apparently. The data reveals that 3-NO_2, 3-bromo and 4-fluoro substitution on phenyl thiazole have increased the anti-BuChE activity remarkably. No substitution or substitution of CH_3 or Br at 4^{th} position or dichloro at 3 & 4^{th} positions of phenylthiazole show moderate anti-BuChE activity. On the other hand 4-Cl, 4-NO_2, 3-OCH_3, 4-CN and 4-OCH_3 substitution on phenylthiazole reduces the inhibitory activity against BuChE. On comparing **8j** and **8g**, 3-NO_2 is found to be more active than 4-NO_2. Between the compounds 8i (3-Br) and 8d (4-Br), the 3-Br derivative is found to be more active. It is useful to note that the substitution at 3-position, (with exception of **8b**) on phenylthiazole results in higher inhibitory activity against BuChE than 4-substituted counterparts.

Table 2.3: IC_{50} value & docking score of compounds **3**, **4**, **7a-l** & **8a-l** against AChE & BuChE

	IC_{50} (µM)		SI	Docking Score	
	AChE	**BuChE**		**AChE**	**BuChE**
3	906.31 ± 49.13	1151.1 ± 133.90	0.78	Inactive	Inactive
4	846.33 ± 25.29	346.68 ± 13.15	2.44	-7.739	-5.856
7a	975.48 ± 29.18	385.69 ± 24.41	2.53	-5.989	-5.624
7b	3112.81 ± 953.42	453.61 ± 71.11	6.86	-5.989	-5.624
7c	829.12 ± 38.17	1089.97 ± 89.12	0.76	-5.916	-5.529
7d	4824.25 ± 989.91	893.92 ± 128.12	5.39	-5.916	-5.765
7e	998.97 ± 40.31	585.43 ± 52.83	1.71	-5.878	-5.840
7f	3514.13 ± 856.19	1730.01 ± 118.59	2.03	-5.539	-5.144
7g	978.85 ± 14.83	1345.52 ± 150.87	0.73	-5.680	-5.044
7h	1859.65 ± 237.09	2280.80 ± 91.71	0.81	-6.287	-6.342
7i	4024.77 ± 728.16	3.54 ± 1.64	1136.00	-6.364	-5.828
7j	3223.9 ± 236.21	23.24 ± 6.00	138.71	-5.510	-5.230
7k	1681.54 ± 68.37	912.14 ± 162.74	1.84	-7.739	-5.856
7l	278.44 ± 83.34	405.56 ± 45.72	0.69	-6.128	-5.937
8a	414.27 ± 21.57	106.25 ± 9.29	3.89	-8.830	-9.077
8b	2008.17 ± 357.47	76.41 ± 4.60	26.28	-9.262	-8.620
8c	1354.68 ± 135.84	713.61 ± 66.48	1.89	-10.97	-7.878
8d	423.78 ± 30.84	321.49 ± 57.75	1.32	-9.642	-6.783
8e	427.83 ± 14.83	240.69 ± 39.62	1.78	-8.804	-7.014
8f	1016.83 ± 56.50	500.8 ± 59.22	2.03	-7.261	-8.732
8g	1767.56 ± 167.81	777.81 ± 49.08	2.27	-7.620	-7.595
8h	1659.79 ± 449.63	476.89 ± 54.96	3.48	-7.964	-8.490
8i	**5231.54 ± 1160.8**	**61.64 ± 1.67**	**84.87**	**-9.165**	**-7.990**
8j	**1642.76 ± 136.93**	**46.47 ± 0.37**	**35.35**	**-6.961**	**-8.893**
8k	1379.91 ± 337.62	553.59 ± 194.15	2.49	-8.151	-9.692
8l	1839.56 ± 209.44	107.32 ± 19.73	17.14	-9.620	-8.570
Donepezil	0.042 ± 0.010	4.66 ± 0.503	155.30	-5.57	-6.92

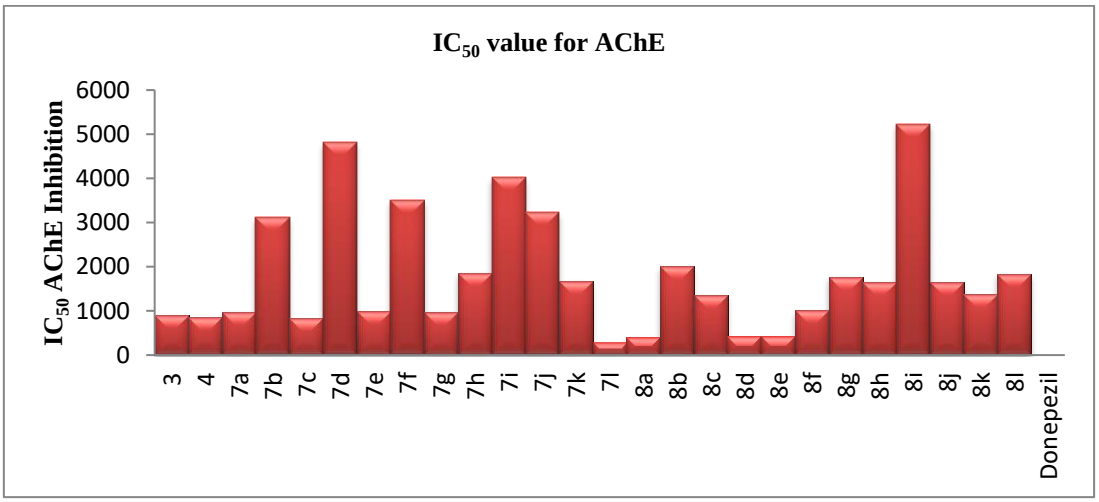


Figure 2.16: IC_{50} value in μM concentration for AChE enzyme (**3, 4, 7a-l** and **8a-l**).

IC_{50} value less than 100 μM concentration is considered significant inhibition; therefore, most of the compounds are inactive against AChE enzyme.

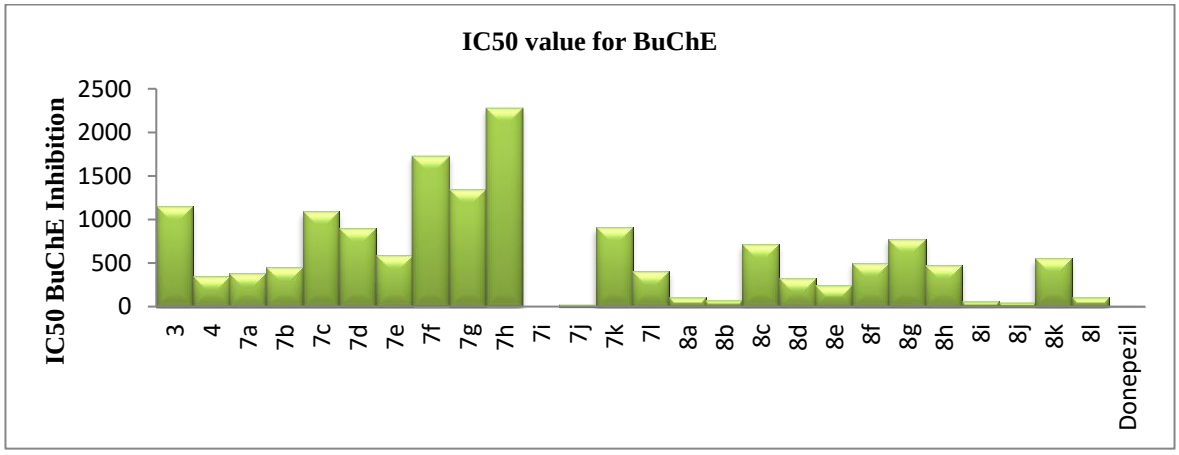


Figure 2.17: IC_{50} value in μM concentration for BuChE enzyme (**3, 4, 7a-l** & **8a-l**, Scheme 2.1). IC_{50} value less than 100 μM concentration is considered significant inhibition against BuChE enzyme.

CHAPTER 3

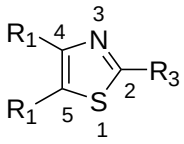
Design, Synthesis and Evaluation of N-Benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)acetamide Derivatives as Cholinesterase Inhibitors

3.1 Introduction

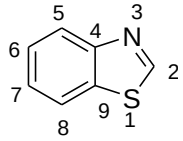
Thiazole is a five-membered heterocyclic molecule possessing two hetero atoms: nitrogen (N) and sulphur (S) with molecular formula, C_3H_3NS (Figure 3.1).[166] Thiazole was first described by A. Hantzsch and J. H. Waber in 1887.[167] This molecule possesses both an electron accepting (C=N) and electron donating group (-S-). The lone pair of electrons present on S atom makes the molecule 6π electron system that fulfills the criteria for aromaticity. The numbering in thiazole starts from the sulphur atom (Figure 3.1). When the thiazole ring fused at the 4, 5 positions with 6-membered benzene ring, the resulting molecules are known as benzothiazoles which are useful bicyclic heterocyclic molecules (Figure 3.1).[168]

Thiazole and its derivatives is the important scaffold in the field of medicinal chemistry and display a wide range of biological activities. This ring is present in many natural and synthetic products with a broad range of biological applications such as antioxidant, antibacterial, antifungal, anti-tubercular, diurectic, anti-inflammatory and anti-cancerous.[104] Vitamin B_1 (thiamine) contains thiazole moiety which helps in the normal functioning of the nervous system by its role in the synthesis of acetylchloline.[104] Bacitracin and penicillin antibiotics also contains this moiety in their structures.[169] Some of the synthetic drugs belonging to this family includes acinitrazole and sulfathiazole[170] (antimicrobial agents), pramipexole[171] (antidepressant), Bleomycin and Tiazofurin[172] (antineoplastic agents), Ritonavir[173] (anti-HIV drug), cinalukast[174] (antiasthmatic drug) and Nizatidine (antiulcer agent) (Figure 3.2).[175] Additionally, extensively thiazole derivatives have been successfully used in the past as potential neuroprotective agents.[176] Tetrahydrobenzothiazoles,[177] phenolic thiazoles[178] and benzothiazoles[179] are well known for their neuroprotective nature. Benzothiazole derivatives developed by Hofmann Le Roche is a potent adenosine receptor ($A_{2A}R$) antagonist and have been used for the treatment of Parkinson disease.[180] The other therapeutic applications of benzothiazoles derivatives includes neurodegenerative disorder treatment, local brain ischemia, central muscle relaxants and cancer.[181] Literature shows that thiazole–triazole linked derivative have shown potent anti-alzheimeric activity.[182] Siddiqui et al in 2009[168] and Mishra et al in 2015[183] reviewed the diverse biological activities of thiazoles towards CNS activities like dopamine receptor ligands, nNOS inhibitors, adenosine receptor ligands, GABA receptor

ligands, glutamate receptor ligands, 5-HT receptor ligands, cannabinoid receptor ligands, opioid receptor ligands, acetylcholine receptor ligands, neuroprotective and anticonvulsant agents.

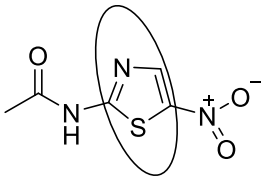


Thiazole

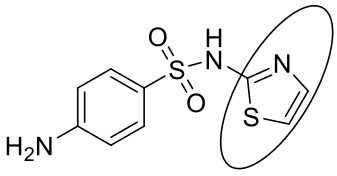


Benzothiazole

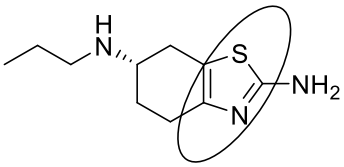
Figure 3.1: Structures of thiazole and benzothiazole



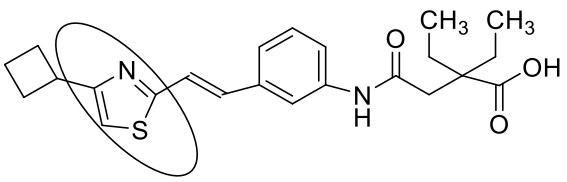
Acinitrazole



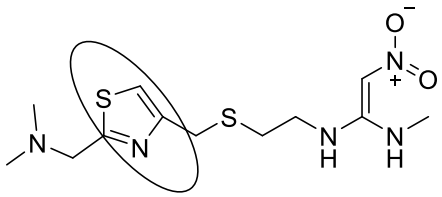
Sulfathiazole



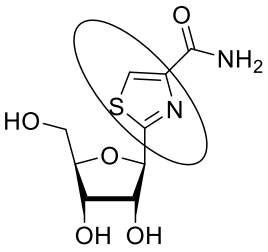
Pramipexole



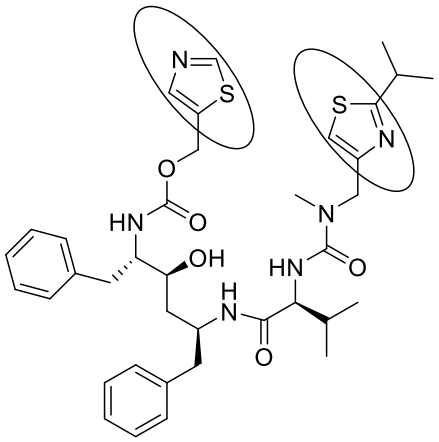
Cinalukast



Nizatidine



Tiazofurin



Ritonavir

Figure 3.2: Thiazole moiety containing drugs

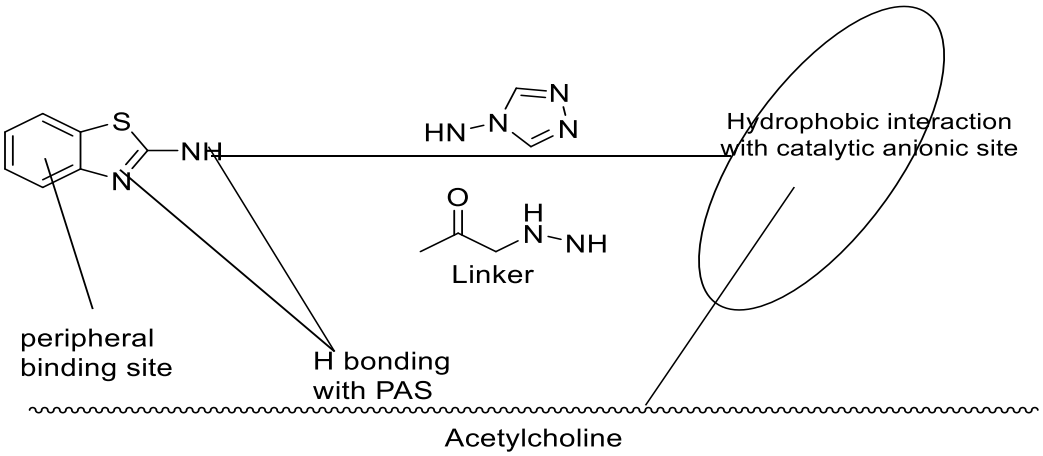
In the 1950s, a number of 2-aminobenzothiazoles were intensively studied. The 2-aminobenzothiazole scaffold is one of the privileged structure in medicinal chemistry and reported cytotoxic on cancer cells.[184] Several literatures highlighted that the combination of 2-aminobenzothiazole with other heterocyclic molecule lead to new drug molecule which allow to achieve new pharmacological action profile towards target with lower toxicity.

The benzothiazoles derivatives are used in neurodegenerative disorder treatment, local brain ischemia, central muscle relaxants and cancer.[181] Literature also reveals that thiazole–triazole linked derivatives have shown potent anti-alzheimeric activity, Also, Vitamin B_1 (thiamine) contains thiazole moiety that helps in the normal functioning of the nervous system by synthesizing acetylchloline. So, this chapter deals with the design, synthesis and evaluation of benzothizole-triazole conjugates as anti-alzheimeric agents (Figure 3.3). The preliminary docking studies of fragments had indicated that benzothiazole and triazole interacted favourably with the active site of AChE and BuChE. Therefore, the compounds with these moieties were designed, synthesized and evaluated.

3.2 Experimental

3.2.1 Preliminary *in silico* interaction studies of benzothiazole and triazole moieties with AChE and BuChE

Preliminary docking studies of the benzothiazole and triazole moieties with cholinesterase enzymes such as AChE (1EVE) and BuChE (4TPK) was performed using Glide module of Schrodinger Suite (Small-Molecule Drug Discovery Suite 2017-3, Schrödinger, LLC, New York, NY, 2017). Favourable interactions of these fragments with these enzymes (AChE and BuChE) were identified. These fragments were interacted in different parts of the active site. On the basis of these results, we designed and synthesized N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)acetamide derivatives. Further, the synthesized novel compounds were validated by doing *in silico* and *in vitro* experimental studies.



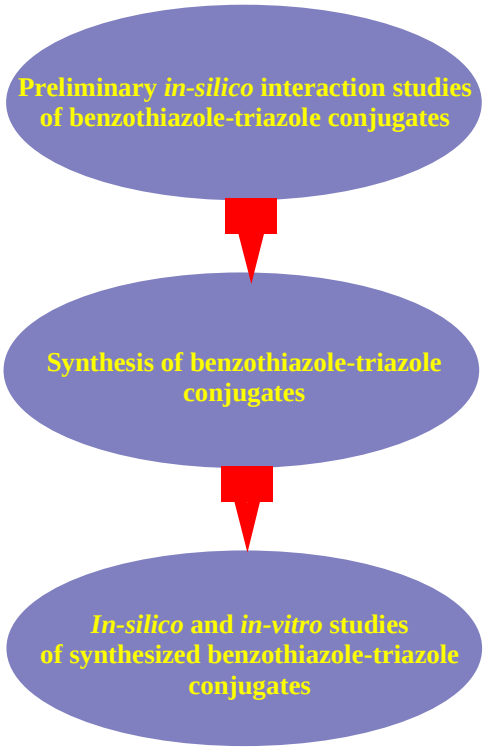


Figure 3.3: Flow chart diagram showing the steps in design, synthesis and evaluation of benzothiazole-triazole conjugates as anti-Alzheimeric agents

3.2.2 Synthesis of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino) acetamide derivatives (16a-i)

All commercially available solvents and reagents were purchased from reputed company and were used without further purification. Melting points were determined on a laboratory capillary

melting apparatus and are uncorrected. FTIR spectra were recorded on a Perkin Elmer Spectrum Version 10.5.3 FTIR spectrophotometer. The ν_{max} are expressed in cm^{-1}, and the chemical shifts are expressed in ppm. ^{1}H and ^{13}C NMR were recorded on a Bruker spectrophotometer and Jeol spectrophotometer (400/100MHz) using TMS as internal standard. The abbreviations s, d, t, q, m and bs stand for singlet, doublet, triplet, quartet, multiplet and broad singlet respectively. The elemental analysis was measured by PerkinElmer 2400. Thin-layer chromatography was performed on aluminium-coated silica plates purchased from Merck.

Synthesis of compounds **16a-i** has been achieved by following three schemes 3.1-3.3. Scheme 3.1 deals with the synthesis of 2-aminobenzothiazole derivatives whereas scheme 3.2 shows the synthetic pathway of substituted triazoles. The covalent linking of triazole and benzothiazole is given in scheme 3.3.

Synthesis of 2-aminobenzothiazole (10)

A solution of aniline (**9**, 4.5 mL, 50 mmol) in glacial acetic acid (50 mL) was taken in a round bottom flask (250 mL), and then potassium thiocynate (KSCN) (4.8 g, 50 mmol) was added to the solution. The reaction mixture was kept at freezing mixture of ice and salt and was mechanically stirred till dissolution. Then Br_2 (2.5 mL in 4 mL glacial acetic acid, 50 mmol) was added from the dropping funnel at such rate the temperature does not raise beyond 5°C. After all bromine was added, the solution was stirred for 4 hours at room temperature. The progress of reaction was monitored by thin layer chromatography (TLC) (hexane: ethyl acetate, 7:3, v/v). After completion of reaction, the resulting crude solid product was filtered and washed with glacial acetic acid. The solid was dried and dissolved in hot water, and neutralized with aqueous ammonia solution (25%). The resulting precipitate was dried and purified by recrystallisation with ethanol to get a white solid as pure product.

Yield: 78%; Mp.: 126-128°C (Lit. Mp.[185] 129°C); FTIR (KBr): 3395, 3269, 3055, 2727, 1917, 1628, 1522, 1338, 1104, 887, 739 685 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.00 (2H, s, NH), 7.20-7.65 (4H, m, ArH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 118, 121, 125, 131, 153, 166, 169.

Synthesis of N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (11)

A solution of 2-aminobenzothiazole (**10**, 5 g, 33.3 mmol) in 15 mL of tetrahydrofuran (THF) was taken in a round bottom flask (100 mL). Further, 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) (1 mL, 6.6 mmol) was added to the solution. The reaction mixture was kept at 0°C. A

dropping funnel was fitted to the flask, and a solution of chloro acetylchloride (3.2 mL, 40 mmol, in 2 mL THF) was taken in a dropping funnel and added drop wise to the reaction mixture. The reaction mixture was allowed to come at room temperature and stirred for 6 hours. The progress of the reaction was monitored by TLC (hexane: ethyl acetate, 7:3, v/v). After completion of the reaction, crude solid product was obtained which was filtered and washed with water. The formed product was further re-crystallized using absolute ethanol.

Yield: 83%; Mp.: 142-145^{o}C (Lit. Mp.[186] 145^{o}C); FTIR (KBr): 3372, 3255, 3164, 2986, 2852, 2735, 1692, 1596, 1442, 1396, 1269, 1176, 1015, 983, 865, 772, 677 cm^{-1}; ^{1}H NMR (DMSO-$d_{6,}$ 400 MHz) δ: 4.47 (2H, s, CH_2), 7.32-8.01 (4H, m, ArH), 12.75 (1H, bs, NH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 43, 121, 124, 126, 131, 148, 158, 166; Anal. calc. for $C_9H_7N_2OSCl$: C, 47.69; H, 3.11; N, 12.36; S, 14.15; found C, 47.62; H, 3.08; N, 12.29; S, 14.09.

This reaction was performed with different aryl amines to check the versatility of the method (Table 3.3)

2-Chloro-N-(6-chlorobenzothiazole-2-yl)acetamide (11a)

YIeid: 76%; Mp.: 210-213^{o}C; FTIR (KBr): 3248, 2945, 2743, 1692, 1645, 1595, 1554, 1378, 1275,781 cm^{-1}; ^{1}H NMR (DMSO-$d_{6,}$ 400 MHz) δ: 4.46 (2H, s, CH_2), 7.35-8.05 (3H, m, ArH), 12.78 (1H, bs, NH); Anal. calc. for $C_9H_6N_2OSCl_2$: C, 41.40; H, 2.32; N, 10.73; S, 12.28; found C, 41.33; H, 2.29; N, 10.68; S, 12.23.

2-Chloro-N-(4-phenylthiazol-2-yl)acetamide (11b)

Yield: 86%; Mp.: 180-181^{o}C; FTIR (KBr): 3354, 2967, 2765, 1678, 1572, 1442, 1327, 1264, 1140, 1025, 849, 722, 686, 575 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.23 (2H s, CH_2), 6.75(1H, s, CH of thiazole), 7.28-7.81 (5H, m, ArH), 10.20 (1H, bs NH); Anal. calc. for $C_{11}H_9N_2OSCl$: C, 52.28; H, 3.59; N, 11.08; S, 12.69; found C, 52.22; H, 3.54; N, 11.02; S, 12.60.

2-Chloro-N-[4-(4-fluorophenyl)thiazol-2-yl]acetamide (11c)

Yield: 85%; Mp.: 134-136^{o}C; FTIR (KBr): 3362, 2998, 2742, 1680, 1576, 1488, 1266, 1161, 1067, 831, 707, 519 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.25 (2H, s, CH_2), 6.67 (1H, s, CH of thiazole), 7.09 (2H, d, J = 8.8 Hz, ArH), 7.76 (2H, d, J = 8.8 Hz, ArH), 9.34 (1H, bs, NH); Anal.

calc. for $C_{11}H_8N_2OSFCl$: C, 48.80; H, 2.98; N, 10.35; S, 11.84; found C, 48.76; H, 2.91; N, 10.32; S, 11.80.

2-Chloro-N-[4-(4-chlorophenyl)thiazol-2-yl]acetamide (11d)

Yield: 85%; Mp.: 188-191^oC; FTIR (KBr) : 3373, 2985, 2864, 1692, 1543, 1478, 1312, 1291, 1177, 1084, 1012, 841, 761, 670, 593 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 4.27 (2H, s, CH_2), 6.73 (1H, s, CH of thiazole), 7.36 (2H, d, J = 8.4 Hz, ArH), 7.73 (2H, d, J = 8.4 Hz, ArH), 9.75 (1H, bs, NH); Anal. calc. for $C_{11}H_8N_2OSCl_2$: C, 46.01; H, 2.81; N, 9.76; S, 11.17; found C, 45.97; H, 2.80; N, 9.72; S, 11.09.

2-Chloro-N-[4-(4-bromophenyl)thiazol-2-yl]acetamide (11e)

Yield: 86%; Mp.: 206-208^oC; FTIR (KBr): 3472, 2973, 2885, 1678, 1586, 1515, 1465, 1326, 1268, 1072, 843, 712, 652, 521 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 4.12 (2H, s, CH_2), 6.69 (1H, s, CH of thiazole), 7.20 (2H, d, J = 7.6 Hz, ArH), 7.68 (2H, d, J = 7.6 Hz, ArH), 9.04 (1H, bs, NH); Anal. calc. for $C_{11}H_8N_2OSBrCl$: C, 39.84; H, 2.43; N, 8.45; S, 9.67; found C, 39.78; H, 2.38; N, 8.41; S, 9.62.

2-Chloro-N-(4-*p*-tolylthiazol-2-yl)acetamide (11f)

Yield: 90%; Mp.: 148-150^oC; FTIR (KBr): 3340, 2967, 2872, 2740, 1699, 1567, 1426, 1328, 1268, 1140, 1070, 974, 820, 700, 652, 508 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 2.40 (3H, s, CH_3), 4.20 (2H, s, CH_2), 7.15 (1H, s, CH of thiazole), 7.25 (2H, d, J = 6.8 Hz, ArH), 7.72 (2H, d, J = 6.8 Hz, ArH), 10.23 (1H, bs, NH); Anal. calc. for $C_{12}H_{11}N_2OSCl$: C, 54.03; H, 4.16; N, 10.50; S, 12.02; found C, 53.98; H, 4.10; N, 10.41; S, 11.96.

2-Chloro-N-[4-(4-methoxyphenyl)thiazol-2-yl]acetamide (11g)

Yield: 95%; Mp.: 232-234^oC; FTIR (KBr): 3332, 2980, 2764, 1694, 1538, 1429, 1330, 1255, 1171, 1029, 972, 843, 735, 612, 534 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 3.87 (3H s, OCH_3), 4.27 (2H, s, CH_2), 6.97 (2H, d, J = 8.4 Hz, ArH), 7.08 (1H, s, CH of thiazole), 7.77 (2H, d, J = 8.4 Hz, ArH), 9.93 (1H, bs, NH); Anal. calc. for $C_{12}H_{11}N_2O_2SCl$: C, 50.97; H, 3.92; N, 9.91; S, 11.34; found C, 50.91; H, 3.87; N, 9.89; S, 11.29.

2-Chloro-N-[4-(4-nitrophenyl)thiazol-2-yl]acetamide (11h)

Yield: 75%; Mp.: 295-297^oC; FTIR (KBr): 3315, 2992, 2874, 1688, 1560, 1542, 1436, 1345, 1265, 1180, 1038, 972, 854, 738, 642, 532 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 4.27 (2H, s, CH_2), 7.41 (1H, s, CH of thiazole), 8.04 (2H, d, J = 8.8 Hz, ArH), 8.23 (2H, J = 8.8 Hz, ArH),

9.87 (1H, bs, NH); Anal. calc. for $C_{11}H_8N_3O_3SCl$: C, 44.38; H, 2.71; N, 14.11; S, 10.17; found C, 44.32; H, 2.67; N, 14.06; S, 10.11.

2-Chloro-N-[4-(4-cyanophenyl)thiazol-2-yl]acetamide (11i)

Yield: 76%; Mp.: 276-278^oC; FTIR (KBr): 3325, 2972, 2864,2732,1678, 1560, 1542, 1435, 1345, 1265, 1180, 1038, 854, 738, 642, 532 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 4.24 (2H, s, CH_2), 6.98 (1H, s, CH of thiazole), 7.45 (2H, d, J = 8.4 Hz, ArH), 7.92 (2H, d, J = 8.4 Hz, ArH), 9.84 (1H, bs, NH); Anal. calc. for $C_{12}H_8N_3OSCl$: C, 61.34; H, 4.58; N, 15.90; S, 18.19; found C, 61.29; H, 4.55; N, 15.83; S, 18.11.

2-Chloro-N-[4-(3,4-dichlorophenyl)thiazol-2-yl]acetamide (11j)

Yield: 79% Mp.: 228-230^oC; FTIR (KBr): 3349, 2968, 2854, 1684, 1528, 1415, 1355, 1246, 1132, 1060, 832, 746, 672, 538 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 4.23 (2H, s, CH_2), 7.23 (1H, s, CH of thiazole) 7.61-8.02 (3H, m, ArH), 9.76 (1H, bs, NH); Anal. calc. for $C_9H_8N_2S$: C, 41.08; H, 2.19; N, 8.71; S, 9.97; found C, 40.98; H, 2.12; N, 8.67; S, 9.93.

General procedure for the synthesis of 4-amino-5-phenyl-4H-[1,2,4]triazole-3-thiol derivatives (15)

The derivatives were prepared according to the reported method in literature[187,188] and given in scheme 3.2.

Synthesis of benzohydrazide derivatives (13)

The ester of substituted aromatic acid (**12**, 26 mmol) was dissolved in 30 mL ethanol, and hydrazine hydrate (0.1 mmol) was then added drop-wise to the mixture with stirring. The resulting mixture was allowed to reflux for 6 hours. The completion of the reaction was monitored by TLC (ethyl acetate: petroleum ether, 1:1, v/v). After completion of reaction, the excess ethanol was distilled out and the contents were allowed to cool. The crystals formed were filtered, washed thoroughly with water, and dried. This was used for further reactions without any purification.

Benzohydrazide (13a)

Yield: 64%; Mp.: 110-112^oC; FTIR (KBr): 3298, 3196, 2978, 2874, 1672, 1578, 1476, 1368, 1256, 1178, 1056, 956, 840, 746, 651 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 4.54 (bs, 2H, NH_2),

7.26 (m, 1H, ArH), 7.41 (dd, 2H, J = 8.4 Hz, 3.2 Hz ArH), 7.79 (dd, 2H, J = 8.0 Hz, 2.8 Hz, Ar-H), 9.74 (bs, 1H, NH).

4-Fluorobenzohydrazide (13b)

Yield: 73%; Mp.: 160-162^{o}C; FTIR (KBr): 3278, 3184, 2928, 2865, 1660, 1597, 1451, 1371, 1265, 1093, 990, 830, 729, 686 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.52 (bs, 2H, NH_2), 7.31 (d, 2H, J = 8.0 Hz, ArH), 7.97 (d, 2H, J = 8.0 Hz, ArH), 9.68 (bs, 1H, NH).

4-Chlorobenzohydrazide (13c)

Yield: 68%; Mp.: 166-168^{o}C; FTIR (KBr): 3223, 3194, 2967, 2856, 1678, 1587, 1454, 1367, 1248, 1198, 1078, 967, 840, 735, 646 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.56 (bs, 2H, NH_2), 7.46-7.48 (d, 2H, J = 7.2 Hz, ArH), 7.79-7.80 (d, 2H, J = 7.6 Hz, ArH), 9.56 (bs, 1H, NH).

4-Bromobenzohydrazide (13d)

Yield: 62%; Mp.: 169-170^{o}C; FTIR (KBr): 3270, 3156, 2971, 2884, 1685, 1578, 1436, 1340, 1286, 1194, 1067, 998, 856, 742, 650 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.50 (bs, 2H, NH_2), 7.43 (d, 2H, J = 6.8 Hz, ArH), 7.80 (d, 2H, J = 7.2 Hz, ArH), 9.59 (bs, 1H, NH).

3-Bromobenzohydrazide (13e)

Yield: 63%; Mp.: 154-158^{o}C; FTIR (KBr): 3224, 3146, 2998, 2884, 1690, 1546, 1456, 1360, 1275, 1187, 1076, 995, 887, 640 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.53 (bs, 2H, NH_2), 7.48 (m, 1H, ArH), 7.69 (dd, 1H, J = 8.00 Hz, 2.4 Hz, ArH), 7.79 (dd, 1H, J = 8.00 Hz, 2.4 Hz, ArH), 9.64 (bs, 1H, NH).

4-Methylbenzohydrazide (13f)

Yield: 66%; Mp.: 114-116^{o}C; FTIR (KBr): 3234, 3123, 2987, 2574, 1678, 1598, 1456, 1378, 1276, 1180, 1068, 992, 846, 768, 667 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2. 46 (s, 3H, CH_3) 4.56 (bs, 2H, NH_2), 7.30 (d, 2H, J = 7.7 Hz, ArH), 7.79 (d, 2H, J = 8.00 Hz, ArH), 9.67 (bs, 1H, NH).

4-Methoxybenzohydrazide (13g)

Yield: 64%; Mp.: 134-138^{o}C; FTIR (KBr): 3224, 3176, 2996, 2874, 1685, 1575, 1468, 1340, 1166, 1078, 976, 867, 778, 698 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.83 (s, 3H, OCH_3), 4.51 (bs, 2H, NH_2), 7.02 (d, 2H, J = 8.00 Hz, ArH), 7.85 (d, 2H, J = 8.00 Hz, ArH), 9.74 (bs, 1H, NH).

4-Nitrobenzohydrazide (13h)

Yield: 67%; Mp.: 214-216^{o}C; FTIR (KBr): 3238, 3179, 2989, 2854, 1682, 1538, 1470, 1352, 1274, 1168, 1098, 993, 897, 756, 648 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 6.92 (bs, 2H, NH_2), 7.99 (d, 2H, J = 7.60 Hz, ArH), 8.27 (d, 2H, J = 7.20 Hz, ArH), 9.68 (bs, 1H, NH).

3,4,5-Trimethyoxybenzohydrazide (13i)

Yield: 61%; Mp.: 156-158^{o}C; FTIR (KBr): 3246, 3136, 2978, 2869, 1686, 1575, 1498, 1368, 1265, 1189, 1076, 934, 871, 779, 653 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.69 (s, 3H, OCH_3), 3.86 (s, 6H, OCH_3), 7.01 (bs, 2H, NH_2), 7.21 (d, 1H, J = 3.20 Hz, ArH), 9.78 (bs, 1H, NH).

Synthesis of potassium 2-benzoylhydrazine-1-carbodithioate derivatives (14)

KOH (4.2 g, 75 mmol) was dissolved in absolute ethanol (200 ml). To the above solution, aryl acid hydrazide, (**13**, 50 mmol) was added and the solution was cooled on ice. To this, carbon disulfide (75 mmol) was added in small portions with constant stirring. The reaction mixture was agitated continuously for a period of 15 hours. It was then diluted with anhydrous ether. The precipitated potassium dithiocarbazinate was collected by filtration. The precipitate was further washed with anhydrous ether (100 mL) and evaporated under vacuum. The potassium salt thus obtained was in quantitative yield, and was used in the next step without further purifications.

Synthesis of 4-amino-5-phenyl-4H-[1,2,4]triazole-3-thiol derivatives (15)

A suspension of potassium dithiocarbazinate, (**14**, 100 mmol) in water (5 ml) and hydrazine hydrate (15 ml, 300 mmol) was refluxed for 30 min with occasional shaking. The colour of the reaction mixture changed to green with the evolution of hydrogen sulfide gas (lead acetate paper test and odour). A homogeneous reaction mixture was obtained during the reaction process. The completion of the reaction was monitored with TLC (ethyl acetate: petroleum ether, 1:1, v/v). The reaction mixture was cooled to room temperature, and was diluted with water (100 mL). On acidification with concentrated hydrochloric acid, the required triazole (**15**) was precipitated out. It was filtered, washed thoroughly with cold water, and then recrystallized from ethanol.

4-Amino-5-phenyl-4H-[1,2,4]triazole-3-thiol (15a)

Yield: 60%; Mp.: 196-198ºC; (Lit.[187] Mp.: 198-200ºC); FTIR (KBr): 3412, 3070, 2667, 1640, 1546, 1467, 1366, 1284, 1170, 1069, 966, 840, 732, 640 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 3.35 (1H, s, SH), 7.53-8.03 (m, 7H, ArH); Anal. calc. for $C_8H_8N_4S$: C, 49.98; H, 4.19; N, 29.14; S, 16.68; found: C, 49.90; H, 4.16; N, 29.13; S, 16.60.

4-Amino-5-(4-fluorophenyl)-4H-[1,2,4]triazole-3-thiol (15b)

Yield: 72%; Mp.: 204-206ºC; (Lit.[189] Mp.: 208ºC); FTIR (KBr): 3423, 3091, 2943, 2656, 1615, 1508, 1476, 1350, 1298, 1187, 1073, 970, 846, 728, 691 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 5.68 (bs, 2H, NH_2), 7.28-7.33 (m, 2H, ArH), 7.99-8.11 (2H, m, ArH); Anal. calc. for $C_8H_7N_4SF$: C, 45.71; H, 3.36; N, 26.65; S, 15.25; found: C, 45.67; H, 3.28; N, 26.61; S, 15.20.

4-Amino-5-(4-chlorophenyl)-4H-[1,2,4]triazole-3-thiol (15c)

Yield: 68%; Mp.: 208-210ºC; (Lit.[189] Mp.: 210-212ºC); FTIR (KBr): 3429, 3056, 2972, 2687, 1635, 1528, 1451, 1348, 1287, 1192, 1089, 998, 856, 740, 647 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 5.71 (bs, 2H, NH_2), 7.54-7.61 (m, 2H, ArH), 8.01-8.05 (m, 2H, ArH). Anal. calc. for $C_8H_7N_4SCl$: C, 42.39; H, 3.11; N, 24.72; S, 14.14; found: C, 42.30; H, 3.09; N, 24.68; S, 14.10.

4-Amino-5-(4-bromophenyl)-4H-[1,2,4]triazole-3-thiol (15d)

Yield: 67%; Mp.: 202-204ºC; (Lit.[189] Mp.: 205-206ºC); FTIR (KBr): 3410, 3056, 2978, 2647, 1644, 1587, 1434, 1378, 1243, 1176, 1067, 989, 856, 747, 682 cm^{-1}; ^1H NMR [$(CD_3)_2CO$, 400 MHz] δ: 5.49 (bs, 2H, NH_2), 7.69-7.75 (m, 2H, ArH), 8.10-8.14 (m, 2H, ArH), 12.82 (bs, 1H, SH); Anal. calc. for $C_8H_7N_4SBr$: C, 35.44; H, 2.60; N, 20.66; S, 11.82; found: C, 35.41; H, 2.58; N, 20.62; S, 11.76.

4-Amino-5-(3-bromophenyl)-4H-[1,2,4]triazole-3-thiol (15e)

Yield: 63%; Mp.:208-210ºC; (Lit.[189] Mp.: 212ºC); FTIR (KBr): 3433, 3073, 2987, 2667, 1647, 1578, 1467, 1389, 1254, 1162, 1058, 948, 823, 749, 692 cm^{-1}; ^1H NMR [$(CD_3)_2CO$, 400 MHz] δ: 5.51 (bs, 2H, NH_2), 7.72-7.76 (m, 2H, ArH), 8.17 (m, 1H, ArH), 8.39 (m, 1H, ArH), 12.89 (bs, 1H, SH); Anal. calc. for $C_8H_7N_4SBr$: C, 35.44; H, 2.60; N, 20.66; S, 11.82; found: C, 35.43; H, 2.54; N, 20.61; S, 11.79.

4-Amino-5-(4methylphenyl)-4H-[1,2,4]triazole-3-thiol (15f)

Yield: 71%; Mp.:195-198C; (Lit.[189] Mp.: 201ºC); FTIR (KBr): 3423, 3062, 2939, 2637, 1636, 1540, 1429, 1338, 1280, 1162, 1072, 938, 891, 749, 662 cm^{-1}; ^1H NMR [$(CD_3)_2CO$, 400 MHz] δ:

2.43 (s, 3H, CH_3), 5.51 (bs, 2H, NH_2), 7.82 (d, 2H, J = 8.00 Hz, ArH), 8.02 (d, 2H, J = 8.40 Hz, ArH), 12.79 (bs, 1H, SH); Anal. calc. for $C_9H_{10}N_4S$: C, 52.41; H, 4.89; N, 27.16; S, 15.54; found: C, 52.39; H, 4.84; N, 27.10; S, 15.49.

4-Amino-5-(4-methoxyphenyl)-4H-[1,2,4]triazole-3-thiol (15g)

Yield: 69%; Mp.:210-212^oC; (Lit.[190] Mp.: 215^oC); FTIR (KBr): 3434, 3059, 2981, 2649, 1648, 1563, 1498, 1372, 1259, 1183, 1071, 917, 893, 749, 628 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3,80 (s, 3H, OCH_3), 5.78 (bs, 2H, NH_2), 7.24 (d, 2H, J = 8.00 Hz, ArH), 7.83 (d, 2H, J = 8.40 Hz, ArH), 12.69 (bs, 1H, SH). Anal. calc. for $C_9H_{10}N_4OS$: C, 48.63; H, 4.54; N, 25.21; S, 14.42; found: C, 48.58; H, 4.51; N, 25.17; S, 14.40.

4-Amino-5-(4-nitrophenyl)-4H-[1,2,4]triazole-3-thiol (15h)

Yield: 76%; Mp.: 178-182^oC; FTIR (KBr): 3430, 3109, 2998, 2689, 1641, 1546, 1476, 1353, 1292, 1139, 1074, 948, 879, 738, 661 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 5.98 (bs, 2H, NH_2), 8.00 (d, 2H, J = 7.60 Hz, ArH), 8.24 (d, 2H, J = 7.20 Hz, Ar-H), 12.89 (bs, 1H, SH). Anal. calc. for $C_8H_7N_5O_2S$: C, 40.50; H, 2.97; N, 29.52; S, 13.51; found: C, 40.47; H, 2.92; N, 29.49; S, 13.48.

4-Amino-5-(3,4,5-trimethoxyphenyl)-4H-[1,2,4]triazole-3-thiol (15i)

Yield: 63%; Mp.: 215-220^oC; (Lit.[191] Mp.: 221^oC); FTIR (KBr): 3428, 3102, 2976, 2667, 1638, 1556, 1482, 1348, 1249, 1173, 1068, 928, 840, 728, 652 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.67 (s, 3H, OCH_3), 3.86 (s, 6H, OCH_3), 6.02 (bs, 2H, NH_2), 7.39 (2H, d, J = 2.80 Hz Ar-H), 12.03 (bs, 1H, SH); Anal. calc. for $C_{11}H_{14}N_4O_3S$: C, 46.80; H, 5.00; N, 19.85; S, 11.36; found: C, 46.75; H, 4.99; 19.78; S, 11.32.

General procedure for the synthesis of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide derivatives (Scheme 3.3, 16a-i)

A solution of substituted triazoles (**15**, 2.6 mmol) in 6 mL dry acetone or acetonitrile and triethyl amine (0.4 mL, 2 mmol) were taken in a round bottom flask (50 mL). To this N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (**11**, 2.6 mmol) was added. The reaction mixture was stirred at 50^oC for 4 hours. The progress of reaction was monitored by TLC (CH_2Cl_2: methanol, 9:1, v/v). After completion of reaction, the solvent was removed under reduced pressure and reaction mixture was extracted with ethyl acetate (3×40 mL). The organic layer was dried over

anhydrous sodium sulphate, and the excess of solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (60-120 mesh) using chloroform: methanol 3:1 v/v) as eluent.

N-Benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide (16a)

Yield: 75%; Mp.: 221-224^{o}C; FTIR (KBr): 3361, 3064, 2995, 2064, 1682, 1597, 1553, 1485, 1318, 1256, 1082, 756 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,) δ: 2.67 (s, 2H, CH_2), 3.50 (s, 1H, SH), 7.31-8.00 (m, 9H, ArH), 12.82 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 36, 121, 122, 123, 124, 126(3C of Ar), 129 (2C of Ar), 132 (2C of Ar), 163, 165, 167 (C=O); Anal. calc. for $C_{17}H_{14}N_6OS_2$: C, 53.39; H, 3.69; N, 21.97; S, 16.77; found C, 53.32; H, 3.64; N, 21.93; S, 16.71.

N-Benzothiazol-2-yl-2-[3-(4-fluorophenyl)-5-mercapto-[1,2,4]triazol-4-ylamino]acetamide (16b)

Yield: 68%; M.p.: 242-246^{o}C; IR (KBr): 3320, 3164, 2958, 2012, 1679, 1610,1548, 1480, 1324, 1276, 1086, 750 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,) δ: 2.87 (s, 2H, CH_2), 3.38 (s, 1H, SH), 7.28-8.21 (m, 8H, ArH), 12.75 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 38, 114, 117, 120, 122, 124, 125, 127, 131, 148, 155, 162, 164, 169, 173; Anal. calc. for $C_{17}H_{13}N_6OS_2F$: C, 50.99; H, 3.27; N, 20.99; S, 16.01; found C, 50.93; H, 3.13; N, 20.96; S, 15.99.

N-Benzothiazol-2-yl-2-[3-(4-chlorophenyl)-5-mercapto-[1,2,4]triazol-4-ylamino]acetamide (16c)

Yield: 62%; M.p.: 258-260^{o}C; IR (KBr): 3332, 3176, 3062, 2938, 1928, 1673, 1620, 1555, 1476, 1439, 1417, 1234, 1093, 829, 746, 718, 677 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.76 (s, 2H, CH_2), 3.36 (s, 1H, SH), 7.26-8.19 (m, 8H, ArH), 12.65 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 36, 116, 122, 124, 126, 127, 129, 131, 133, 149, 152, 166, 169, 172; Anal. calc. for $C_{17}H_{13}N_6OS_2Cl$: C, 48.98; H, 3.14; N, 20.16; S, 15.38; found C, 49.94; 3.08; N, 20.10; S, 15.32.

N-Benzothiazol-2-yl-2-[3-(4-bromophenyl)-5-mercapto-[1,2,4]triazol-4-ylamino]acetamide (16d)

Yield: 58%, M.p.: 278-281^{o}C; IR (KBr): 3321, 3165, 3042, 2942, 1936, 1686, 1612, 1548, 1472, 1430, 1232, 1086, 832, 735, 694 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.82 (s, 2H, CH_2), 3.42 (s, 1H, SH), 7.21-8.02 (m, 8H, ArH), 12.63 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 39,

117, 120, 122, 124, 126, 131, 132, 133, 150, 165, 169, 175; Anal. calc. for $C_{17}H_{13}N_6OS_2Br$: C, 44.26; H, 2.84; N, 18.22; S, 13.90; found C, 44.21; H, 2.80; N, 18.19; S, 13.86.

N-Benzothiazol-2-yl-2-[3-(3-bromophenyl)-5-mercapto-[1,2,4]triazol-4-ylamino]acetamide (16e)

Yield: 53%, M.p.: 241-245^oC; IR (KBr): 3329, 3156, 3048, 2947, 1948, 1678, 1618, 1568, 1478, 1236, 1094, 746, 675 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.67 (s, 2H, CH_2), 3.56 (s, 1H, SH), 7.19-8.06 (m, 8H, ArH), 12.78 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 38, 118, 120, 122, 123, 125, 126, 127, 129, 131, 132, 136, 152, 154, 166, 169, 173; Anal. calc. for $C_{17}H_{13}N_6OS_2Br$: C, 44.26; H, 2.84; N, 18.22; S, 13.90; found C, 44.23; H, 2.80; N, 18.21; S, 13. 83.

N-Benzothiazol-2-yl-2-[3-(4-methylphenyl)-5-mercapto-[1,2,4]triazol-4-ylamino]acetamide (16f)

Yield: 62%, M.p.: 232-236^oC; IR (KBr): 3346, 3168, 3036, 2946, 1928, 1688, 1626, 1540, 1465, 1245, 1087, 766, 640 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.32 (s, 3H, CH_3), 2.86 (s, 2H, CH_2), 3.46 (s, 1H, SH), 7.34-7.99 (m, 8H, ArH), 12.78 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 20, 36, 119, 122, 125, 126, 128, 130, 131, 132, 151, 154, 167, 168, 172; Anal. calc. for $C_{18}H_{16}N_6OS_2$: C, 54.53; H, 4.07; N, 21.20; S, 16.17; found C, 54.48; H, 3.98; N, 21.19; S, 16.15.

N-Benzothiazol-2-yl-2-[3-(4-methoxylphenyl)-5-mercapto-[1,2,4]triazol-4-ylamino] acetamide (16g)

Yield: 57%, M.p.: 256-258^oC; IR (KBr): 3336, 3146, 3028, 2968, 1967, 1679, 1614, 1529, 1457, 1240, 1076, 748, 656 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.72 (s, 2H, CH_2), 3.67 (s, 1H, SH), 3.83 (s, 3H, OCH_3), 7.36-8.12 (m, 8H, ArH), 12.72 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 38, 52, 115, 119, 122, 123, 125, 126, 129, 131, 150, 159, 168, 173; Anal. calc. for $C_{18}H_{16}N_6O_2S_2$: C, 52.41; H, 3.91; N, 20.37; S, 15.54; found C 52.38; H, 3.87; N 20.32; S, 15.48.

N-Benzothiazol-2-yl-2-[3-(4-nitrophenyl)-5-mercapto-[1,2,4]triazol-4-ylamino]acetamide (16h)

Yield : 48%, M.p.: 296-298^oC; IR (KBr): 3340, 3160, 2958, 1950, 1685, 1618, 1542, 1473, 1352, 1248, 1056, 773, 642 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.85 (s, 2H, CH_2), 3.67 (s, 1H, SH), 7.34-814 (m, 8H, ArH), 12.89 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 39,

120, 123, 126, 127, 129, 132, 138, 149, 153, 155, 169, 170, 176; Anal. calc. for $C_{17}H_{13}N_7O_3S_2$: C, 47.77; H, 3.07; N, 22.94; S, 15.00; found C, 47.72; H, 3.04; N, 22.90; S, 14.96.

N-Benzothiazol-2-yl-2-[3-(3,4,5-trimethoxyphenyl)-5-mercapto-[1,2,4]triazol-4-ylamino] acetamide (16i)

Yield: 44%, M.p.: 241-245^oC; FTIR (KBr): 3397, 3156, 2942, 1939, 1681, 1606, 1560, 1448, 1241, 1046, 765, 637 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 2.88 (s, 2H, CH_2), 3.66 (s, 1H, SH), 3.72 (s, 3H, OCH_3), 3.84 (s, 6H, OCH_3), 7.31-8.14 (m, 6H, ArH), 12.92 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 36, 59, 115, 118, 120, 123, 124, 125, 126, 129, 148, 152, 164, 166, 171; Anal. calc. for $C_{20}H_{20}N_6O_4S_2$: C, 50.84; H, 4.27; N, 17.79; S, 13.57; found C, 50.79; H, 4.21; N, 17.71; S, 13.55.

3.2.3 *In- silico* (Docking) studies

Geometries of the compounds **10**, **15a-i**, and **16a-i** were optimized at the B3LYP/6-31G* level using Gaussian 09 quantum chemistry software (http://gaussian.com/). The global minima of the structures were verified using vibrational frequencies. Crystal structure of the protein AChE (PDB Id: 1EVE) was downloaded from protein data bank (PDB: www.rcsb.org). Though many structures of AChE are available, but the above protein structure from *Tetronarce californica* organism was opted as assay used for *in vitro* experiment was also carried on enzyme from the same organism. Similarly for BuChE structure PDB Id (4TPK) was used.

Before docking, the ligand molecules and enzymes were prepared by Glide 'ligprep' and 'Protein preparation' modules respectively. The ligand was refined in torsional space using the force field OPLS3 (Glide XP) with a distance-dependent dielectric model. Finally, a small number of poses are minimized within the field of the receptor with full ligand flexibility. The Glide module of Schrodinger uses high throughput virtual screening (HTVS), standard Precision (SP) and Xtra precision (XP) docking methodologies. As the last one provided more appropriate results, the current study provided XP docking score for all the ligands (Table 3.4).

3.2.4 *In-vitro* experimental studies

Inhibition of acetylcholinesterase (AChE) and butrylcholinesterase (BuChE) activity assay

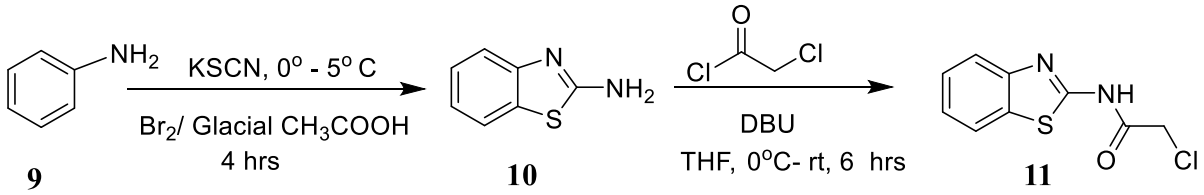
The synthesized molecules were tested for AChE and BuChE inhibitory activities according to the method described by Najafi et al, 2017[145] with some modifications. Enzyme inhibition assay was performed in a 96-well plate by using Ellman's reagent 5,5'-dithio-bis-[2-nitrobenzoic acid] (DTNB) method. Briefly, 25 µL AChE/BuChE (25 mU in 100 µM PBS) was incubated with 75 µL DTNB (100 µM PBS containing 600 µM $NaHCO_3$) for 5 min at room temperature. To this, 25 µL of test compounds (1 – 1000 µM), and 50 µL PBS (pH 7.4) were added. The reaction mixture was then incubated for 15 min at room temperature. Reaction was initiated by adding 25 µL of acetylthiocholine iodide and butylthiocholine (75 mM in PBS) for AChE and BuChE inhibitory assay respectively. Change in absorbance was recorded spectrophotometrically during the experimental duration of 4 min at 412 nm by using UV-spectrophotometer. A blank reaction was run simultaneously, which was having 25 µL solvent (1% DMSO) in place of drugs. Percent inhibition of AChE activity was calculated by using following equation. Similar method was also used to determine the inhibition of BuChE activity.

$$\%\text{AChE/BuChE inhibition} = \frac{(Absorbance\ of\ control - Absorbance\ of\ test) \times 100}{Absorbance\ of\ control}$$

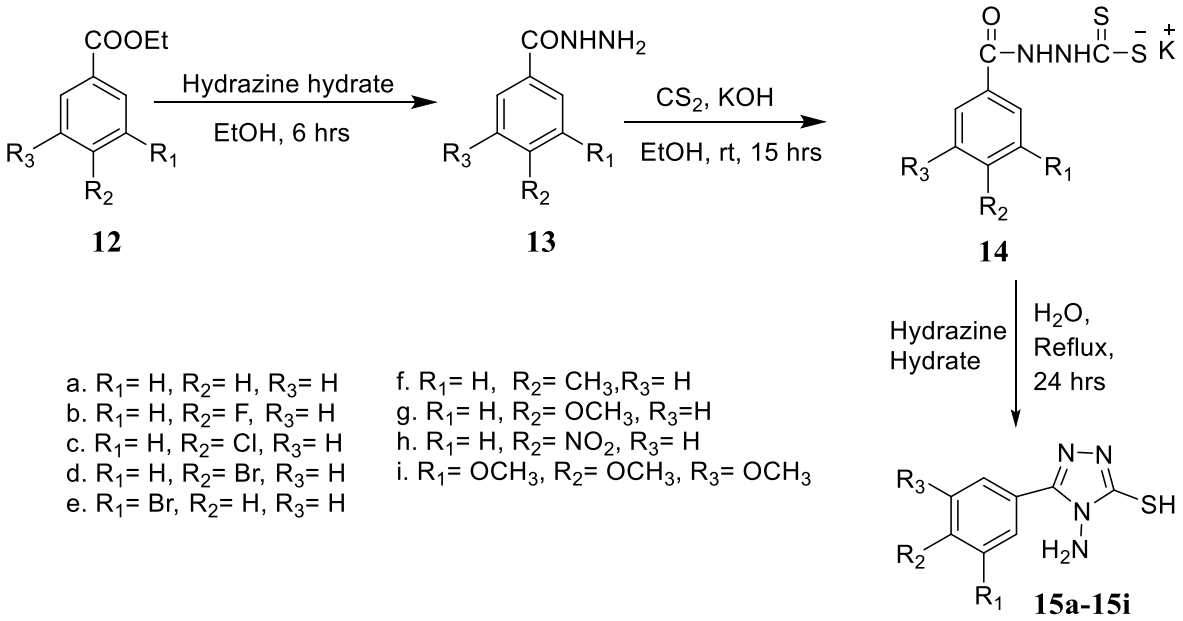
3.3 Results and discussion

3.3.1 Synthesis of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide derivatives

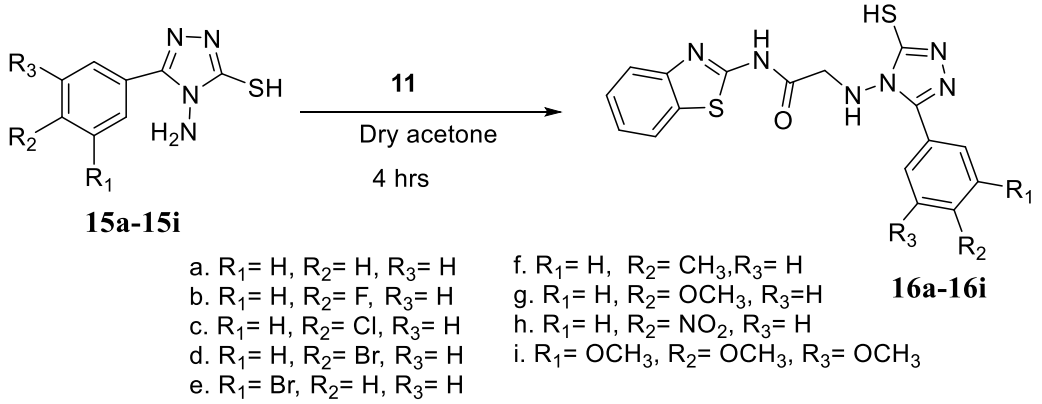
The synthesis of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide derivatives (**16a-i**) were achieved by using schemes 3.1, 3.2 and 3.3. The synthesized compounds were characterized by FTIR, ^{1}H NMR,^{13}C NMR and elemental analysis.



Scheme 3.1: Synthesis of N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (**11**)



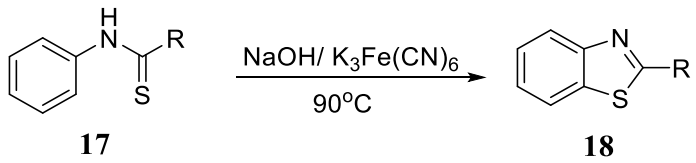
Scheme 3.2: Synthesis of 4-amino-5-phenyl-4H-[1,2,4]triazole-3-thiol derivatives (**15a-i**)



Scheme 3.3: Synthesis of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide derivatives (**16a-i**)

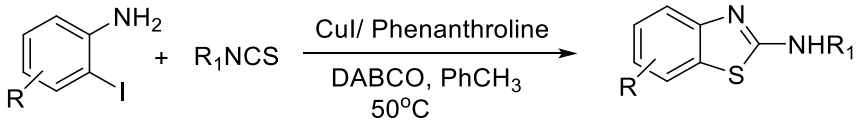
Synthesis of 2-aminobenzothiazole (10)

2-Aminobenzothiazole (**10**) are important starting materials for many useful and biologically active heterocycles.[192-195] There have been several reports for their synthesis.[196] Kim, et.al reported its synthesis this molecule using oxidising agent potassium ferricyanide in aqueous sodium hydroxide [$NaCN/K_3Fe(CN)_6$] (Scheme 3.4).[192] The compound **17** was also cyclized using sodium hydride (NaH) in the presence of N-methylpyrolidinone (NMP) at 140°C.[193] The 2-aminobenzothiazoles were successfully synthesized by Qiuping et al using 2-iodobenzenamine and isothiocynate as starting material.[194] This reaction was carried out by using CuI as catalyst in presence of 1,4-diazobicyclo(2,2,2)-octane (DABCO) in toluene at 50°C (Scheme 3.5).



17 **18**

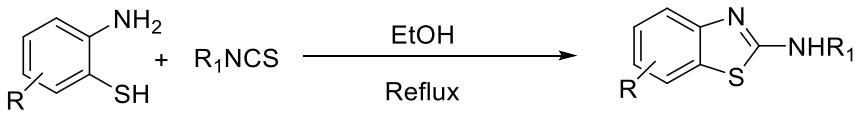
Scheme 3.4: Synthesis of 2-alkylbenzothiazole by using $NaOH/K_3Fe(CN)_6$



19 **20** **21**

Scheme 3.5: Synthesis of 2-N-alkylbenzothiazole by using CuI/DABCO

Tweit, et al. reported the synthesis of substituted 2-aminobenzothiazole by refluxing alkyl isothiocynate and 2-aminothiol in alcohol as solvent (Scheme 3.6).[195]



22 **20** **21**

Scheme 3.6: Synthesis of 2-(N-alkyl)benzothiazole

We synthesized the compound **10** from aniline and potassium thiocynate.[182] The potassium thiocynate was added to the solution of aniline in glacial acetic acid. Then bromine (Br_2) in glacial acetic acid was added with the help of dropping funnel. During addition of Br_2, the temperature of reaction mixture was maintained below 5°C. Br_2 in this reaction are acting as an oxidizing agent and is used for cyclization.[196] After addition of all bromine, the reaction mixture was allowed to come at room temperature and stirred for 4 hours. The solid mass was separated and filtered, and then was washed with glacial acetic acid to remove the unreacted Br_2. After washing the solid residue was dried at vacuum under reduced pressure and subsequently dissolved in hot water. The resulting solution was neutralized by adding 25% ammonia solution. The white precipitate of 2-aminobenzothiazole was obtained which was characterized by spectroscopic techniques. The absorption at 3395 and 1522 cm^{-1} in the IR spectrum of 2-aminothiazole have been assigned for N-H stretching of NH_2 and C=N stretching of thiazole respectively (Figure 3.4). In the ^1H NMR spectrum, the aromatic protons of benzothiazole appeared in the region 7.00-7.65 ppm (Figure 3.5). The peaks at 118, 121, 125, 131, 153, 166 and 169 in ^{13}C NMR spectrum further confirms the formation of 2-aminobenzothiazole (Figure 3.6).

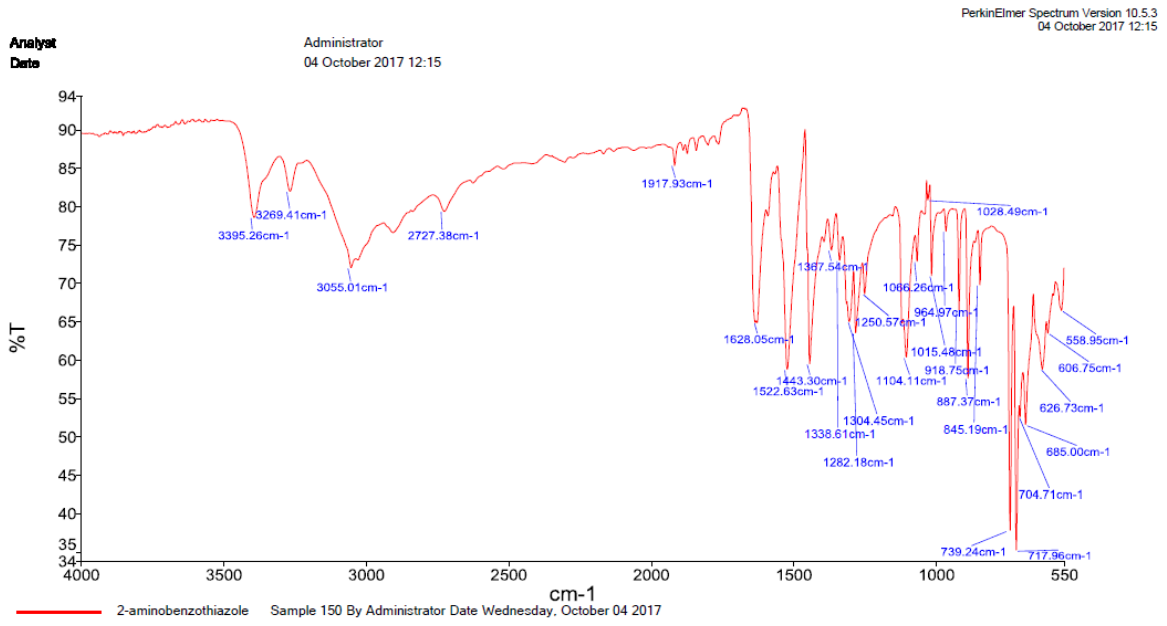


Figure 3.4: FTIR spectrum of 2-aminobenzothizole (**10**)

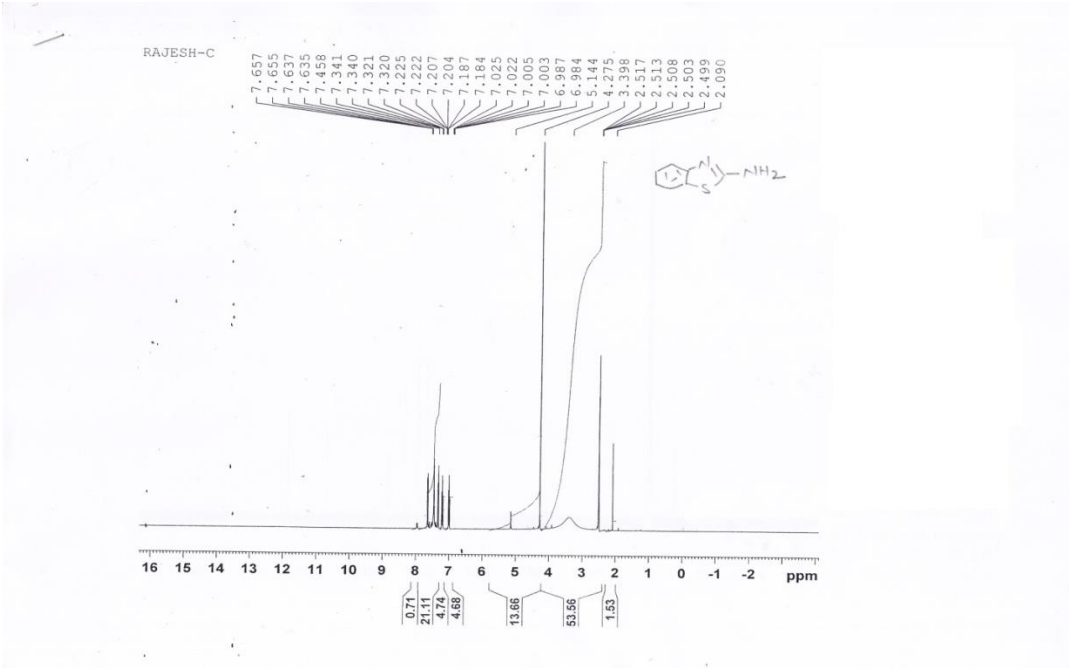


Figure 3.5: ^{1}H NMR spectrum of 2-aminobenzothiazole (**10**)

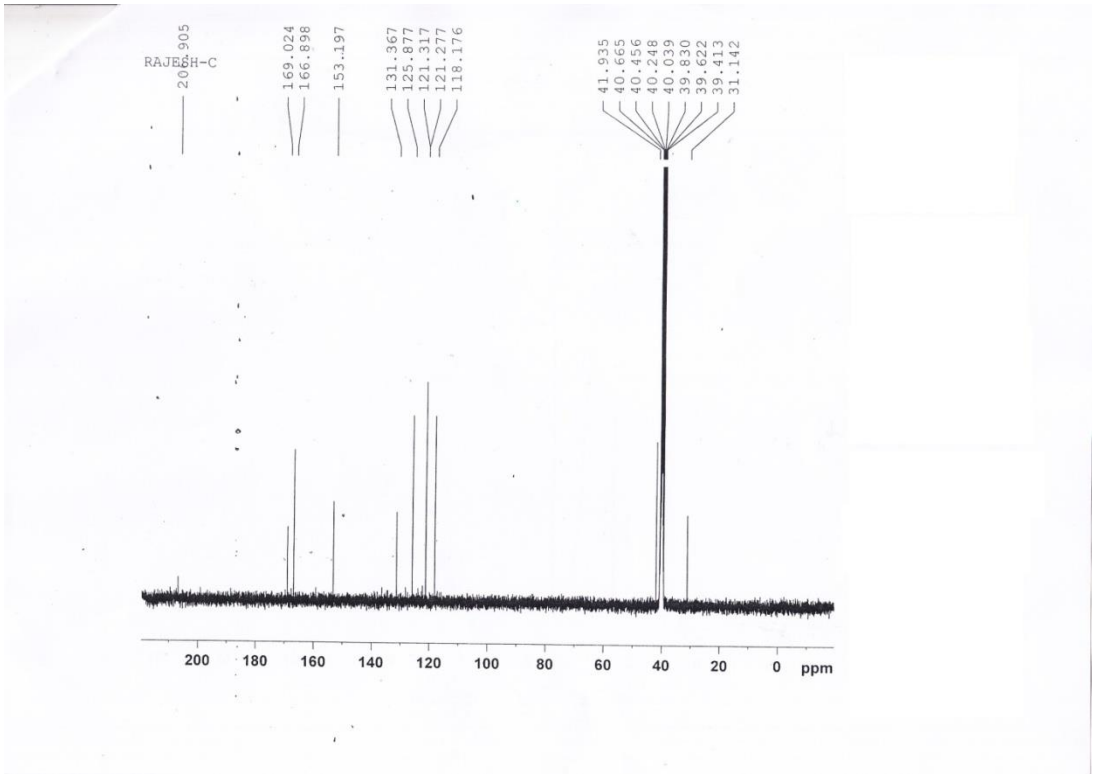


Figure 3.6: ^{13}C NMR spectrum of 2-aminobenzothiazole (**10**)

Synthesis of N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (11)

The synthesis of N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (**11**) was achieved by reacting 2-aminobenzothiazole with chloro acetylchloride in the presence of 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU). Amide bond formation is the most common reaction and plays a vital role in organic synthesis.[197] A large number of synthetic and natural molecules are known which possess this functional group. The synthetic chemists are always looking for better and easier methods for the formation of amide bond.[198-202] The condensation of an amine or aniline with carboxylic acid or its derivatives is commonly employed method for amide bond formation.[198-202] For the synthesis of compound **11**, getting the quantitative yield using the reported methods is a major challenge. Some of the important reported methods for the synthesis of 2-chloroacetamide in solution phase using a various solvents with different bases include triethylamine (TEA) in DMF,[203] TEA in DCM,[204] toluene,[205] K_2CO_3 in benzene,[206] TEA in THF[207], TEA in dioxane and so on.[208] In spite of their potential utility, many of these reported methods suffer from drawbacks such as harsh reaction conditions, long reaction times, unsatisfactory yields, tedious product isolation procedures and needs purification by column chromatography.[208] As a part of our ongoing effort towards the synthesis of biologically active compounds, we herein developed an efficient high yielding synthetic protocol for the one-pot synthesis of amides from aryl amines and chloro acetylchloride using DBU as non-nucleophilic base in THF solvent (Table 3.1). This method gave 75 to 95% yields in 3-6 hours at room temperature (rt) for the synthesis of amides like N-phenylacetamides from substituted aryl amines (**9-9h**), N-benzothiazol-2-ylacetamide (**11**) and N-(4-phenylthiazol-2-yl)acetamides from substituted 4-phenylthiazole-2-amines (**7a-7l**) by DBU. The reactions have also been performed in TEA and DABCO using different solvent systems. The combination of DBU and THF gave best result (Table 3.1). This method ensures the wide substrate scope with excellent yields. The products were isolated and purified by recrystallization. DBU is commercially available and cheap homogenous catalyst. It is a sterically hindered bicyclic amidine base and especially useful where side reactions due to nucleophilicity of basic nitrogen are a problem.[209-211] It is one of the strongest organic neutral base (pKa = 12) in which the +M effect of the adjacent nitrogen stabilizes the protonated species. It has been used in many organic reactions including amide bond formations in recent years.[212] In a typical reaction, aniline (6 mmol) was dissolved in THF (5 ml) and then DBU (1.2 mmol) was added (Scheme 3.7). The reaction mixture was placed on the freezing mixture of ice and salt, and

mechanically stirred for 15 min. After that the chloroacetyl chloride (6.1 mmol) was added from dropping funnel at such rate so that the temperature does not rise beyond 5^{o}C. The reaction mixture was further stirred at room temperature for 3 hours. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured into cold water. The compound was precipitated out which was filtered and washed with water. The precipitate was dried and recrystallized using ethanol. The product, N-phenylacetamide was obtained as a solid powder with 86% yield (Table 3.1 and 3.2). The same procedure was also repeated for the substrates 2-aminobenzothiazole and 2-amino-4-phenylthiazole to check the versatility of the process. The optimization of catalysts DBU, DABCO (1,4-diazobicyclo(2,2,2)-octane) and TEA was also performed for aniline in the different solvent systems, and the same ratio applied to all other substrates under optimized reaction conditions (Table 3.1). The reactions in the bases like TEA (triethylamine) and DABCO remained non-completed even after performing the reaction for the longer time.

The reactant, aryl amines in both the cases was not consumed completely even after stirring for 10 hours at room temperature as observed in TLC. Hence, the products were separated by using column chromatography leading to low yield in comparison to DBU. The summary of comparative studies for different bases in the different solvent is given in table 3.1. The comparison of % isolated yield in case of DABCO and DBU for 6 mmol of aniline is given in figure 3.7 According to the proposed mechanisms, DBU provides significant acceleration compared to other amine bases. This suggests that DBU is not only acting as a base rather playing another role also. There are different mechanisms postulated to explain the role of DBU in these types of reactions.[213] According to our observation, the most suitable catalytic mechanism is the displacement of chloride ion by DBU and hence activates the carbonyl for attack by the lone pair of nitrogen present on aryl amines (Figure 3.8). The synthesized compounds were characterized by spectroscopic technique and melting point for known compounds. The absorption at 3228 and 1689 cm^{-1} in the IR spectrum of compound **11** have been assigned for N-H stretching for NH and C=O stretching for amide bond respectively (Figure 3.9). In the ^{1}H NMR spectrum, a singlet at 4.47 ppm is for the CH_2 proton, which is present in between C=O and Cl atom. A multiplet in the 7.31-8.01 ppm region is due to four aromatic protons and a broad singlet at 12.72 ppm is due to NH proton (Figure 3.10). The peaks at 43, 121, 122, 124, 126, 131, 148, 158 and 166 in ^{13}C NMR spectrum for aromatic carbon and

carbonyl carbon atoms respectively (Figure 3.11), further confirm the formation of compound **11**.

Table 3.1: Optimization of catalysts and solvents for compounds **9**, **10** & **7a**.

Entry	Compd. No.	Solvent	% yield by using different catalysts		
			DBU	DABCO	Et_3N
1	**9**	THF	86	68	70
2	**10**	THF	83	60	62
3	**7a**	THF	86	63	64
4	**9**	1,4-dioxane	75	70	68
5	**10**	1,4-dioxane	71	64	69
6	**7a**	1,4-dioxane	74	66	68
7	**9**	Benzene	58	50	56
8	**10**	Benzene	52	No reaction	50
9	**7a**	Benzene	51	No reaction	51
10	**9**	DCM	72	62	65
11	**10**	DCM	70	58	61
12	**7a**	DCM	70	59	64
13	**9**	DMF	74	74	69
14	**10**	DMF	71	69	64
15	**7a**	DMF	73	68	65

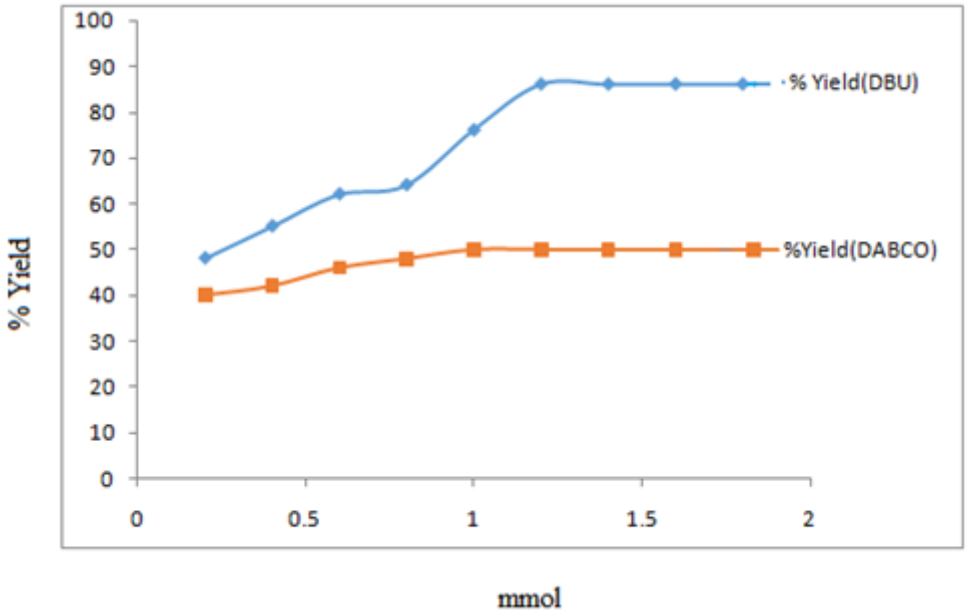
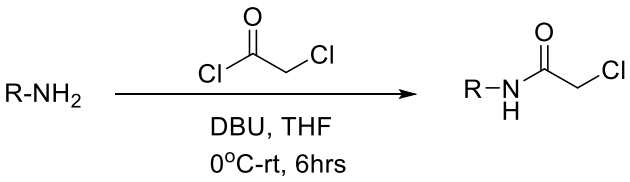


Figure 3.7. Comparative % yield optimization with DBU & DABCO catalysts for aniline

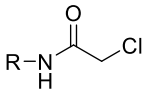
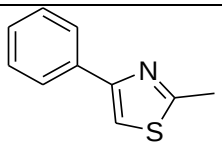
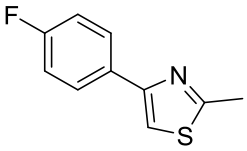
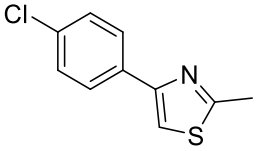
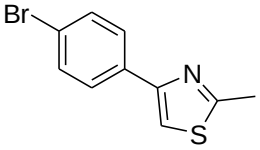
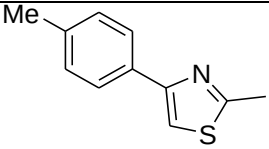
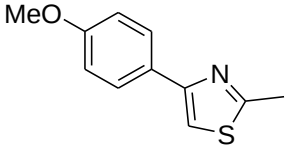


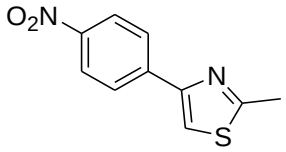
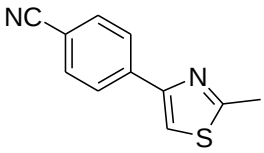
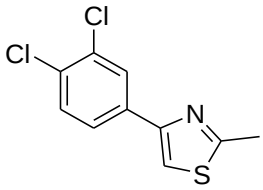
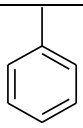
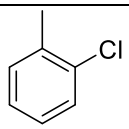
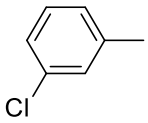
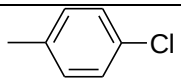
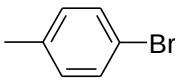
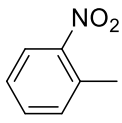
Scheme 3.7: Reaction of aryl amine with chloroacetyl chloride in the presence of DBU

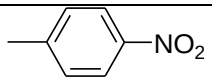
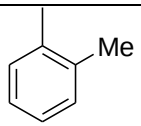
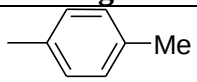
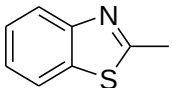
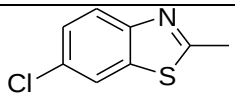
Synthesis of 4-amino-5-phenyl-4H-[1,2,4]triazole-3-thiol derivatives (15a-i)

4-Amino-5-phenyl-4H-[1,2,4]triazole-3-thiol (**15a-i**) compounds were synthesized using literature methods[187] starting from esters of benzoic acid and their derivatives (Scheme 3.2). The reactions between benzoates (**12**) and hydrazine hydrate gave the corresponding hydrazide derivatives (**13**). The formed hydrazide derivatives (**13**) were further treated with carbon disulfide under basic conditions and stirred at room temperature for 15 hours to form the corresponding disulfide salts (**14**), which were used for subsequent reaction without purification. The formed salts were then reacted with hydrazine hydrate in water. The mixture was refluxed for 24 hours to form the corresponding triazole derivatives (**15**).

Table 3.2: Amidation of chloroacetyl chloride with different aryl amines **7a-h**, **7l**, **9**, **9a-i, 10,** and **10a**

R	Time (hrs)	% Yield of products 	Mp (°C)	Lit mp[ref]
 **7a**	4	86 (**11b)**	181	-
 **7b**	5	85 (**11c**)	134-136	-
 **7c**	5	85 (**11d)**	188-181	-
 **7d**	5	86 (**11e)**	206	-
 **7e**	4	90 (**11f**)	149	-
 **7f**	4	95 (**11g)**	234	-

 **7g**	6	75 (**11h**)	295-297	-
 **7h**	6	76 (**11i**)	276-278	-
 **7l**	6	79 (**11j**)	230	-
 **9**	3	86	136	134[214]
 **9a**	3	82	69-70	73[214]
 **9b**	3	80	100	98-100[215]
 **9c**	3	85	176	178[215]
 **9d**	3	85	182	180-184[215]
 **9e**	5	76	98	96-98[216]

 **9f**	5	79	180	178-180[216]
 **9g**	4	86	98-100	105-107[214]
 **9i**	4	88	164	164-166[214]
 **10**	6	83 (**11**)	145	-
 **10**	6	76 (**11a**)	213	-

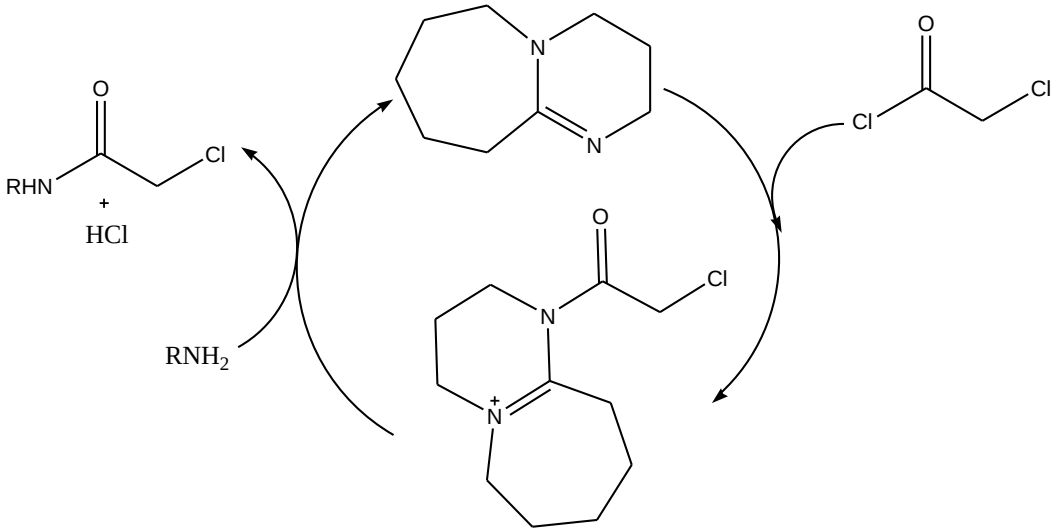


Figure 3.8: Proposed catalytic cycle for amidation of chloroacetyl chloride with different aryl amines using DBU

The formation of triazole derivatives (**15a-i**) were confirmed by spectroscopic techniques. The absorptions at 3412 and 1546 cm^{-1} in the IR spectrum of 4-amino-5-phenyl-4H-[1,2,4]triazole-3-thiol were assigned for NH_2 and C=N stretching for triazole respectively. In the ^1H NMR spectrum, the broad singlet at 3.35 ppm is for SH proton, while aromatic protons appeared in the region 7.53-8.03 ppm (Figure 3.12).

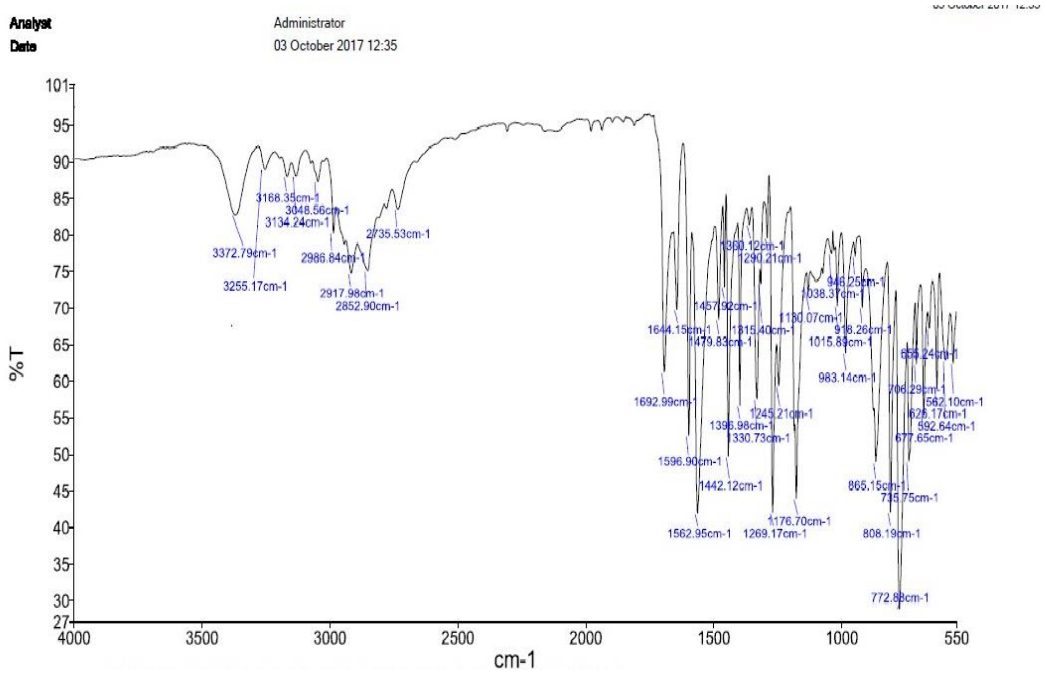


Figure 3.9: FTIR spectrum of N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (**11**)

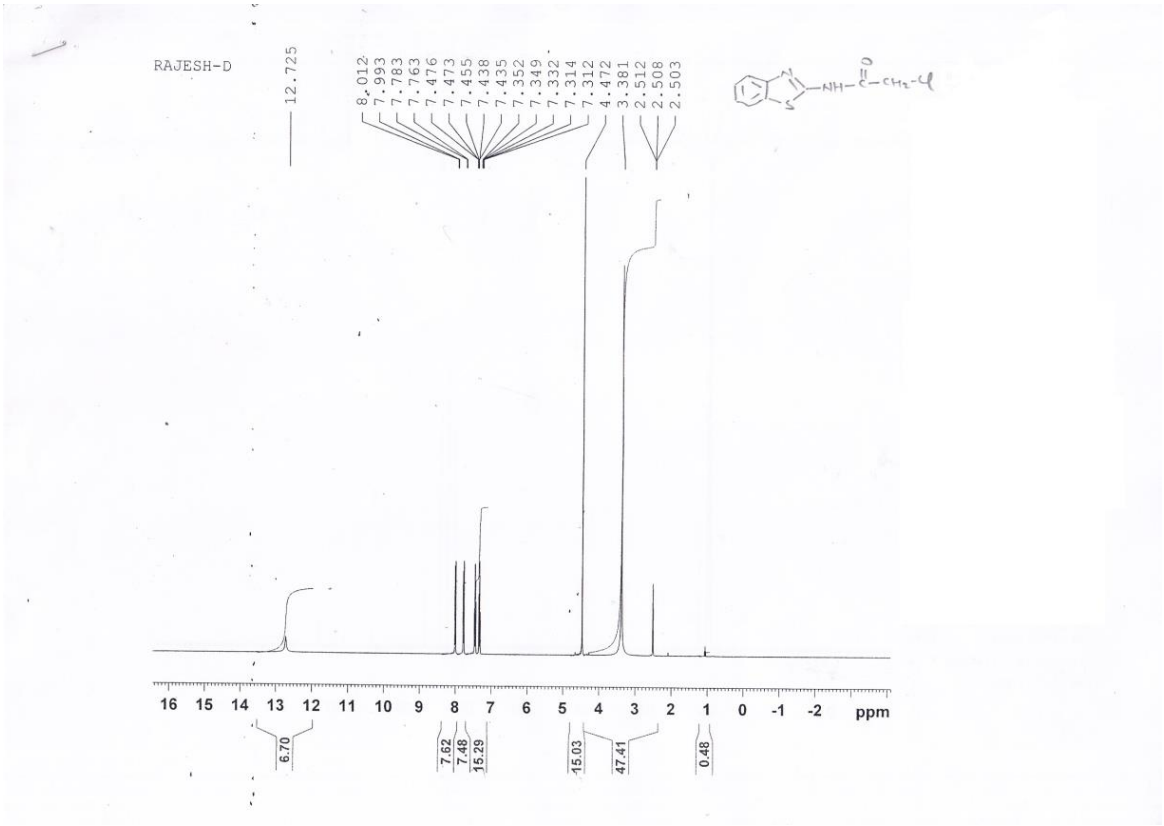


Figure 3.10: ^{1}H NMR spectrum of N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (**11**)

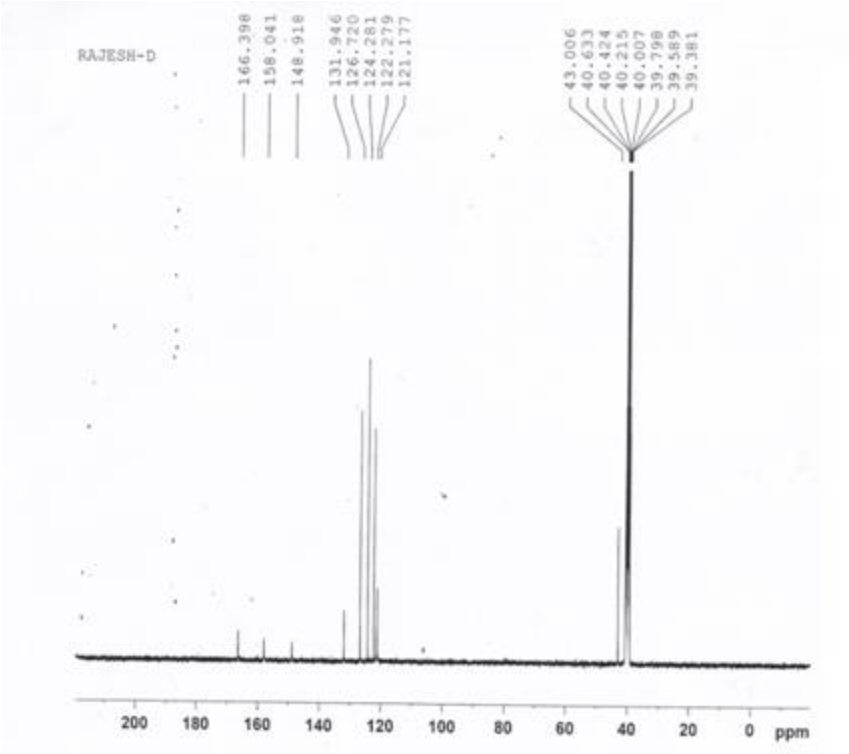


Figure 3.11: ^{13}C NMR spectrum of N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (**11**)

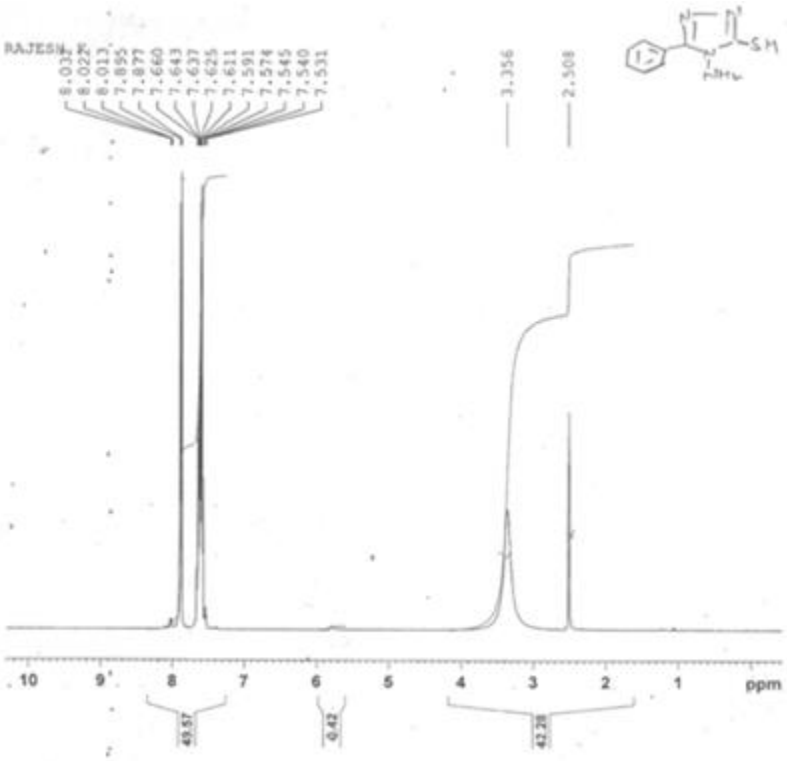


Figure 3.12: ^{1}H NMR spectrum of 4-amino-5-phenyl-4H-[1,2,4]triazole-3-thiol (**15a**)

Synthesis of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)acetamide derivatives (16a-i)

The synthesis of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide derivatives was achieved by following scheme 3.3. The products were formed by nucleophilic substitution reaction of NH_2 of triazoles (**15**) to chloroacetamide derivative of 2-aminobenzothiazole (**11**). For this reaction, the triazole was dissolved in dry acetone or CH_3CN, and then added weak base triethyl amine. The reaction mixture was stirred at 50°C. After dissolution of triazole, the compound **11** was added and stirred the reaction mixture for 4 hours. After completion of reaction, the product was purified by column chromatography and characterized by FTIR, NMR spectroscopy and elemental analysis. The absorptions at 3361, 1682 and 756 cm^{-1} in IR spectrum are assigned for NH stretching, C=O stretching of amide and C-S stretching respectively (Figure 3.13). In the ^1H NMR spectrum, a singlet at 2.67 ppm is for CH_2 proton; another singlet at 3.50 ppm is for SH proton attached to triazole ring; multiplet in the region 7.31-8.00 ppm is for 9 aromatic protons; and a broad singlet at 12.62 ppm is for NH proton adjacent to carbonyl group (Figure 3.14). These proton NMR peaks confirms the formation of products. The ^{13}C NMR spectrum peaks at 36, 121-132, and 163-167 ppm further confirms the formation of product (Figure 3.15).

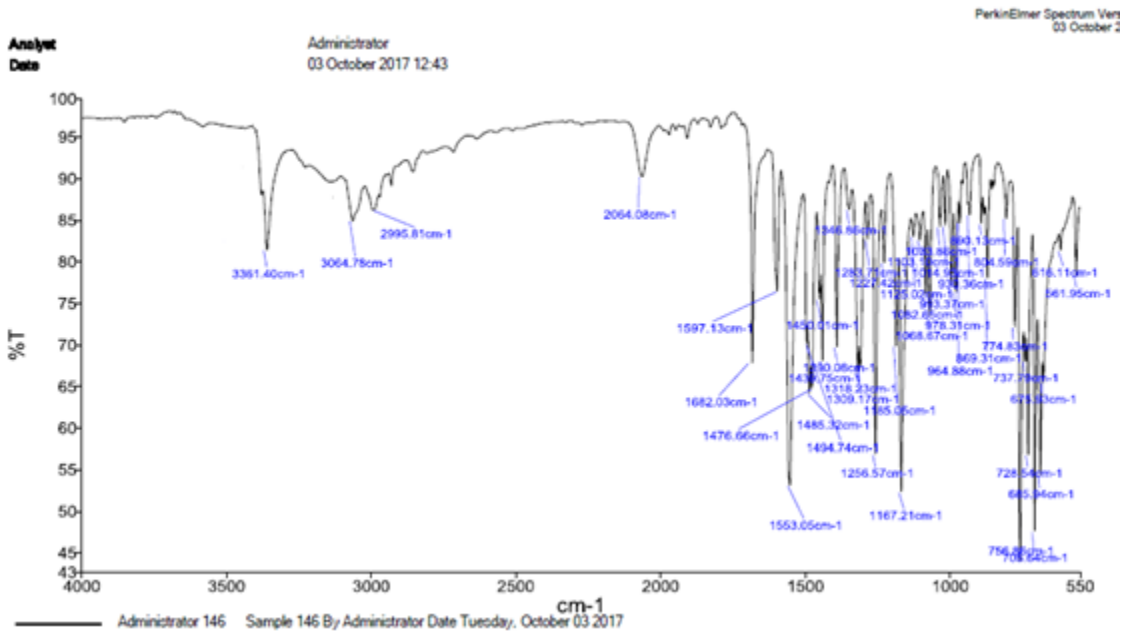


Figure 3.13: FTIR spectrum of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide (**16a**)

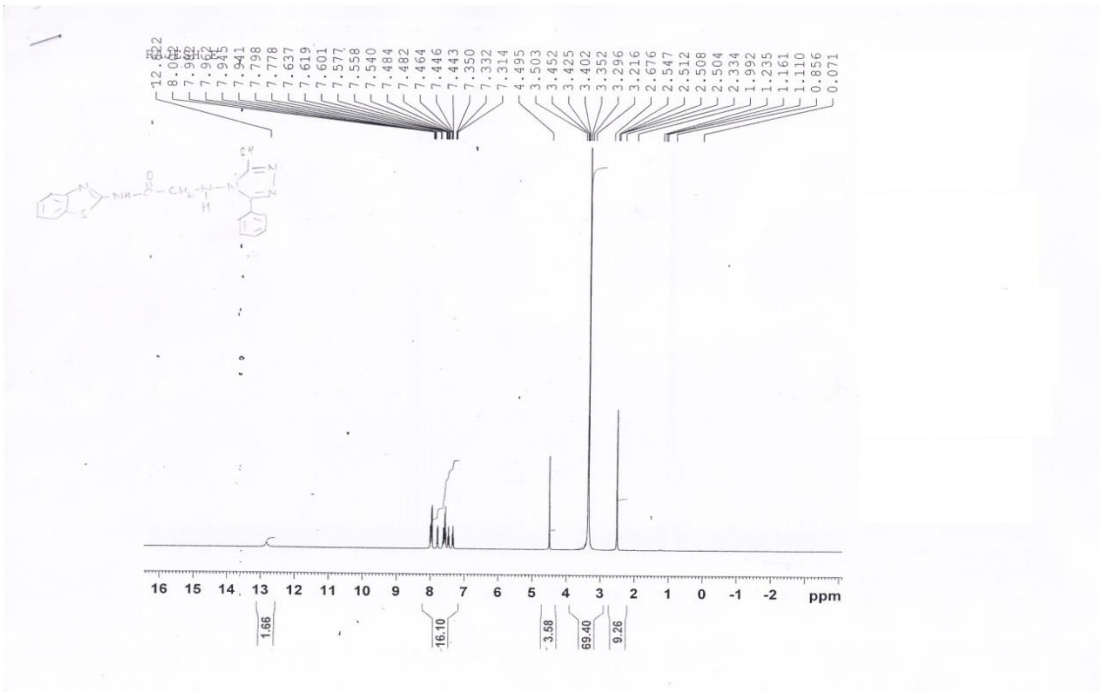


Figure 3.14: ^{1}H NMR spectrum of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)acetamide (**16a**)

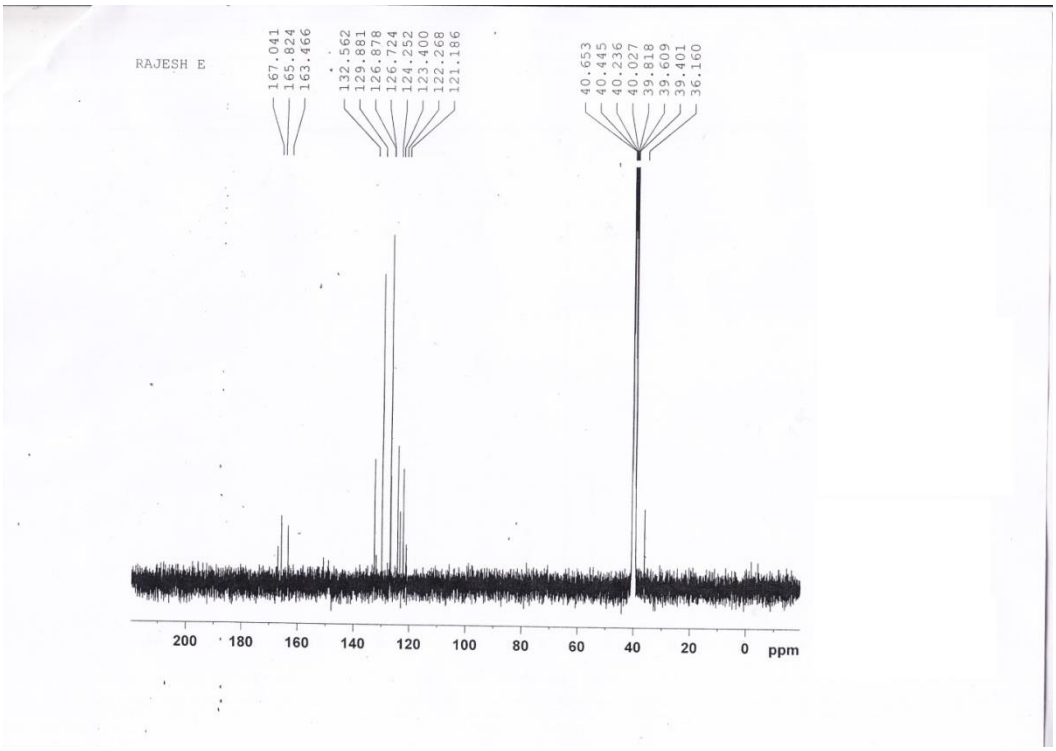


Figure 3.15: ^{13}C NMR spectrum of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)acetamide (**16a**)

3.3.2 *In-silico* interaction analysis

Potential binding profile of the novel synthesized compounds **(16a-i)** and compounds (**10, 15a-i**) into AChE and BuChE enzymes was studied by performing docking studies. In spite of diverse series of compounds, the docking scores of these molecules are quite high for almost all molecules. This may be an indication of inaccurate score calculation. *In-vitro* results have also indicated that none of the molecule is active against AChE (Table 3.3). However, a range of docking scores indicating favorable to unfavorable interactions are obtained from docking of diverse compounds against BuChE. Further, the docking results of BuChE are correlating well with the *in vitro* experimental studies (Table 3.3). Analysis of the docked structure revealed that the Trp 231 and Phe 329 makes an π-π interaction with benzene ring of benzothiazole moiety. Phe 329 also makes π-π interaction with the thiazole ring, the nitrogen atom of thiazole and oxygen atom of carbonyl makes a H-bond with Ser 198. His 438 makes an π-π interaction with phenyl ring present at 5-position of triazole and SH present at 2-position makes H-bond with Thr 120 in **16a (**Figure 3.16). In many compounds, we also observed hydrophobic and aromatic interactions among the compounds and enzyme indicating compounds good binding affinity with the BuChE. The docking results are in consistent with the in-vitro results (Table 3.3).

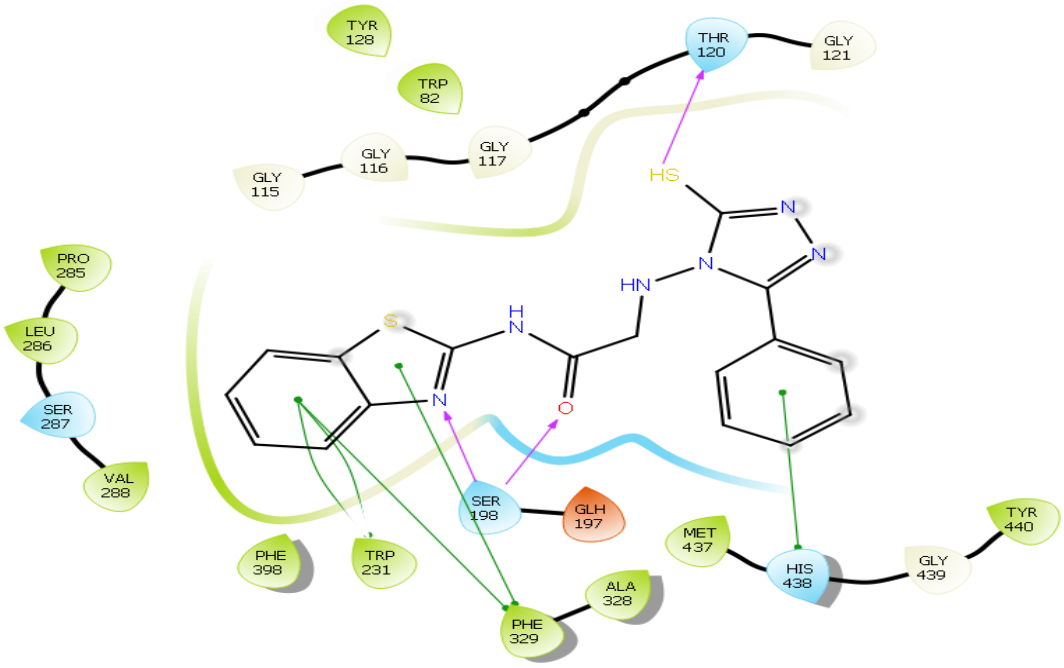


Figure 3.16: Interactions of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)acetamide with active site of BuChE (**16a**)

3.3.3 *In-vitro* inhibition studies of AChE and BuChE

The inhibiton activity of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)acetamide derivatives against AChE & BuChE are given in table 3.3. Based on the IC_{50} value, the synthesized compounds showed poor to no activity towards AChE, but a remarkably high activity towards BuChE. The better inhibition is being displayed by **16a**, **16b** and **16f** with IC_{50} value of 25.18, 95.52 and 83.25 μM respectively. The synthesized compounds are composed of two fragments: 4-amino-5-phenyl-4H-[1,2,4]triazole-3-thiol and 2-aminobenzothiazole joined *via* acetamide linkers. The IC_{50} value of 2-aminobenzothiazole (**10**) was found to be 691.26 μM and of 4-amino-5-phenyl-4H-[1,2,4]triazole-3-thiol (**15a**) 604.25 μM. On coupling both the moieties, the activity increases apparently. The data reveals that unsubstituted or no substitution on phenyl ring of triazole, and 4-fluoro or 4-methyl substitution on phenyl ring of triazole have increased the anti BuChE activity remarkably. Substitution of 4-Cl, 4-Br, 4-NO_2, 3-Br and 3,4,5-trimethoxy at phenyl ring in triazole showed moderate anti BuChE activity. On comparing **16d** and **16e**, 4-Br is found to be more active than 3-Br. It is useful to note that the substitution at 4-position on phenyl ring of triazole results in higher activity against BuChE than 3-substituted counterparts. It has also been also found that the compound **10** and substituted triazole (with exception **15b**) alone are inactive against both the enzyme.

Table 3.3: The IC_{50} value and docking score of synthesized compounds **10**, **15a-i** and **16a-i** against AChE and BuChE

	IC_{50} (μM)		**Docking Score**	
	AChE	**BuChE**	**AChE**	**BuChE**
10	2903.66 ± 234.97	691.26 ± 215.36	Inactive	Inactive
15a	1320.4 ± 140.09	604.25 ± 110.08	-5.63	-5.27
15b	974.86 ± 130.99	168.47 ± 56.24	-6.14	-6.43
15c	643.56 ± 9.47	1548.52 ± 68.89	-5.83	-5.97
15d	216.06 ± 7.84	1200.91 ± 103.98	-6.34	-5.98
15e	671.28 ± 71.53	1023.59 ± 23.28	-5.89	-5.67
15f	501.41 ± 0.82	1525.55 ± 366.47	-5.77	-5.96
15g	1247.40 ± 374.15	1441.27 ± 218.14	-6.24	-5.68
15h	748.89 ± 343.53	1382.56 ± 5.69	Not Docked	-5.27
15i	789.87 ± 21.55	1141.64 ± 466.62	Not Docked	Not Docked
16a	**606.43 ± 21.60**	**25.18 ± 22.10**	**-8.16**	**-9.79**
16b	**542.67 ± 60.02**	**95.52 ± 10.34**	**-6.82**	**-9.49**
16c	261.25 ± 19.31	403.66 ± 4.95	-7.52	-8.49
16d	534.36 ± 43.06	181.73 ± 60.56	-7.06	-9.42
16e	859.40 ± 32.62	479.80 ± 14.71	-8.91	-9.87
16f	**975.42 ± 81.72**	**83.25 ± 16.74**	**-7.06**	**-9.42**
16g	788.59 ± 27.59	ND	Not Docked	Not Docked
16h	3449.1 ± 556.77	318.01 ± 64.73	-5.95	-7.66
16i	520.67 ± 65.25	472.75 ± 0.04	Not Docked	Not Docked
Donepezil	0.042 ± 0.010	4.66 ± 0.503	155.30	-5.57

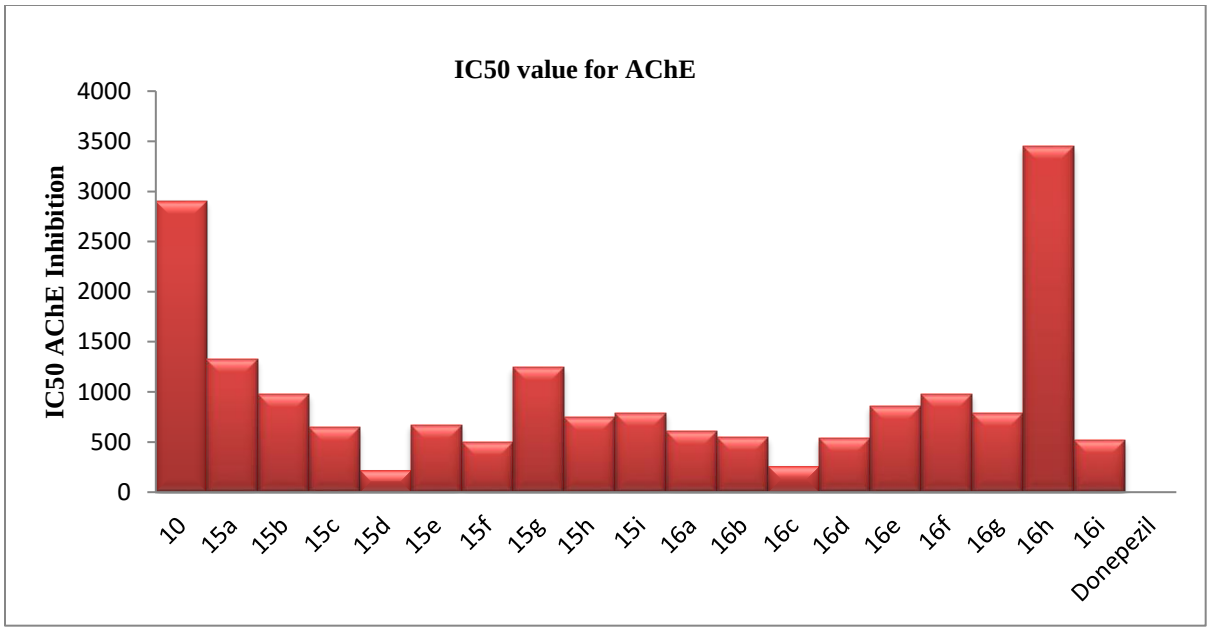


Figure 3.17: IC_{50} value in µM of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)acetamide derivatives against AchE enzyme. IC_{50} value less than 100 µM concentration is considered significant inhibition against AChE enzyme.

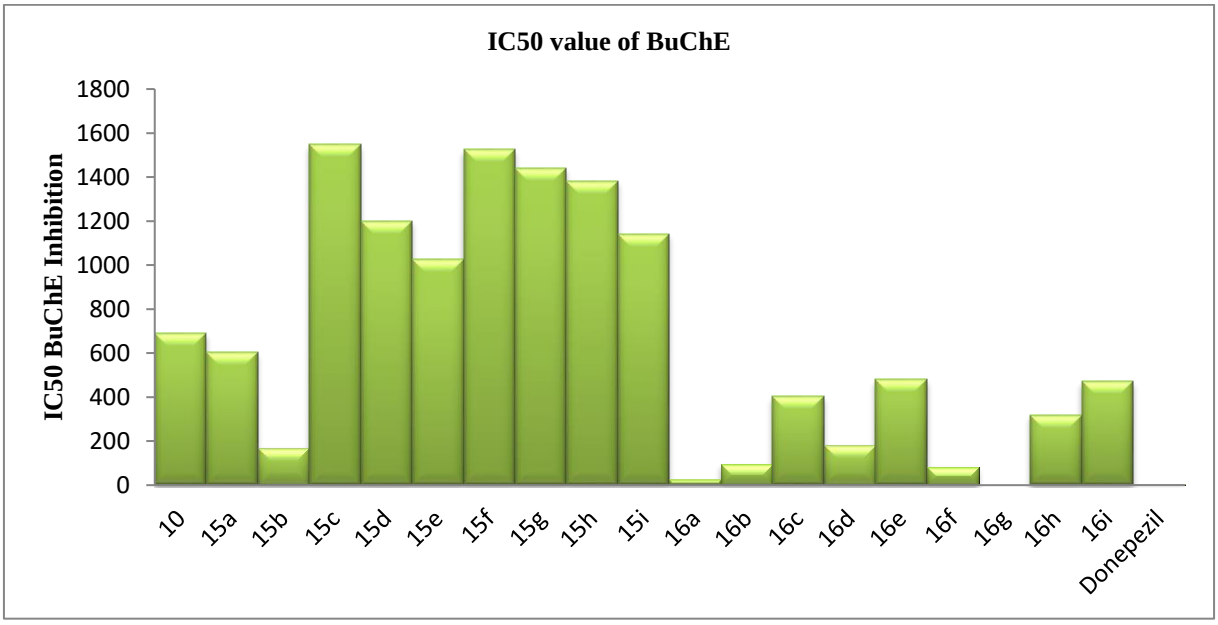


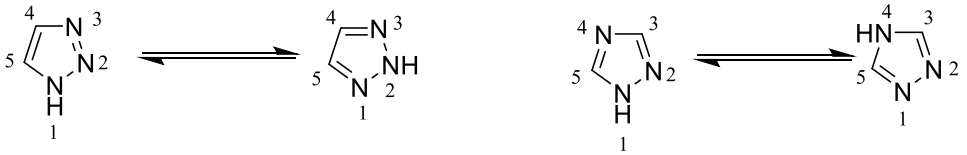
Figure 3.18: IC_{50} value in µM of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)acetamide derivatives against BuChE enzyme. IC_{50} value less than 100 µM concentration is considered significant inhibition against BuChE enzyme.

Chapter 4

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

4.1 Introduction

Triazoles are five membered heterocyclic compounds possessing three nitrogen atoms in the ring with molecular formula $C_2H_3N_3$.[217] The name of triazole compounds was first time described in 1885 by Bladin.[218,219] These compound exist in two isomeric forms: 1,2,3-triazoles and 1,2,4-triazoles (Figure 4.1).[218,219] Tautomerism is also possible in both the structural isomers of triazoles. 1,2,3-triazoles have two tautomeric forms, 1*H*-1,2,3-triazole and 2*H*-1,2,3-triazole whereas 4*H*-1,2,4-triazoles and 1*H*-1,2,4-triazoles are the tautomeric forms of 1,2,4-triazoles (Figure 4.1). The stability of triazole nucleus is due to the delocalization of its π electrons and aromatic nature (Figure 4.2). An aromatic sextet is formed by the contribution of one π electron from each double bonds present in the ring and the remaining two electrons from the lone pair of electrons at the nitrogen atom. This type of unique structure provide triazole derivatives to readily bind with a variety of enzymes and receptors in biological system through interactions such as hydrogen bonds, coordination bonds, ion-dipole, π-π stacking, cation-π, van der Waals force etc, and thus display a wide range of biological activities.[220-222] The 1,2,4-triazole derivatives have potential towards antibacterial, antifungal,[223,224] antimycobacterial,[225] anti-inflammatory,[226] analgesic,[227] anticancer,[228] antihypertensive,[229] anticonvulsant,[230] antiviral,[231] antidepressant,[232] antiasthmatic,[233] diuretic[234] and hypoglycemic[235] activities (Figure 4.3). The triazole ring have been used as an attractive linker to combine different pharmacophore fragments to produce new bifunctional drug molecules which are providing an efficient and convenient pathway to develop various bioactive and functional molecules.[236-238] A large number of triazole-based derivatives have been synthesized and investigated for their biological activities, [237, 239-242]



1H-1,2,3-triazole 2H-1,2,3-triazole 1H-1,2,4-triazole 4H-1,2,4-triazole

Figure 4.1: Isomeric forms of triazoles

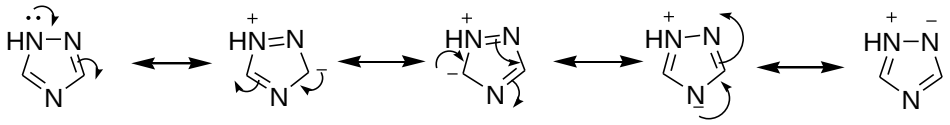


Figure 4.2: Resonating structures of 1,2,4-triazoles

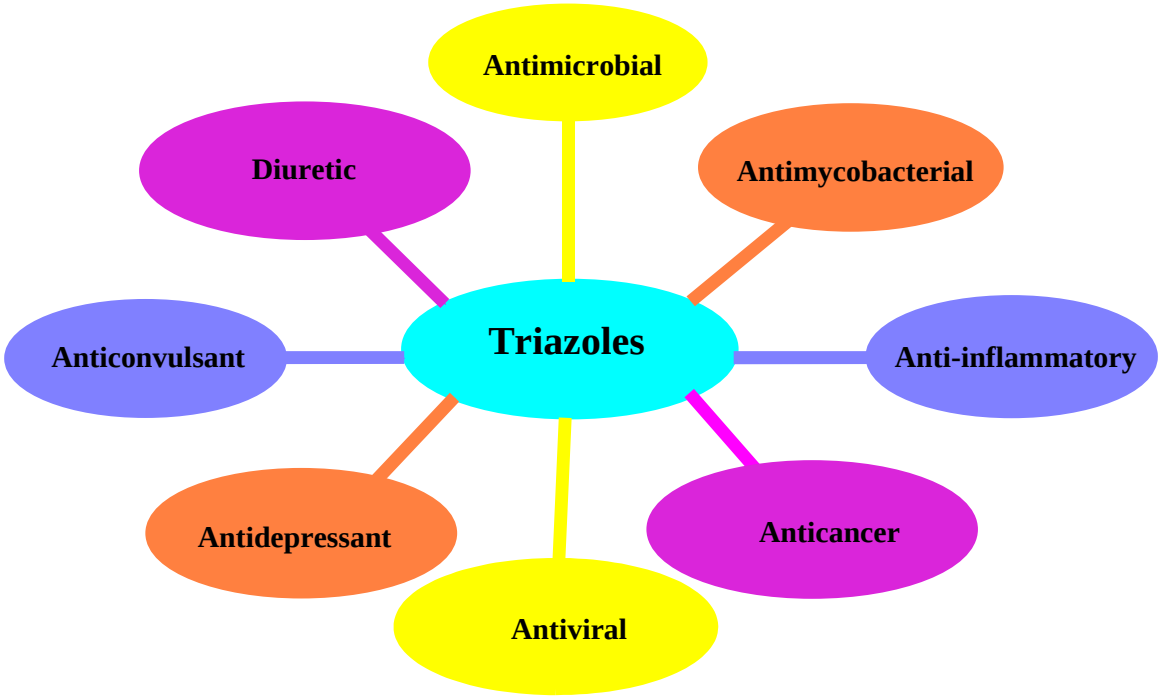


Figure 4.3: Pharmacological applications of triazole

Hypothesis of proposing coumarin-triazole conjugate as cholinesterase inhibitor

Coumarins, triazoles and their derivatives have been extensively studied as they exhibit antioxidative and antidepressant properties. Moreover, coumarin and thiazole derivatives are easy to synthesize and they possess good solubility, low cytotoxicity and excellent cell permeability. Therefore, it is expected that hybrid of coumarin-triazole would improve the potency as compared to coumarin or triazole alone. So, a series of coumarin-triazole conjugates (**26a-i**) using triazole as linker were designed on the basis of preliminary *in-silico* studies (Figure 4.4). These compounds were synthesized and then their activity was evaluated through *in-silico* and *in-vitro* studies. The substitution was further introduced at the 3rd position of coumarin as shown in figure 4.5.

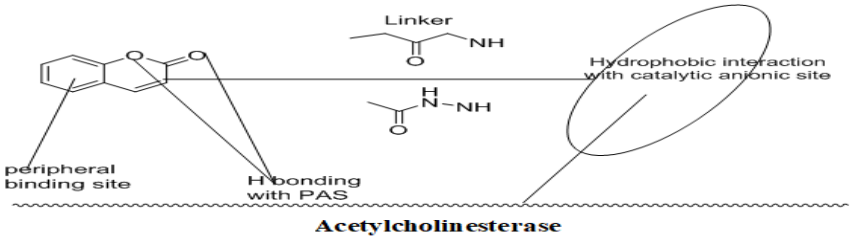


Figure 4.4: Possible interaction of triazole with AChE or BuChE. This conjugate interaction is used for design and synthesis potential lead molecules.

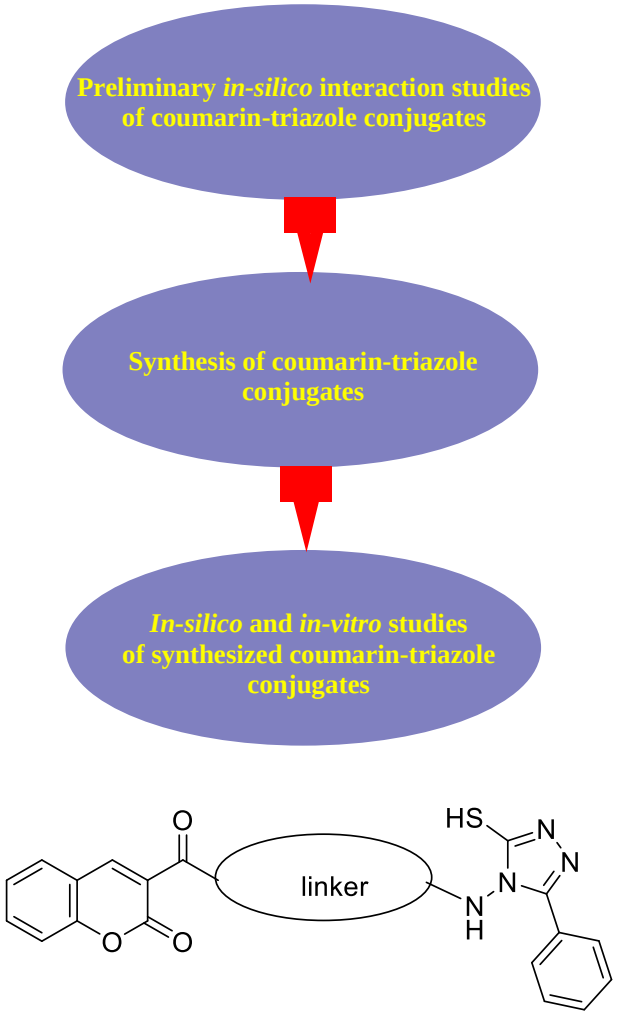


Figure 4.5: Flow chart indicating design, synthesis and evaluation of coumarin-triazole conjugates as cholinesterase inhibitor

4.2 Experimental

4.2.1 Preliminary *in-silico* studies of coumarin-triazole conjugates

Preliminary docking studies of the coumarin and triazole fragments with cholinesterase enzymes such as AChE (1EVE) and BuChE (4TPK) was performed using Glide module of Schrodinger. Favourable interactions of these fragments with these enzymes (AChE and BuChE) were identified. These fragments were interacted in different parts of the active site. On the basis of these results, we designed and synthesized N-(3-mercapto-5-phenyl-4H-1,2,4-triazole-4-yl)-2-oxo-2H-chromene-3-carboxamide derivatives (**26a-i**). These synthesized novel compounds were validated by carrying out *in-silico* and *in-vitro* studies.

4.2.2 Synthesis of N-(3-mercapto-5-phenyl-4H-1,2,4-triazole-4-yl)-2-oxo-2H-chromene-3-carboxamide derivatives (26a-i)

All commercially available solvents and reagents were purchased from reputed company and were used without further purification. Melting points were determined on a laboratory capillary melting apparatus and are uncorrected. FTIR spectra were recorded on a Perkin Elmer Spectrum Version 10.5.3 FTIR spectrophotometer. The ν_{max} are expressed in cm^{-1}. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker spectrophotometer and Jeol spectrophotometer (400/100MHz) using TMS as internal standard. The chemical shifts are expressed in ppm. The abbreviation s, d, t, q, m and bs stand for singlet, doublet, triplet, quartet, multiplet and broad singlet respectively. The elemental analysis was measured by PerkinElmer 2400. Thin-layer chromatography was performed on aluminium-coated silica plates purchased from Merck.

Synthesis of compound **26a-i** has been achieved by using the scheme 4.1. For this, we used the synthesized substituted triazoles of chapter 3 (Scheme 3.2) which are coupled with 2-oxo-2H-chromene-3-carboxylic acid (**25**).

Synthesis of 2-oxo-2H-chromene-3-carboxylic acid ethyl ester (24)

A solution of diethylmalonate (**23**, 3.6 mL, 23.5 mmol) in 15 mL ethanol was taken in a round bottom flask (250 mL). The solution was kept at $0^{o}C$ and further peperidine (0.2 mL, 2.0 mmol) was added. The resulting reaction mixture was stirred at $0^{o}C$ for 5 min followed by addition of salicylaldehyde (**1**, 2.5 mL, 23.5 mmol). The reaction mixture was allowed to come at room

temperature and stirred for 4 hours. The progress of reaction was monitored by TLC (hexane: ethyl acetate, 7:3, v/v). After completion of reaction, the excess of solvent was removed under reduces pressure and extracted with ethyl acetate: dil HCl: water (5:1:4, 3×40 ml). The organic layer was dried over anhydrous sodium sulphate and was concentrated under reduced pressure. The residue was purified by column chromatography (silica gel 60-120 mesh as stationary phase and hexane: ethyl acetate, 4:1, v/v, as a mobile phase) to get the pure product.

Yield: 90%; Mp.: 90-94^{o}C (Lit Mp.:[243] 91-92^{o}C); FTIR (KBr): 3066, 2980, 2916, 1760, 1615, 1563, 1451, 1373, 1295, 1111, 1033, 982, 878, 785, 629 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.40 (t, 3H, CH_3), 4.40 (q, 2H, CH_2), 7.31-7.66 (m, 4H, ArH), 8.52 (s, 1H, H of pyran ring).

Synthesis of 2-oxo-2H-chromene-3-carboxylic acid (25)

A solution of 2-oxo-2H-chromene-3-carboxylic acid ethyl ester (**24**, 3 g, 13.7 mmol) in 6 mL absolute ethanol were taken in a round bottom flask (250 mL), and then added aq NaOH (0.5%, 25 mL). The reaction mixture was set for refluxing for 2 hours. The colour of reaction mixture was changed to orange. The progress of reaction was monitored by TLC (chloroform: methanol, 8:2, v/v). After completion of reaction HCl was added to make the solution acidic (pH = 2). Off white precipitate was obtained which was filtered and washed with water (3×50 mL). The product was further recrystalized by using absolute ethanol.

Yield: 86%; Mp: 188-190^{o}C (Lit Mp.[244] 189-192^{o}C); FTIR (KBr): 3450, 3059, 2781, 1738, 1672, 1565, 1489, 1373, 1299, 1145, 1039, 985, 882, 767, 645 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.39-7.92 (m, 4H, ArH), 8.75 (s, 1H, H of pyran ring), 13.24 (bs, 1H, COOH).

General procedure for the synthesis of N-(3-mercapto-5-phenyl-4H-1,2,4-triazole-4-yl)-2-oxo-2H-chromene-3-carboxamide derivatives (26a-i)

A solution of 2-oxo-2H-chromene-3-carboxylic acid (**25**, 0.52 mmol) in 3 mL DMF was taken in a round bottom flask (25 mL). The solution was kept on ice bath and added HOBt (0.52 mmol). The resulting mixture was stirred at 0-5^{o}C for 20 min. After cooling, EDC (0.78 mmol) was added. The reaction mixture was stirred for half an hour to get white precipitate of EDU. The reaction mixture was removed from ice bath and substituted triazoles (**12**, 0.63 mmol) was added. The reaction mixture was refluxed for 24 hours. The progress of reaction was monitored by TLC (hexane: ethyl acetate, 8:2, v/v). After completion of reaction the residue was extracted with ethyl acetate (3×50 ml). The organic layer was dried over anhydrous sodium sulphate and

concentrated under reduced pressure. The residue obtained was purified by silica gel column chromatography (Silica gel 60-120 mesh as stationary phase and hexane: ethyl acetate, 1:1, v/v, as mobile phase) to get the desired products.

N-(3-mercapto-5-phenyl-4H-1,2,4-triazole-4-yl)-2-oxo-2H-chromene-3-carboxamide (26a)

Yield: 67%; Mp.: 188-192^{o}C.; FTIR (KBr): 3308, 3136, 2960, 2834, 1737, 1682, 1580, 1451, 1374, 1256, 1134, 1027, 923, 864, 742, 646 cm^{-1}; ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) δ: 1.36 (bs, 1H, SH), 7.22-7.49 (m, 7H, ArH), 8.01-8.03 (m, 2H, ArH), 8.72 (s, 1H, Hof pyran ring); ^{13}C NMR (DMSO-*d*$_{6,}$ 100 MHz) δ: 115, 118, 120, 124, 126 (2C, ArH), 129 (2C, ArH), 131, 132 (2C), 133, 148, 151, 163, 164, 169; Anal. calc. for $C_{18}H_{12}N_4O_3S$: C, 59.33; H, 3.32; N, 15.38; S, 8.80; found C, 59.28; H, 3.29; N, 15.34; S, 8.76.

N-[3-(4-fluorophenyl)-5-mercapto-4H-1,2,4-triazol-4-yl]-2-oxo-2H-chromene-3-carboxamide (26b)

Yield: 58%; Mp.: 194-198^{o}C.; FTIR (KBr): 3317, 3118, 2997, 2828, 1714, 1682, 1576, 1462, 1320, 1248, 1112, 1027, 942, 841, 740, 692 cm^{-1}; ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) δ: 1.39 (bs, 1H, SH), 7.28-7.98 (m, 8H, ArH), 8. 71 (s, 1H, H of pyran ring); ^{13}C NMR (DMSO-*d*$_{6,}$ 100 MHz) δ: 114, 116, 118, 120, 123 (2C, ArH), 126, 128 (2C, ArH)), 134 (2C, ArH), 142, 159, 163, 166, 169; Anal. calc. for $C_{18}H_{11}N_4O_3SF$: C, 56.54; H, 2.90; N, 14.65; S, 8.38; found C, 56.49; H, 2.87; N, 14.61; S, 8.30.

N-[3-(4-chlorophenyl)-5-mercapto-4H-1,2,4-triazol-4-yl]-2-oxo-2H-chromene-3-carboxamide (26c)

Yield: 61%; Mp.: 202-206^{o}C.; FTIR (KBr): 3323, 3125, 2987, 2836, 1721, 1676, 1582, 1476, 1328, 1242, 1110, 1018, 936, 845, 731, 672 cm^{-1}; ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) δ: 1.34 (bs, 1H, 7.32-7.88 (m, 8H, ArH), 8.70 (s, 1H, H of pyran ring); ^{13}C NMR (DMSO-*d*$_{6,}$ 100 MHz) δ: 113, 116, 118, 121, 124 (2C), 127, 131(2C, ArH), 135 (2C ArH), 140, 156, 159, 163, 167; Anal. calc. for $C_{18}H_{11}N_4O_3SCl$: C, 54.21; H, 2.78; N, 14.05; S, 8.04 found C, 54.17; H, 2.69; N, 13.98; S, 8.01.

N-[3-(4-bromophenyl)-5-mercapto-4H-1,2,4-triazol-4-yl]-2-oxo-2H-chromene-3-carboxamide (26d)

Yield: 56%; Mp.: 210-214; FTIR (KBr): 3316, 3128, 2994, 2831, 1731, 1681, 1587, 1452, 1333, 1236, 1124, 1032, 938, 836, 735, 631 cm^{-1}; ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) δ: 1.37 (bs, 1H, SH),

7.39-7.96 (m, 8H, ArH), 8.71 (s, 1H, H of pyran ring); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 112, 115, 119, 122, 126 (2C, ArH), 129, 134 (2C, ArH), 137 (2C, ArH), 142, 154, 157, 160, 169; Anal. calc. for $C_{18}H_{11}N_4O_3SBr$: C, 48.77; H, 2.50; N, 12.64; S, 7.23; found C, 48.72; H, 2.44; N, 12.61; S, 7.19.

N-[3-(3-bromophenyl)-5-mercapto-4H-1,2,4-triazol-4-yl]-2-oxo-2H-chromene-3-carboxamide (26e)

Yield: 52%; Mp.: 194-196ºC.; FTIR (KBr): 3312, 3126, 2952, 2830, 1726, 1685, 1584, 1472, 1321, 1246, 1122, 1032, 942, 833, 741, 679cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.36 (bs, 1H, SH), 7.39-8.01 (m, 8H, ArH, 8.72 (1H, H of pyran ring); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 112, 114, 117, 120, 123, 126 (2C, ArH), 128 (2C, ArH), 133 (2C, ArH), 136 (2C, ArH), 148, 152, 160, 166, 169; Anal. calc. for $C_{18}H_{11}N_4O_3SBr$: C, 48.77; H, 2.50; N, 12.64; S, 7.23; found C, 48.70; H, 2.45; N, 12.60; S, 7.20.

N-[3-(4-methylphenyl)-5-mercapto-4H-1,2,4-triazol-4-yl]-2-oxo-2H-chromene-3-carboxamide (26f)

Yield: 57%; Mp.: 201-206ºC.; FTIR (KBr): 3326, 3099, 2967, 2847, 1722, 1677, 1589, 1467, 1328, 1241, 1118, 1029, 939, 831, 745, 672 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.35 (bs, 1H, SH), 2.32(s, 3H, CH_3), 7.37-7.97 (m, 8H, ArH), 8.70 (s, 1H, H of pyran); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 23, 113, 114, 118, 120, 124 (2C, ArH), 128 (2C, ArH), 132 (2C, ArH), 135, 147, 156, 161, 165, 168; Anal. calc. for $C_{19}H_{14}N_4O_3S$: C, 60.31; H, 3.73; N, 14.81; S, 8.47; found C, 60.28; H, 3.69; N, 14.76; S, 8.42.

N-[3-(4-methoxyphenyl)-5-mercapto-4H-1,2,4-triazol-4-yl]-2-oxo-2H-chromene-3-carboxamide (26g)

Yield: 56%; Mp.: 214-218ºC.; FTIR (KBr): 3307, 3131, 2967, 2831, 1728, 1680, 1578, 1461, 1319, 1254, 1110, 937, 842, 738, 687 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.38 (bs, 1H, SH), 3.78 (s, 3H, OCH_3), 7.12-7.94 (m, 8H, ArH), 8.72 (s, 1H, H of pyran ring); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 54, 112, 114, 118, 121, 123 (2C, ArH), 129 (2C, ArH), 132 (2C, ArH), 134, 145, 158, 162, 165, 169; Anal. calc. for $C_{19}H_{14}N_4O_4S$: C, 57.86; H, 3.58; N, 14.21; S, 8.13 found C, 57.79; H, 3.52; N, 14.18; S, 8.09.

N-[3-(4-nitrophenyl)-5-mercapto-4H-1,2,4-triazol-4-yl]-2-oxo-2H-chromene-3-carboxamide (26h)

Yield: 60%; Mp.: 235-240^{o}C.; FTIR (KBr): 3316, 3096, 2987, 1721, 1682, 1570, 1464, 1348, 1265, 1121, 930, 839, 731, 678 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.36 (bs, 1H, SH), 7.36-8.19 (m 8H, ArH), 8.71 (s, 1H, H of pyran ring); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 112, 114, 118, 121, 124 (2C ArH), 128, 131 (2C, ArH), 135, 148, 157, 161, 164 (C=O); Anal. calc. for $C_{18}H_{11}N_5O_5S$: C, 52.81; H, 2.71; N, 17.11; S, 7.83; found C, 52.77; H, 2.69; N, 17.08; S, 7.76.

N-[3-(3,4,5-trimethoxyphenyl)-5-mercapto-4H-1,2,4-triazol-4-yl]-2-oxo-2H-chromene-3-carboxamide (26i)

Yield: 50%; Mp.: 165-170^{o}C.; FTIR (KBr): 3311, 3126, 2994, 1719, 1676, 1575, 1468, 1351, 1276, 1101, 1086, 928, 822, 746, 671 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.38 (bs, 1H, SH), 3.68 (s, 3H, OCH_3), 3.86 (s, 6H, OCH_3), 6.93 (d, 2H, J = 3.6 Hz), 7.41-7.96 (m, 4H, ArH), 8.72 (s, 1H, H of pyran ring); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 54 (C, of OCH_3), 62 (2C, of OCH_3), 110, 114, 116, 118, 120, 124, 126, 129 (2C, of ArH), 138, 154 (2C, of ArH), 156, 162, 169 (C=O); Anal. calc. for $C_{21}H_{18}N_4O_6S$: C, 55.50; H, 3.99; N, 12.33; S, 7.05; found C, 55.44; H, 3.96; N, 12.29; S, 7.01.

4.2.2 *In- silico* (Docking) studies

Geometries of the compounds **25** and **26a-i** were optimized at the level B3LYP/6-31G* using Gaussian 09 quantum chemistry software (http://gaussian.com/). The global minima of the structures were verified using vibrational frequencies. Crystal structure of the protein AChE (PDB Id: 1EVE) was downloaded from protein data bank (PDB: www.rcsb.org). Though many structures of AchE are available, but the above protein structure from *Tetronarce californica* organism was opted as assay used for *in vitro* experiment was also carried on enzyme from the same organism. Similarly for BuChE structure PDB Id (4TPK) was used.

Before docking the ligand molecules and enzymes were prepared by Glide 'ligprep' and 'Protein preparation' modules respectively. The ligand was refined in torsional space using the force field OPLS3 (Glide XP) with a distance-dependent dielectric model. Finally, a small number of poses were minimized within the field of the receptor with full ligand flexibility. The Glide module of Schrodinger uses high throughput virtual screening (HTVS), standard Precision

(SP) and Xtra precision (XP) docking methodologies. As the last one provided more appropriate results, the current study provided XP docking score for all the ligands (Table 4.1).

4.2.3 *In-vitro* experimental studies

Inhibition of acetylcholinesterase (AChE) and butrylcholinesterase (BuChE) activity assay

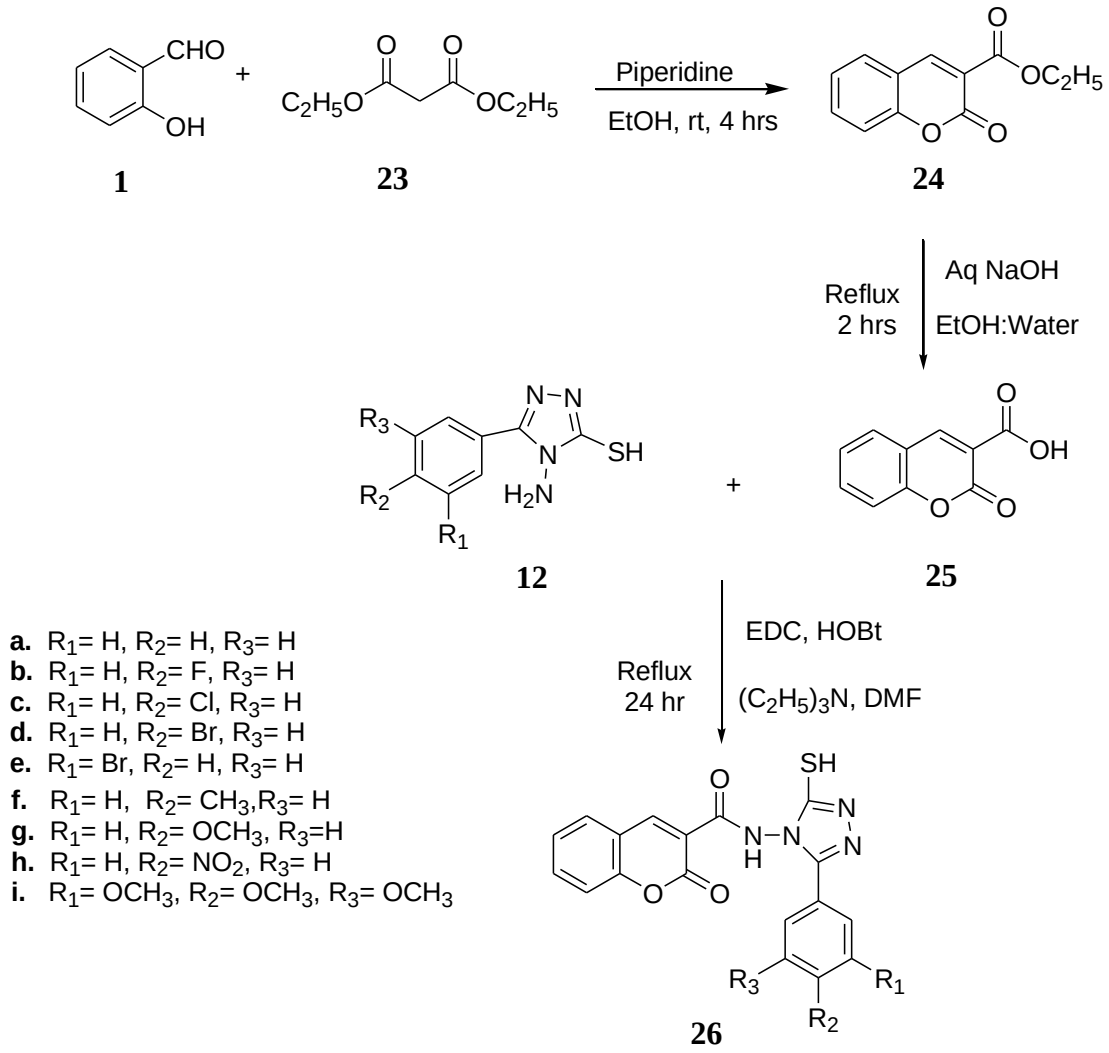
In this section the synthesized molecules were tested for AChE and BuChE inhibitory activity according to the method previously described by Najafi et al.[145] Enzyme inhibition assay was performed in a 96-well plate by using DTNB method. Briefly, 25 µL AChE/BuChE (25 mU in 100 µM PBS) was incubated with 75 µL DTNB (in 100 µM PBS, having 600 µM $NaHCO_3$) for 5 min at room temperature. To this, 25 µL of test compounds (1 – 1000 µM), and 50 µL PBS (pH 7.4) were added. The reaction mixture was then incubated for 15 min at room temperature. Reaction was initiated by adding 25 µL of acetylthiocholine iodide and butylthiocholine (75 mM in PBS) for AChE and BuChE inhibitory assay respectively. Change in absorbance was recorded spectrophotometrically during the experimental duration of 4 min at 412 nm by using UV-spectrophotometer. A blank reaction was run simultaneously, which was having 25 µL solvent (1% DMSO) in place of drugs. Percent inhibition of AChE activity was calculated by using following equation

$$\%\text{AChE/BuChE inhibition} = \frac{(Absorbance\ of\ control - Absorbance\ of\ test) \times 100}{Absorbance\ of\ control}$$

4.3 Results and discussion

4.3.1 Synthesis of N-(3-mercapto-5-phenyl-4H-1,2,4-triazole-4-yl)-2-oxo-2H-chromene-3-carboxamide derivatives (26a-i)

The synthesis of N-(3-mercapto-5-phenyl-4H-1,2,4-triazole-4-yl)-2-oxo-2H-chromene-3-carboxamide derivatives (**26a-i**) have been achieved by schemes 4.1. The synthesized compounds were characterized by FTIR, ^{1}H NMR, ^{13}C NMR and elemental analysis.



a. R_1= H, R_2= H, R_3= H
b. R_1= H, R_2= F, R_3= H
c. R_1= H, R_2= Cl, R_3= H
d. R_1= H, R_2= Br, R_3= H
e. R_1= Br, R_2= H, R_3= H
f. R_1= H, R_2= CH_3, R_3= H
g. R_1= H, R_2= OCH_3, R_3=H
h. R_1= H, R_2= NO_2, R_3= H
i. R_1= OCH_3, R_2= OCH_3, R_3= OCH_3

Scheme 4.4: Synthesis of 2-oxo-2H-chromene-3-carboxylic acid (3-mercapto-5-phenyl-[1,2,4]triazole-4-yl)-amide derivatives (**26**)

Synthesis of 2-oxo-2H-chromene-3-carboxylic acid ethyl ester (24)

The synthesis of coumarin derivatives was already discussed in chapter 2. In this chapter we follow the same procedure for the synthesis of 2-oxo-2H-chromene-3-carboxylic acid ethyl ester (**24**). In present chapter we have taken diethylmalonate instead of ethylacetoacete for the synthesis of **24**. Piperidine, which is a mild base having pKb value 2.9 abstract proton from the active methylene group of diethylmalonate to give resonance stabilized carbanion which attacks the carbonyl group of salicylaldehyde to undergo nucleophilic addition reaction and subsequently dehydration to give the desired crude product **24**. The impure product was purified by column chromatography. The product was further recrystalized from absolute ethanol and

characterized by spectroscopic data. The absorption at 3066, 2980 and 1760 cm^{-1} in the FTIR spectrum of **24** have been assigned for C-H stretching of ArH, C=O stretching of α, β unsaturated ester respectively (Figure 4.6). In the ^{1}H NMR spectrum, a triplet at 1.40 ppm for three CH_3 protons adjacent to OCH_2, a quartet at 4.40 for two OCH_2 protons, a singlet at 8.64 ppm for one H proton of pyran ring and the four aromatic protons appeared in the region 7.40-7.96 ppm (Figure 4.7).

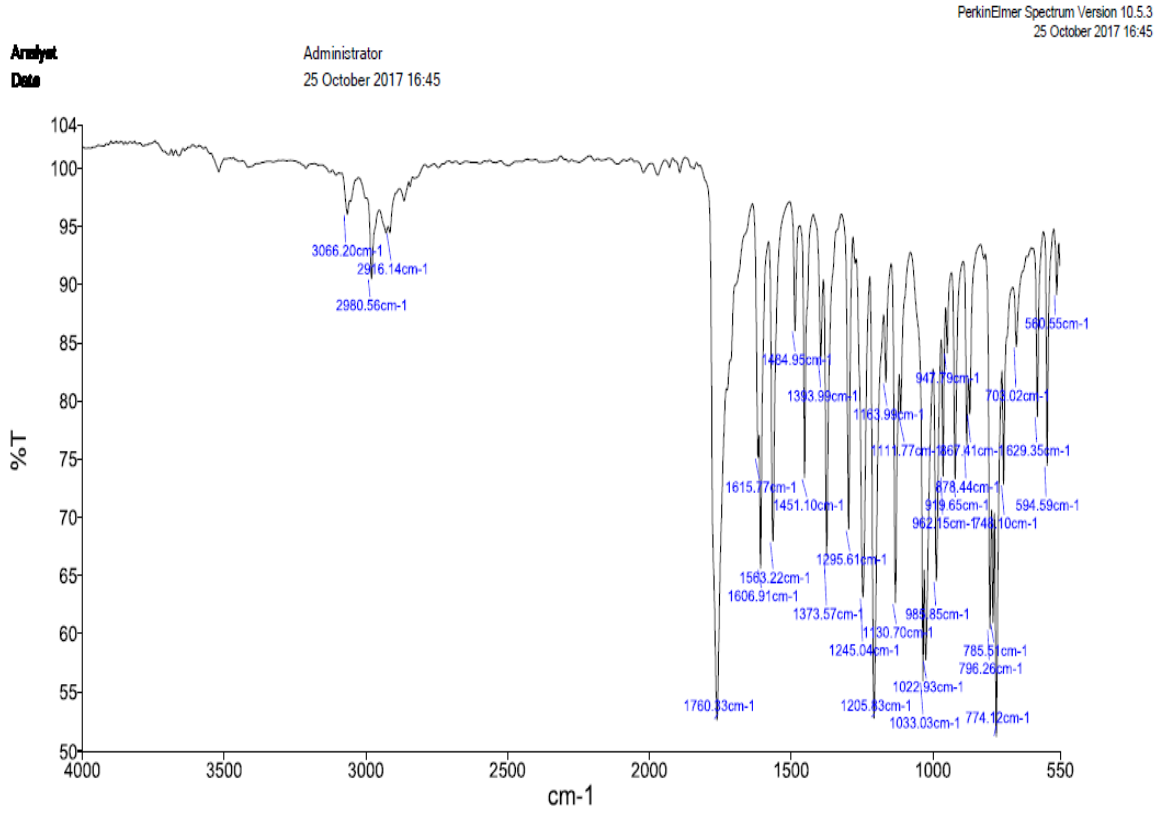


Figure 4.6: FTIR spectrum of 2-oxo-2H-chromene-3-carboxylicacidethyl ester (**24**)

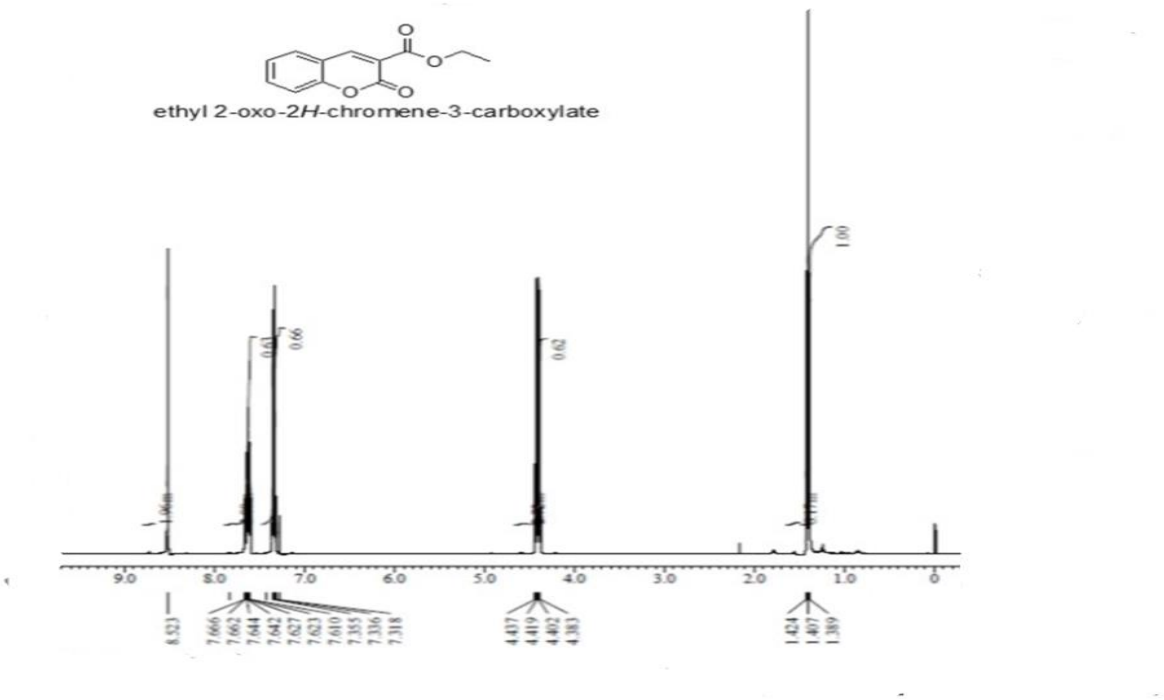


Figure 4.7: ^{1}H NMR spectrum of 2-oxo-2H-chromene-3-carboxylicacidethyl ester (**24**)

Synthesis of 2-oxo-2H-chromene-3-carboxylic acid (25)

The 2-oxo-2H-chromene-3-carboxylic acid was synthesized by basic hydrolysis of **24**. The known process reported for the hydrolysis of ester is acidic hydrolysis or basic hydrolysis. Initially, we followed the both procedure for the hydrolysis of ester but we found that hydrolysis under acidic condition, gave low yield of product as compared to the hydrolysis under basic condition. A solution of compound **24** was taken in 6 mL of absolute ethanol and the (0.5%, 25 mL) NaOH was added, after addition of all NaOH, the reaction mixture was set for refluxing for 2 hours. During hydrolysis the colour of reaction mixture was changed from yellow to orange. After completion of reaction the product was precipitated by addition of concentrated solution of HCl. The product was washed with water (4×50 ml) and dried. This was further characterized by spectroscopic data. The absorption at 3450, 3059 and 1738 cm^{-1} in the FTIR spectrum of **25** have been assigned for O-H stretching for carboxylic group, C-H stretching of ArH and C=O stretching of carboxylic group respectively (Figure 4.8). In the ^{1}H NMR spectrum the four aromatic protons appeared in the region 7.39-7.92 ppm, a singlet at 8.75 ppm is for one proton of

pyran ring and a broad singlet at 13.24 ppm for COOH protons further confirms the presence of carboxylic group which are formed after the hydrolysis of ester, (Figure 4.9).

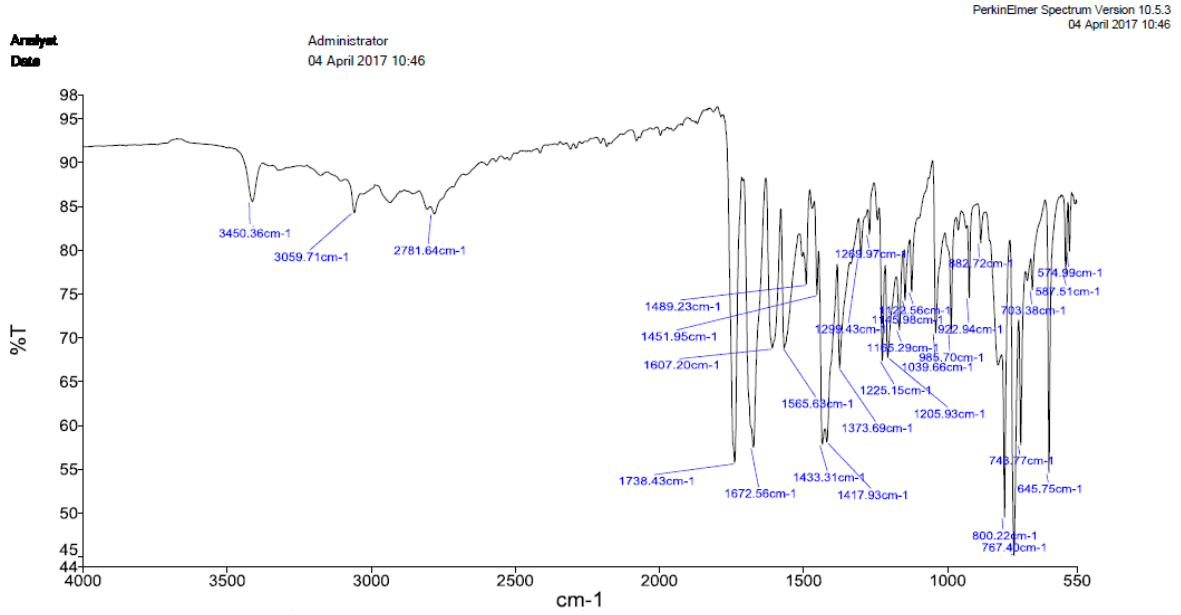


Figure 4.8: FTIR spectrum of 2-oxo-2H-chromene-3-carboxylic acid (**25**)

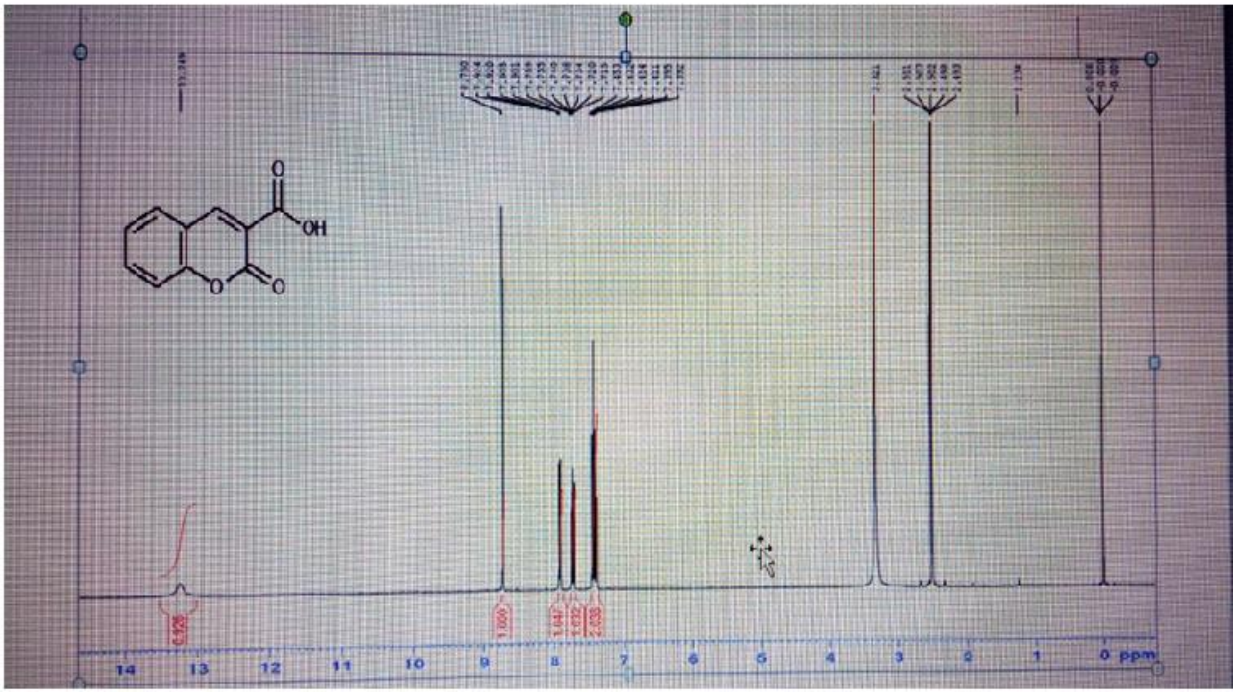


Figure 4.10: ^{1}H NMR spectrum of 2-oxo-2H-chromene-3-carboxylicacid (**25**)

Synthesis of N-(3-mercapto-5-phenyl-4H-1,2,4-triazole-4-yl)-2-oxo-2H-chromene-3-carboxamide(26a-i)

N-(3-mercapto-5-phenyl-4H-1,2,4-triazole-4-yl)-2-oxo-2H-chromene-3-carboxamide (**26a-i**) were synthesized by reaction between compound (**25**) and substituted triazoles (**12a-i**) in presence of EDC/HoBt (Scheme 4.1). The coupling took place through amide bond formation using 1-ethyl-3-(3′-dimethylaminopropyl)-carbodiimidehydrochloride (EDC) as coupling agents in the presence of additives hydroxybezotriazole (HOBt). Several coupling reagent have been reported in literature for reaction between carboxylic acid and amines, in which carboxylic group are activated by variety of activating agent.[245] The use of N,N′-dicyclohexylcarbodiimide (DCC), for the formation of amide and other peptide bonds was first reported by Sheehan and Hess in 1955.[246] The additive used to stop the isomerization leading to the formation of N-aceylurea from O-aceylurea. In absence of additive this isomerisation reduces yield of the product. The first step involves the reaction of carboxylic group with EDC to form O-acylurea. This O-aceyl urea on reaction with additives give anhydride which on reaction with amine in presence of mild base triethyl amine gives the coupled product as shown in mechanism, figure 4.10. The products were purified by column chromatography and characterized by spectroscopic techniques including elemental analysis. The absorption at 3308, 3136, 2960and 1682 cm^{-1} in the FTIR spectrum of N-(3-mercapto-5-phenyl-4H-1,2,4-triazole-4-yl)-2-oxo-2H-chromene-3-carboxamide (**26a**) have been assigned for N-H stretching, C-H stretching of ArH and C=O stretching for amide group respectively (Figure 4.11). In the ^{1}H NMR spectrum, a broad singlet in the region 1.36 ppm is for one SH proton, a multiplet in the region 7.22-7.49 is for seven aromatic protons and multiplet in the region at 8.01-8.03 ppm is for two aromatic protons and singlet at 8.72 ppm is for proton at pyran ring confirms the formation of product (Figure 4.12). The spectrum 115, 118, 120, 121, 124, 126, 129, 131, 132, 133, 148, 151, 163, 164, 169 in ^{13}C NMR further confirms the formation of product (Figure 4.13). Similarly, the other compounds **26b** to **26i** were synthesized and characterized.

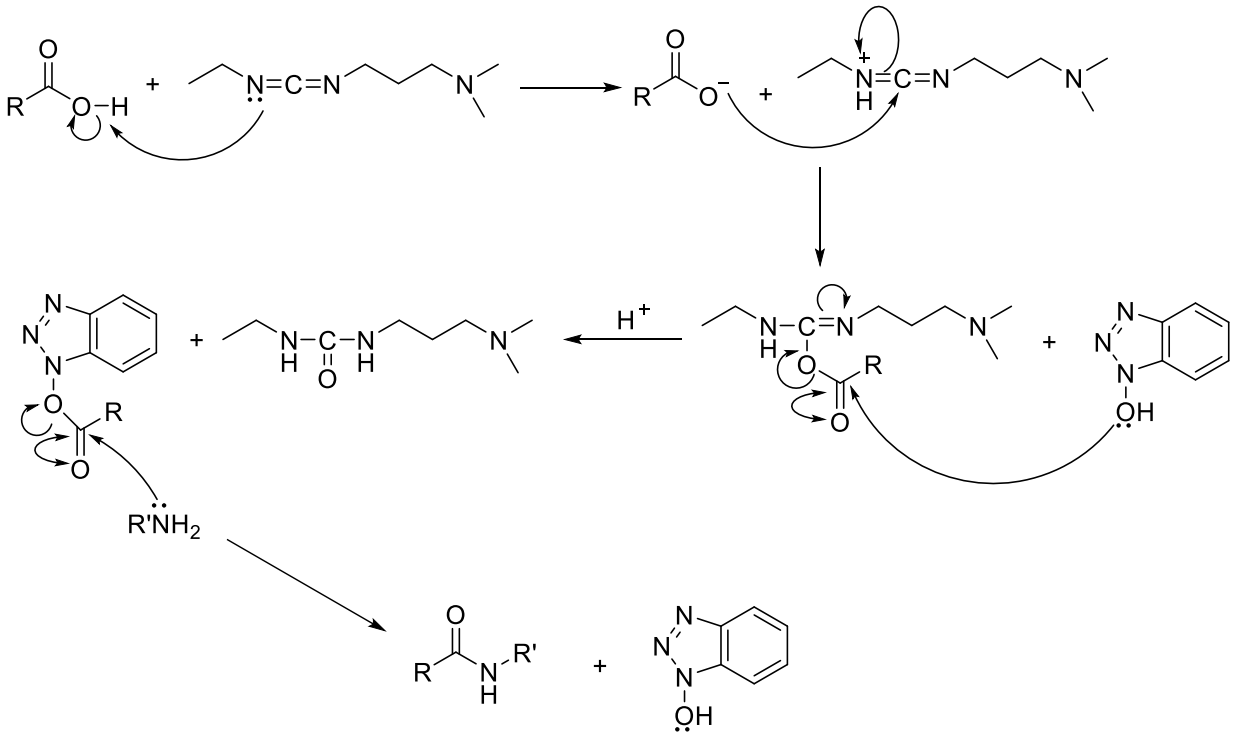


Figure 4.10: Mechanism for synthesis of N-(3-mercapto-5-phenyl-4H-1,2,4-triazole-4-yl)-2-oxo-2H-chromene-3-carboxamide (**26**)

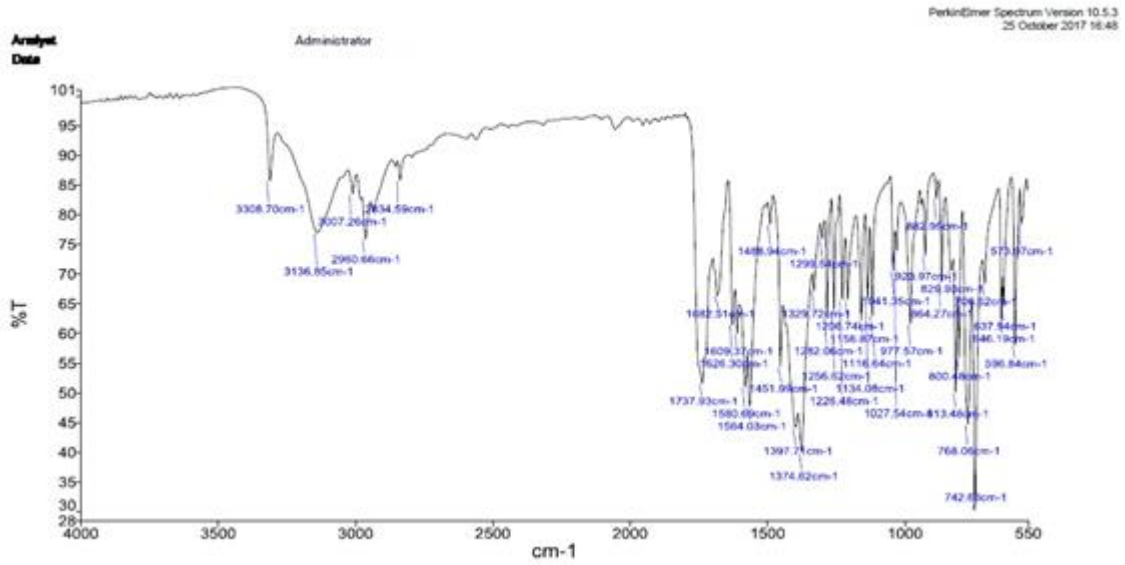


Figure 4.11: FTIR spectrum of N-(3-mercapto-5-phenyl-4H-1,2,4-triazole-4-yl)-2-oxo-2H-chromene-3-carboxamide (**26a**)

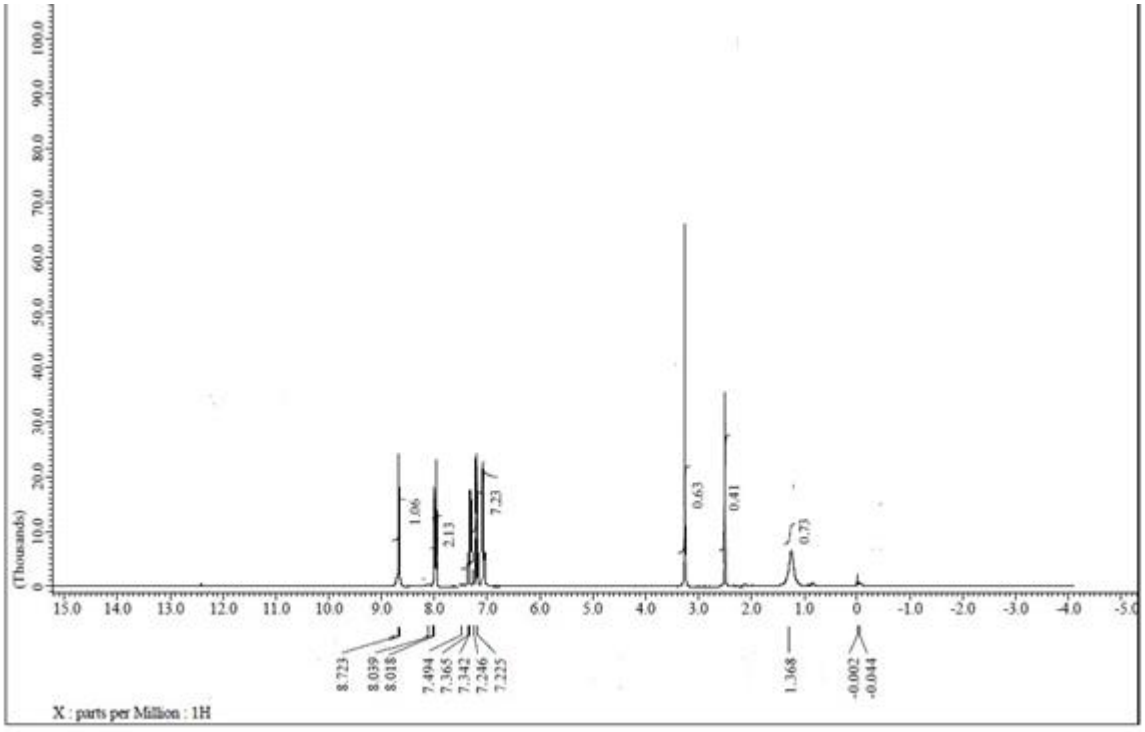


Figure 4.12: ^{1}H NMR spectrum of N-(3-mercapto-5-phenyl-4H-1,2,4-triazole-4-yl)-2-oxo-2H-chromene-3-carboxamide (**26a**)

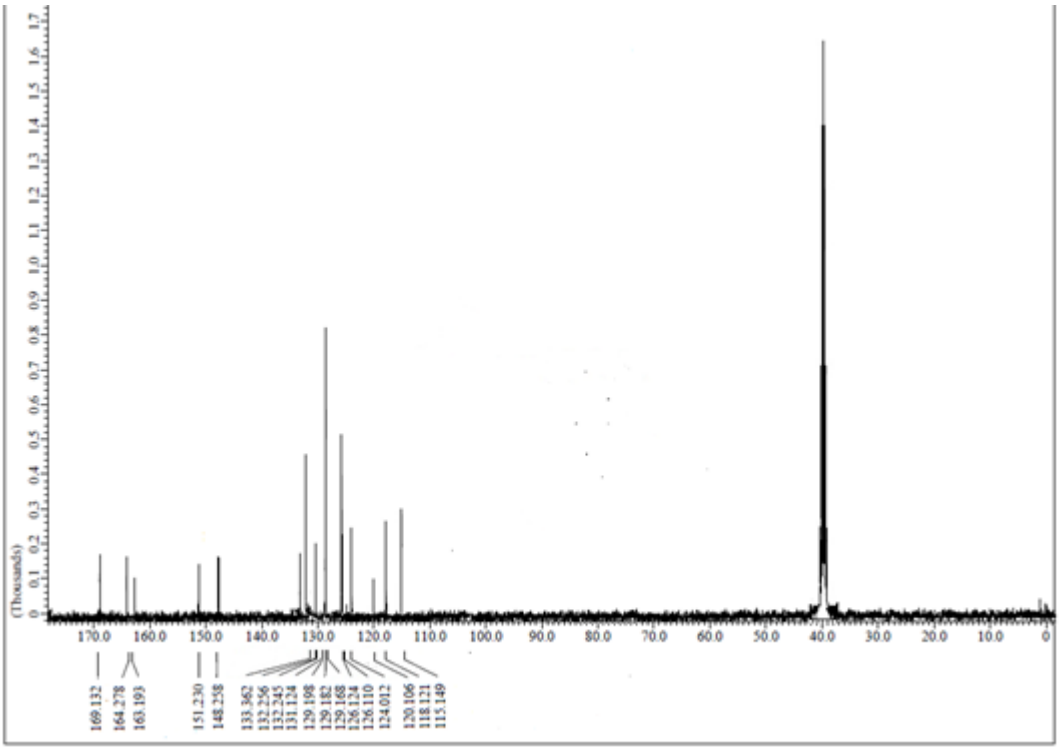


Figure 4.13: ^{13}C NMR spectrum of N-(3-mercapto-5-phenyl-4H-1,2,4-triazole-4-yl)-2-oxo-2H-chromene-3-carboxamide (**26a**)

4.3.2 *In-silico* interaction analysis

Potential binding affinity of the novel synthesized compounds (**26a-i**) with AChE and BuChE enzymes have been studied by performing docking studies. In spite of diverse series of compounds, the interactions of these molecules are quite low for almost all molecules. This may be an indication of inaccurate score calculation. *In vitro* results have also indicated that none of the molecule is active against AChE as well as BuChE (Table 4.1). The compound series shows less interaction with AChE as well as BuChE. Further, the docking results of AChE and BuChE show some correlation with the *in vitro* experimental studies. Analyses of the docked structure revealed that in active site of BuChE, only Phe329 makes π- π interaction with 4-fluoro phenyl ring of **26b** (Figure 2.14). In these compounds, though hydrophobic interactions were observed, but these are not so prominent as compared to outcomes reported in previous studies.

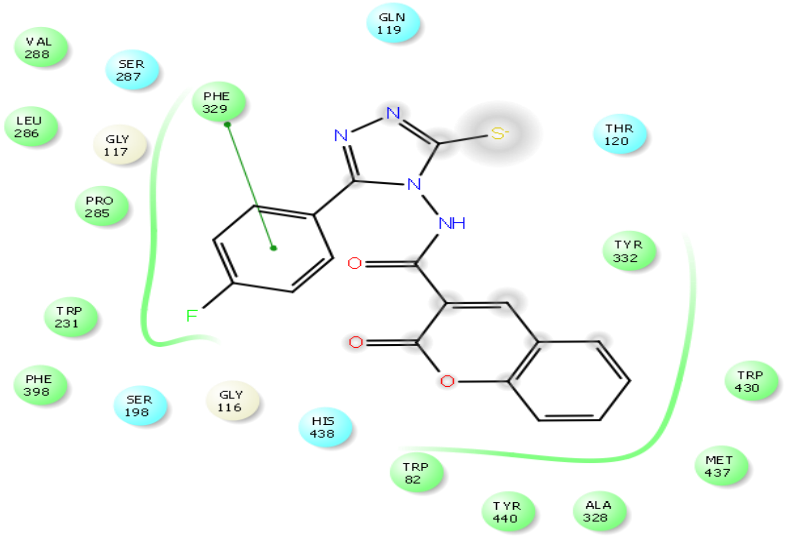


Figure 4.14: Interaction of N-[3-(4-fluorophenyl)-5-mercapto-4H-1,2,4-triazol-4-yl]-2-oxo-2H-chromene-3-carboxamide (**26b**) with active site of BuChE

4.3.3 *In-vitro* inhibition studies of AChE & BuChE

The inhibition activity of N-(3-mercapto-5-phenyl-4H-1,2,4-triazole-4-yl)-2-oxo-2H-chromene-3-carboxamide derivatives against AChE & BuChE are given in table 4.1. Based on the IC_{50} value, the synthesized compounds showed poor to no activity towards both enzymes

AChE and BuChE. Out of these only 4-fluoro derivative (**26b**) shows little activity towards BuChE.

Table 4.1: The IC_{50} value and docking score of compounds **25, 26a-i** against AChE and BuChE

S. No.	AChE	BuChE	Docking Score	
			AChE	**BuChE**
24	840.25 ± 96.38	794.05 ± 188.26	Not Docked	Not Docked
26a	651.78 ±14.73	ND	-7.56	-7.96
26b	**1190.50 ± 2.29**	**252.16 ± 40.76**	**-6.37**	**-10.25**
26c	ND	ND	-5.77	-10.78
26d	ND	848.81 ± 146.57	-5.97	-10.35
26e	**1754.80 ± 718.01**	**273.18 ± 28.91**	**-6.47**	**-9.89**
26f	ND	431.96 ± 23.82	-5.49	-10.38
26g	**ND**	**313.24 ± 5.12**	**-6.46**	**-9.45**
26h	1030 ± 247.15	405.70 ± 28.24	-5.44	-9.38
26i	ND	ND	-5.95	-8.12
Donepezil	0.042 ± 0.010	4.66 ± 0.503	-5.57	-6.92

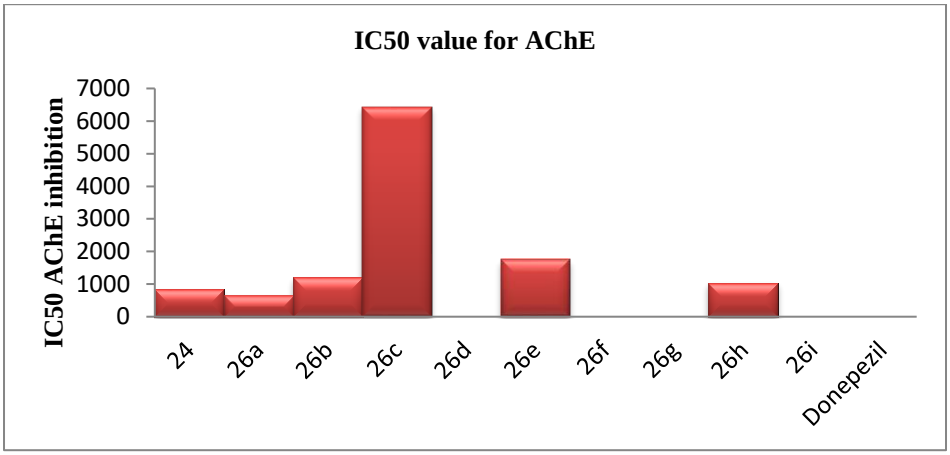


Figure 4.15: *In-vitro* studies of N-(3-mercapto-5-phenyl-4H-1,2,4-triazole-4-yl)-2-oxo-2H-chromene-3-carboxamide derivatives towards AChE (**25**, **26a-i**)

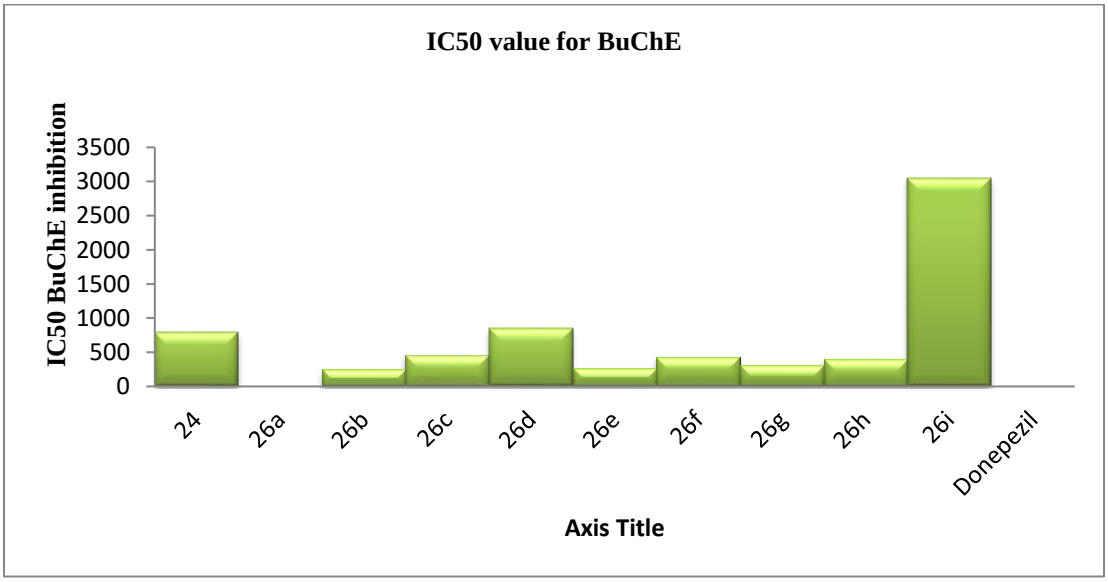


Figure 4.16: *In-vitro* studies of N-(3-mercapto-5-phenyl-4H-1,2,4-triazole-4-yl)-2-oxo-2H-chromene-3-carboxamide derivatives towards BuChE (**25**, **26a-i**)

Chapter 5

Conclusions and Future Prospects

Alzheimer's disease (AD) is a progressive neurological disorder that slowly destroys memory and thinking skills. This is the most common cause 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. 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. The cause and progression of AD is not well understood. However, researchers correlate it partly with the genetic, lifestyle and environmental factors. The only known method for diagnosis is the brain autopsy. However, physician diagnosed 90 percent of AD cases by mental and behavioral tests and also physical examinations of individuals. The complete treatment of AD is still far away. The available drugs only slow down the progression of disease. Several hypotheses have been put forward on the basis of careful observations and experimentations. The most known hypothesis includes cholinergic hypothesis, amyloid hypothesis and MAO hypothesis. The work embedded in this thesis is based on cholinergic hypothesis.

In this thesis, novel heterocyclic molecules were designed and synthesized which were further validated and evaluated towards AChE and BuChE activity. Based on the known heterocyclic fragments interacting with the cholinesterase enzyme, conjugates of coumarin-thiazole, benzothiazole-triazole and coumarin-triazole were designed, synthesized and evaluated.

The twelve novel coumarin-thiazole conjugates were synthesized as 3-[2-(4-phenylthiazol-2-ylamino)-acetyl]-chromen-2-one derivatives (**8a-l**) based on the preliminary *in-silico* studies. The synthesis was carried out in multisteps. First step involved the synthesis of 3-acetyl-2H-chromen-2-one which was brominated to produce the mono bromo derivative (**4**). In another step, the derivatives of 2-amino-4-phenylthiazoles (**7a-l**) were prepared. In this step, we have developed a novel methodology for the synthesis of **7a-l** by using THF as a solvent of choice. The reaction of **4** and **7** in the presence of potassium carbonate gave 3-[2-(4-phenylthiazol-2-ylamino)-acetyl]-chromen-2-one derivatives (**8a-l**). The synthesized compounds were validated by performing *in-silico* and *in-vitro* studies. *In-silico* docking results were consistent with *in-vitro* IC_{50} value for BuChE. However, remarkably high activities towards BuChE are observed and docking results are comparable with *in vitro* IC_{50} values. The best activities are being displayed by 3-{2-[4-(3-nitrophenyl)thiazol-2-ylamino]acetyl}chromen-2-

one (**8j**), 3-{2-[4-(3-bromophenyl) thiazol-2-ylamino]acetyl}chromen-2-one (**8i)**, and 3-{2-[4-(4-fluorophenyl)thiazole-2-ylamino]acetyl}chromen-2-one (**8b**) molecules having IC_{50} value of 46.47, 61.64 and 76.41 μM respectively. Molecule with IC_{50} value less than 100 μM or less is considered as lead molecule for the modification of more potent drugs. *In-vitro* studies and docking score also shows that the molecule **3**, **4** and some 2-amino-4-phenylthiazole derivatives provide less activity towards AChE as well as BuChE. On the other hand, when **4** was coupled with **7a-l** to form **8a-l**, the activities towards AChE and BuChE have been increased.

The novel benzothiazole-triazole conjugates were synthesized as N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)acetamide derivatives (**16a-i**) based on the preliminary *in-silico* studies. The benzothiazole and triazole derivatives were synthesized separately and then combined through covalent amide linkage. We have also developed a novel methodology for the synthesis of N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (**11**) derivatives. The method was validated for aromatic amines as one-pot process using chloroacetyl chloride in DBU as catalyst and tetrahydrofuran (THF) as solvent at room temperature. The synthesized benzothiazole-triazole conjugates (**16a-i**) were validated for cholinesterase inhibitors. *In-silico* docking results were consistent with *in- vitro* IC_{50} value for BuChE. Based on the IC_{50} values, the synthesized compound with less than 100 μM has been considered as lead molecule. Among derivatives synthesized, N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide (**16a**), N-benzothiazol-2-yl-2-[3-(4-fluorophenyl)-5-mercapto-[1,2,4]triazol-4-ylamino]acetamide (**16b**) and N-benzothiazol-2-yl-2-[3-(4-methylphenyl)-5-mercapto-[1,2,4] triazol-4-ylamino]acetamide (**16f**) have IC_{50} value 25.18, 95.52 and 83.25, respectively. These molecules are more active towards BuChE; they me be considered as lead molecules.

The coumarin-triazole conjugates were synthesized as N-(3-mercapto-5-phenyl-4H-1,2,4-triazole-4-yl)-2-oxo-2H-chromene-3-carboxamide derivatives (**26a-i**). The designing of these molecules were similar to compound **8**. The structure of the compounds was chosen on the basis of the preliminary *in-silico* studies. For synthesis of this conjugate, coumarin and triazole molecules were linked through the amide linkage. The coupling took place with the help of 1-ethyl-3-(3′-dimethylaminopropyl)-carbodiimidehydrochloride (EDC) as coupling agents in the presence of additives hydroxybezotriazole (HOBt). *In-silico* and *in-vitro* studies indicate that these novel compounds show poor activity towards AChE and BuChE. The results also revealed

that the coumarin-thiazole conjugates having liker atom with methylene as spacer is remarkably high active than the coumarine-triazle conjugates.

The work carried out by us provided some good lead molecules which can be taken up for further derivatization and validation. From these generated data, structure-activity relationship (SAR) may be developed and this SAR can be used for designing of new lead molecules which are expected to provide activity in μM/nm range. Using activity information, the new target specific molecule can be designed which is based on thiazole and triazole moieties. The molecular simulation methods may be explored to design better potent drugs.

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Patent – 01

1. Ram Singh, **Deepak Mishra**, Atiya Fatima, Chittaranjan Rout, Vineet Mehta, Udayabanu Malairaman, Mamta Chaudhary; Novel Thiazole Compounds, Process For Preparing The Same and Pharmaceutical Formulation Thereof. Ref No PAT134/003/17 dated 11-07-2017.

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2. **Deepak Mishra**, Ram Singh and Chittaranjan Rout; A facile amidation of chloroacetyl chloride using DBU; *International Journal of ChemTech Research*, 10(3), 365-372, **2017**. [*ISSN*: 0974-4290 IF = 0.598 (2014)].
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Conferences – 05

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2. A. Fatima, **D. Mishra**, C. Rout and R. Singh; Synthesis and Characterization of Isoflavone based molecule as Antiestrogens; Indian Roadshow Workshop - 4 November **2014** organized by Royal Society of Chemistry at IIT Delhi, India.
3. **D. Mishra**, Geetanjali, C. Rout and R. Singh; Synthesis and Characterization of selected Imidazole Derivatives as potential Anti-Alzheimeric Agents; The 102nd *Indian Science Congress* 2015 in association with the University of Mumbai, 3-7 January **2015**.
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5. **D. Mishra**, Babita, C. Rout and R. Singh; Synthesis of selected Triazole Derivatives as potential Anti-Alzheimeric Agent; 6th International symposium entitled CTDDR 2016" held on 25-28 February **2016**, organized by CDRI , Lucknow, India.