IN-SILICO DOCKING, DESIGN AND SYNTHESIS OF CERTAIN BENZOTRIAZOLE DERIVATIVES AND STUDY OF THEIR ANTI-ALZHEIMER'S ACTIVITY

A Dissertation Submitted to

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MASTER OF PHARMACY

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COIMBATORE - 641 044.

OCTOBER - 2021

CERTIFICATE

This is to certify that the dissertation entitled **"*IN-SILICO* DOCKING, DESIGN AND SYNTHESIS OF CERTAIN BENZOTRIAZOLE DERIVATIVES AND STUDY OF THEIR ANTI-ALZHEIMER'S ACTIVITY",** was carried out by **Ms.THAIYALNAYAKI K (Reg. No. 261915104)** in the Department of **PHARMACEUTICAL CHEMISTRY**, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, which is affiliated to **The Tamil Nadu Dr. M.G.R. Medical University, Chennai** under my direct supervision and guidance to my fullest satisfaction.

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Date:

CERTIFICATE

This is to certify that the pharmacological screening of the dissertation entitled **"*IN-SILICO* DOCKING, DESIGN AND SYNTHESIS OF CERTAIN BENZOTRIAZOLE DERIVATIVES AND STUDY OF THEIR ANTI-ALZHEIMER'S ACTIVITY",** was carried out by **Ms.THAIYALNAYAKI K (Reg. No. 261915104)** in the Department of **PHARMACOLOGY**, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, which is affiliated to **The Tamil Nadu Dr. M.G.R. Medical University, Chennai** to my fullest satisfaction.

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Above all, I humbly submit my dissertation work to ***The Almighty GOD****, who is the source of all the wisdom and knowledge for the completion of my work.*

THAIYALNAYAKI. K

CONTENTS

I. INTRODUCTION

1.1. DRUG DISCOVERY [1-3]

Drug discovery is the process through which potential new therapeutic entities are identified, using a combination of computational, experimental, translational, and clinical models.

Drug discovery is a multifaceted process, which involves identification of a drug chemical therapeutically useful in treating and management of a disease condition. The process of drug discovery includes the identification of drug candidates, synthesis, characterization, screening, and assays for therapeutic efficacy. Small molecule drug discovery programs typically produce massive amounts of data using high-throughput screening techniques where large libraries of chemicals are tested for the ability to modify the target. Modern drug discovery involves the identification of screening hits, Lead identification, and optimization of those hits to increase the affinity, selectivity efficacy/potency, metabolic stability and oral bioavailability.

The goal of a preclinical drug discovery program is to deliver one or more clinical candidate molecules, each of which has sufficient evidence of biological activity at a target relevant to a disease as well as sufficient safety and drug like properties so that it can be entered into human testing. Drug discovery and development is an expensive process due to the high budgets of R&D and clinical trials. It takes almost 12-15 years to develop a single new drug molecule from the time it is discovered when it is available in market for treating patients.

1.2. DRUG DESIGN [4,5]

Drug design is the inventive process of finding new medications based on the knowledge of a biological target. The drug most commonly inhibits the function of a biomolecule such as a protein or an organic molecule which gets activated and in turn results in therapeutic benefit to the patient. Drug design involves the design of molecules that are complementary in shape and charge to the molecular target with which they interact and bind.

Drug Design can be categorized as two types:

- Structure based drug design (SBDD)
- Ligand based drug design (LBDD)

1.2.1. STRUCTURE BASED DRUG DESIGN [6,7]

SBDD is the approach where the structural information of the drug target is exploited for the development of its inhibitor. Receptor structure is a prerequisite for this method. Most commonly the structure of the receptor is determined by experimental techniques such as X-ray crystallography or NMR. If the structure of the protein drug target is not available, protein structure can be predicted by computational methods like threading and homology modeling.

Threading (also called as fold) is a modeling approach used to model proteins that do not have homologous proteins with known structure. During threading, a given amino acid sequence is searched for compatibility with the structures in a database of known folds. The structure of the query protein is built from these folds.

Homology modeling (also called as comparative) is an approach that relies on a clear relationship or homology between the sequence of the target protein and at least one known structure. The process of homology modeling of proteins consists of the following steps: Identification of homologous protein with known 3D structure that can serve as template; sequence alignment of target and template proteins; generation of model for the target based on the 3D structure of the template and the alignment; model refinement and validation. Homology modeling has become the main alternative to get a 3D representation of the target in the absence of crystal structures.

1.2.2. LIGAND BASED DRUG DESIGN [6,7]

Ligand based drug design is an approach used in the absence of the receptor 3D information and it relies on knowledge of molecules that bind to the biological target of interest. 3D quantitative structure activity relationships (3D QSAR) and pharmacophore modeling are the most important and widely used tools in ligand based drug design. They can provide predictive models suitable for lead identification and optimization.

STEPS INVOLVED IN DRUG DESIGN [8]

Stages of drug discovery and development include:

- Target identification & Target validation
- Lead identification
- Lead optimization
- Docking

TARGET IDENTIFICATION & TARGET VALIDATION

Target Identification is the first step in the discovery of a drug, identification of the biological origin of a disease, and the potential targets for intervention. Target identification starts with isolating the function of a possible therapeutic target (gene/nucleic acid/protein) and its role in the disease. An ideal target should be efficacious, safe, meet clinical and commercial requirements and be 'druggable'. The techniques used for target identification may be based on principles of molecular biology, biochemistry, genetics, biophysics, or other disciplines.

Target validation is the process by which the expected molecular target – for example gene, protein or nucleic acid of a small molecule is certified.

LEAD IDENTIFICATION:

A chemical lead is defined as a synthetically stable, feasible, and drug like molecule active in primary and secondary assays with acceptable specificity, affinity and selectivity for the target receptor. This requires definition of the structure activity relationship as well as determination of synthetic feasibility and preliminary evidence of in vivo efficacy and target engagement.

LEAD OPTIMIZATION:

Lead optimization is the process by which a drug candidate is designed after an initial lead compound is identified. The purpose of lead optimization is to maintain properties in lead compounds. In order to produce a pre-clinical drug candidate, the chemical structures of lead compounds (small molecules or biologics) need to be altered to improve target specificity and selectivity. Pharmacodynamic, pharmacokinetic parameters and toxicological properties are also evaluated.

QIKPROP:

QikProp is a quick, accurate, easy-to-use absorption, distribution, metabolism, and excretion (ADME) prediction program designed by Professor William L. Jorgensen. QikProp predicts physically significant descriptors and pharmaceutically relevant properties of organic molecules, either individually or in batches. In addition to predicting molecular properties, QikProp provides ranges for comparing a particular molecule's properties with those of 95% of known drugs.

DOCKING [9-10]

Molecular docking is an attractive scaffold to understand drug-biomolecular interactions for the rational drug design and discovery, as well as in the mechanistic study by placing a molecule (ligand) into the preferred binding site of the target specific region of the DNA/protein (receptor) mainly in a non-covalent fashion to form a stable complex of potential efficacy and more specificity. The information obtained from the docking technique can be used to suggest the binding energy, free energy and stability of complexes. At present, docking technique is utilized to predict the tentative binding parameters of ligand-receptor complex. The main objective of molecular docking is to attain ligand-receptor complex with optimized conformation and with the intention of possessing less binding free energy. There are various kinds of molecular docking procedures involving either ligand/target flexible or rigid based

Rigid-body docking Vs flexible docking

If the bond angles, torsion angles and bond lengths of the components are not modified at any stage of complex generation, then they are known as rigid body docking. A rigid-body docking is sufficiently good for most docking. Docking procedures which permit flexible docking procedures or conformational change, must intelligently select small subset of possible conformational changes for consideration.

Mechanics of docking

To perform a docking screen, the first requirement is a structure of the protein of interest. Usually the structure has been measured using a biophysical technique such as x-ray crystallography or NMR spectroscopy. The protein structure and a database of potential ligands serve as inputs to a docking program. The success of a docking program is based on two components: Search algorithm and Scoring function.

The Search algorithm

In a docking simulation, the variables to optimize are those that define a binding mode. Namely, the rotation angle for all rotatable (flexible) bonds in the ligand, the position of the ligand within the binding site (translation), and the overall rotation of the ligand with respect to the protein. It is not feasible to do an exhaustive search with this number of variables.

Scoring function:

As the scoring function has to be evaluated for numerous different binding modes, the complexity of the function influences greatly how much time is needed to do the docking simulation. The scoring function should therefore be as simple as possible, while still being able to distinguish between favorable and poor protein-ligand interactions. This score has a good balance between accuracy and evaluation time. The score mimics the potential energy change, when the protein and ligand come together. This means that a very negative score corresponds to a strong binding and a less negative or even positive score corresponds to a weak or non-existing binding.

Various software used:

- AutoDock,
- MOE-DOCK,
- FlexX,
- GOLD,
- Glide,
- ICM,
- QXP/Flo+,
- Surflex.

GLIDE [11,12]

This is carried out using glide dock. Glide searches for favorable interaction between one or more ligand molecule and a receptor molecule, usually a protein. Each ligand acts a single molecule, while the receptor may include more than one molecule, e.g., a protein and a cofactor. Glide a run in rigid or flexible docking mode; the latter automatically generated conformation for each input ligand. The combination of position and orientation of a ligand relative to the receptor, along with it conformation in flexible docking, is referred to as a ligand poses. The ligand poses that Glide generate pass through a series of hierarchical filter that evaluate the ligand to the defined active site, and examine the complimentarily of ligand-receptor interaction using a grid-based method patterned after the empirical Chem Score function. Final scoring is then carried out on the energy-minimized poses.

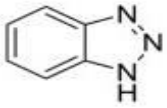
1.3. BENZOTRIAZOLE [13,14](BTA)

Benzotriazole, benzo-fused azoles are a class of heterocyclic compounds of great interest in the Pharmaceutical Chemistry, where Benzotriazole is a benzo-fused triazole moiety. Benzotriazole is easy to introduce into molecules by a variety of condensation, addition, and substitution reaction also have a characteristics such as electron donating in nature, group release, anion director. It is also known as 1*H*-benzo[d]-1,2,3-triazole.

1.3.1. CHEMISTRY OF BENZOTRIAZOLE [14]

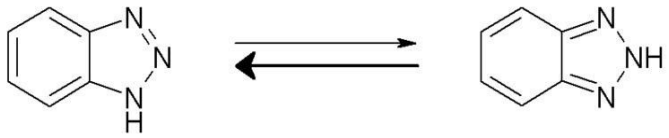
Benzotriazole is bicyclic heterocyclic system consists of three nitrogen atom which is fused with benzene ring. Benzotriazole may be prepared by intramolecular cyclization of O-phenylene diamine with sodium nitrite and acetic acid. This conversion proceeds diazotization of the one amine group. It is a potential chemical scaffold which undergoes chemical transformation to give various products which makes it a synthetically important compound. The structure of Benzotriazole is simple hence synthesizing it in laboratory is feasible.

Physical properties:



Name	: BENZOTRIAZOLE
Chemical Name	: 1*H*-benzo[d]-1, 2, 3 triazole
Molecular formula	: $C_6H_5N_3$
Molecular weight	: 119.1240
Composition	: C (60.50%) H (4.23%) N (35.27%)
Melting point	: 98.5-100°C
Boiling Point	: 350 °C
Nature	: White to brown crystalline powder
Density	: 1.36 g/cm
Solubility	: water g/100 ml is 2 (moderate)
UV absorbance	: 286 nm

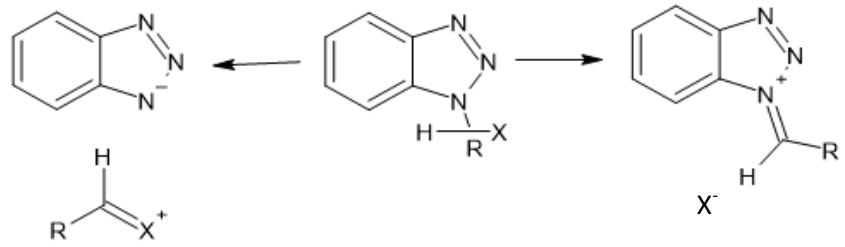
N-Substituted Benzotriazole exist in two tautomeric isomers in 1*H*- and 2*H*- forms in which the 1*H*- form predominates(99%) over the 2*H*- form at room temperature in both gas and solution phase.



1*H*-form 2*H*-Form

Benzotriazole is inexpensive, stable compound. It is soluble in ethanol, benzene, toluene, chloroform, and DMF, sparingly soluble in water but highly soluble in basic solution because it is an acidic appreciable strength with acid pKa 8.2 as well as weak base (pKa < 0) and because of this acid-base property.

Benzotriazole also acts as an electron-donor or a precursor of radicals or carbanions. This molecule also shows not only electron donating but also electron attracting ability, which leads to various synthetic applications. Because of this compounds with and R hetero atom(most commonly N,O and S) attached to a Benzotriazole nitrogen can ionize in two ways, either to form Benzotriazole anion and an immonium, oxonium, tionium cation to give B or to ionize of the hetero atom substituent to give C.



B A C

X = NR^2,OR,SR X = Halogen, OH

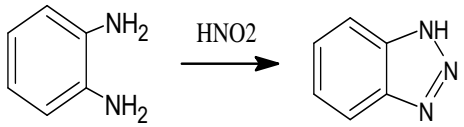
Benzotriazole derivatives are found active against the wide spectrum of target Species. Also Benzotriazole of biological and industrial importance, to find most active sites of Benzotriazole and to investigate target Species.

1.3.2. SYNTHETIC METHODS [15,16]

In 1980, Benzotriazole was first reported as synthetic auxiliary in organic chemistry. Since then benzotriazole is used in the construction of various monocyclic and bicyclic heterocyclic compounds which are difficult to prepare by other methods.

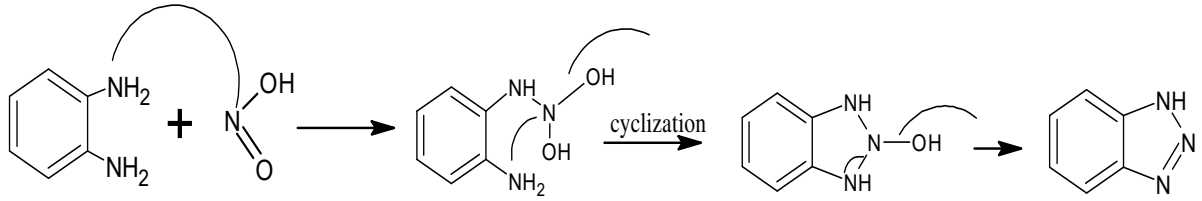
Benzotriazole is particularly versatile synthetic auxiliary because of its attractive properties. There are many distinct routes of preparing Benzotriazole,

I. Benzotriazoles are synthesized by cyclocondensation of o-phenylenediamine with sodium nitrite in acetic acid. Conversion of the diamine into the monodiazonium derivative is followed by spontaneous cyclization.

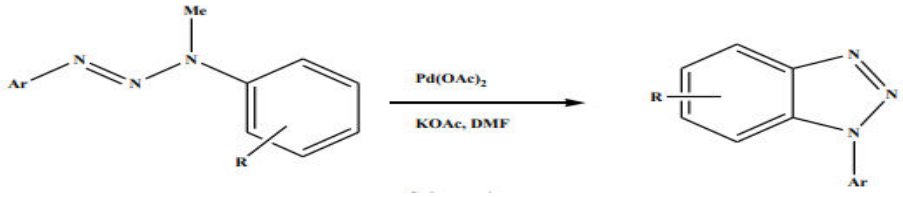


o-phenylenediamine Benzotriazole

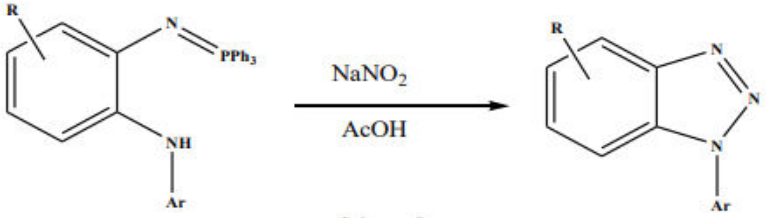
Mechanism



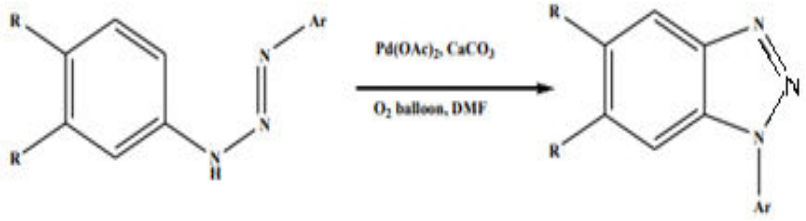
II. The regioselective synthesis of benzotriazoles using 1, 7-palladium migration cyclization dealkylation sequence. These reactions showed high regioselectivity and high yields.



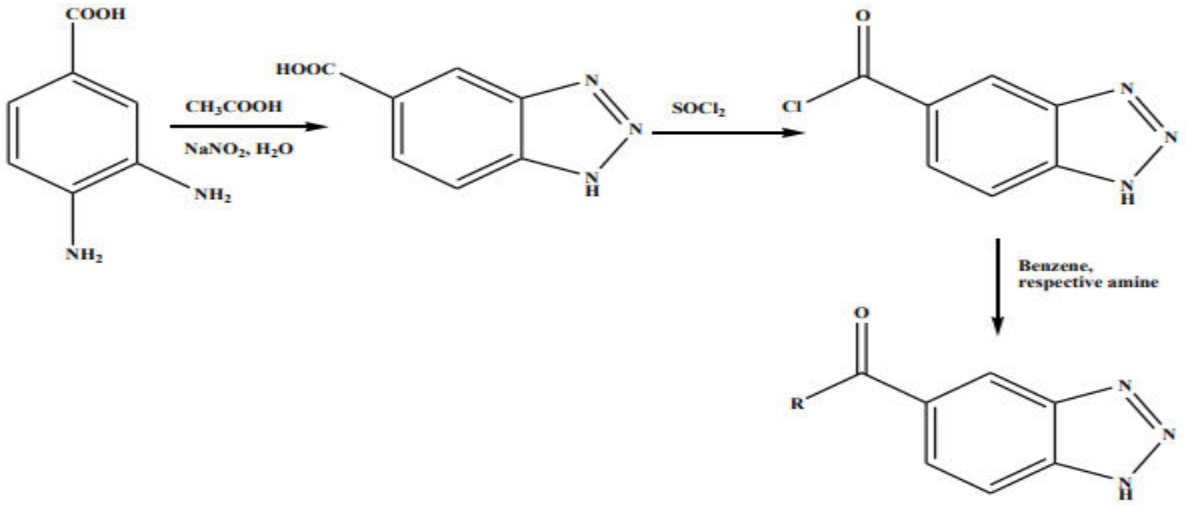
III. Synthesized 1-aryl-1,2,3-benzotriazole via cyclocondensation of 2-(arylamino) aryliminophosphoranes in mild conditions. It involved three-step, halogen-free route of synthesis from simple nitroarenes and arylamines.



IV. Synthesized 1-aryl-1H-benzotriazoles by using catalytic amount of Pd(OAc)2 that affected cyclization at moderate temperature.



V. The conventional and microwave synthesis of Benzotriazole

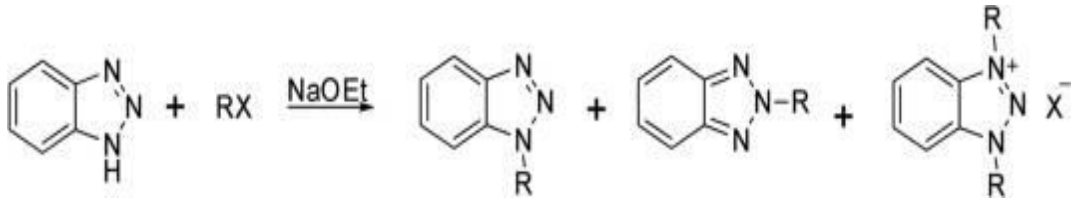


1.3.3. CHEMICAL REACTIONS [48]

I) Formation of Benzotriazole- C bond substitution

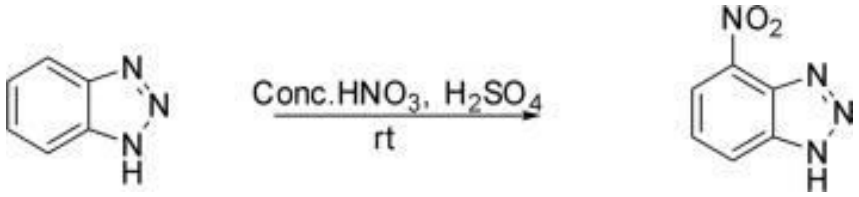
N-alkylation

Alkylation of 1*H*-benzotriazole with alkyl halide using NaOH or NaOEt as a base gave 1-alkylbenzotriazole as a major product and 2-alkylbenzotriazole and 1,3-dialkylbenzotriazolium salts as minor products.



Nitration

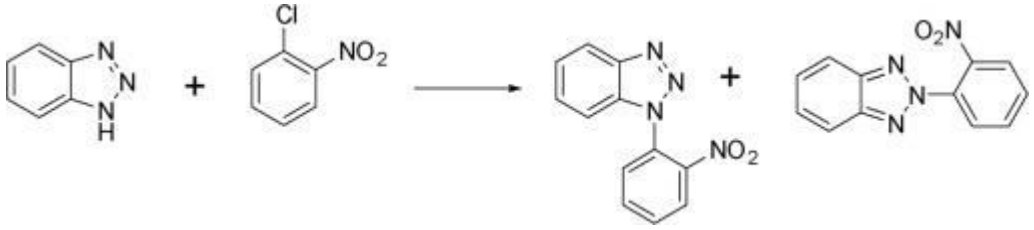
1*H*-Benzotriazole has been nitrated with a mixture of concentrated nitric acid and sulfuric acid at room temperature to give 4-nitro-1*H*-benzotriazole in 50% yields.



II) Formation of Benzotriazole –Ar substitution

N-arylation

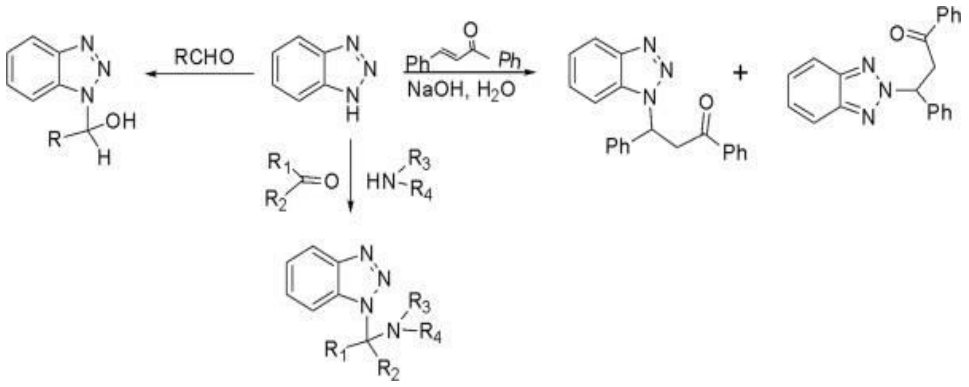
1*H*-Benzotriazole on reaction with activated aryl and heteroaryl halides afforded 1-arylbenzotriazole. Here, 1-chloro-2-nitrobenzene reacting with 1*H*-benzotriazole gave a mixture of 1- and 2-(2-nitrophenyl) Benzotriazole.



III) Formation of Benzotriazole-C bond by addition reaction

Reaction with carbonyl compounds

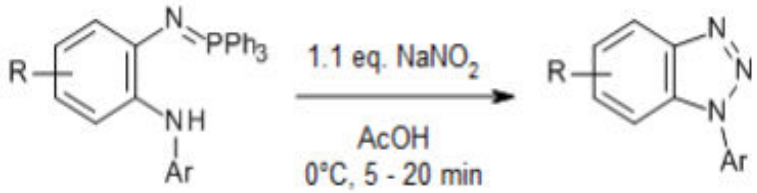
Reaction of 1*H*-benzotriazole with α,β-unsaturated ketones underwent 1,3-conjugated addition to give a mixture of 1-*H*- and 3-(2*H*-benzo[*d*][1,2,3-triazol-2-yl)-1,3-diphenylpropan-1-one,but reaction with aliphatic aldehyde afforded 1-hydroxyalkyl benzotriazole as an addition product. However reaction with ketone in the presence of dialkylamine delivered 1(dialkylaminoalkyl) Benzotriazole.



IV) Condensation reaction

Cyclocondensation of 2-(arylamino)aryliminophosphoranes:

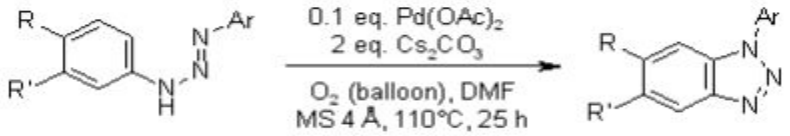
A cyclocondensation of 2-(arylamino)aryliminophosphoranes enables the synthesis of 1-aryl-1,2,3-benzotriazoles under mild conditions. The reaction involves a three-step, halogen-free route starting from simple nitroarenes and arylamines.



V) Cyclization reactions

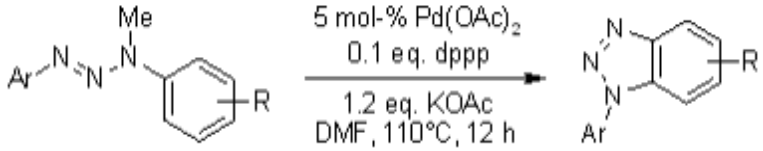
i) C-H activation of aryl triazene:

C-H activation of aryl triazene compounds followed by intramolecular amination in the presence of a catalytic amount of Pd(OAc)2 provides 1-aryl-1H-benzotriazoles at moderate temperature.

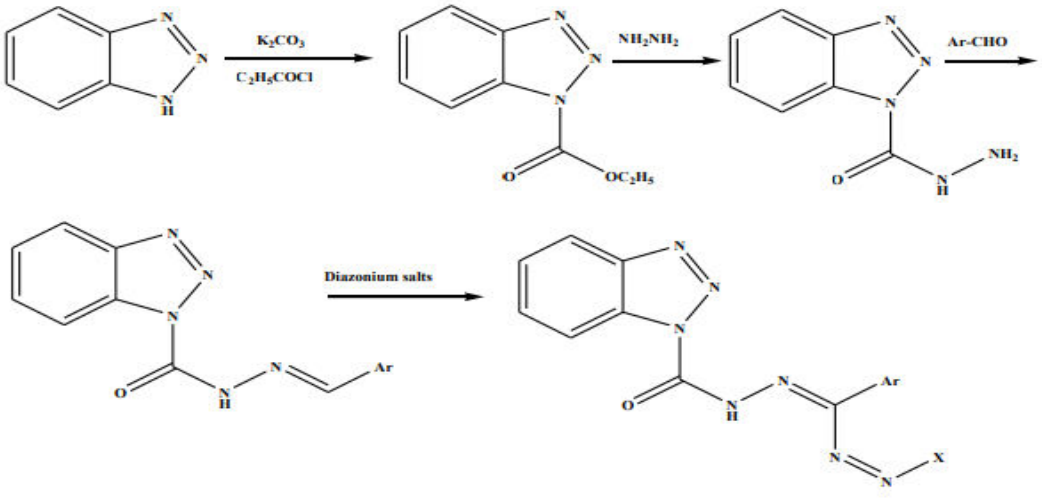


ii) 1,7-palladium migration-cyclization-dealkylation:

A 1,7-palladium migration-cyclization-dealkylation sequence for the regioselective synthesis of benzotriazoles proceed in excellent yields with high regioselectivities.



iii) 1, 2, 3 benzotriazole derivatives using conventional and microwave ultrasonification



1.3.4. PHARMACOLOGICAL ACTIVITY [17]

The *1H*-benzo[d] [1,2,3] triazole can be considered as a privileged structure for its several pharmacological activities. Useful as scaffold for the design of new pharmacologically active compounds, Benzotriazole is undergoing rapid development in the synthesis of heterocycles.

A survey of literature reveals that the benzotriazole nucleus is found to have various pharmacological activities like anti-inflammatory, antimicrobial, antifungal and anticancer activity. It is also known from the literature that molecules containing benzotriazole nucleus possess CNS activity and various other pharmacological activities. It has been considered as prime importance to take up such synthesis of new compounds containing benzotriazole nucleus with a view to get more potent compounds and screen them for CNS activity.

1.4. ALZHEIMER'S DISEASE: [18-21]

Alzheimer's disease (AD), the most common form of dementia, is a chronic neuro degenerative disorder, which is clinically characterized by impairment in memory, complex cognition, language, emotion and behavior. It was defined by Alois Alzheimer in 1906 using criteria of progressive memory loss, disorientation and pathological markers (senile plaques and neurofibrillary tangles) initially it was assumed that AD was rare condition and later it was considered to be an inevitable consequence of aging. These plaques and tangles in the brain are still considered some of the main features of Alzheimer's disease. Another feature is the loss of connections between nerve cells (neurons) in the brain. Neurons transmit messages in the between different parts of the brain, and from the brain to muscles and organs in the body. This damage initially takes place in the parts of the brain involved in the memory including the Entorhinal cortex and hippocampus. It later affects areas in the cerebral cortex such as those responsible for language, reasoning and social behavior. Eventually, many other areas of the brain are damaged.

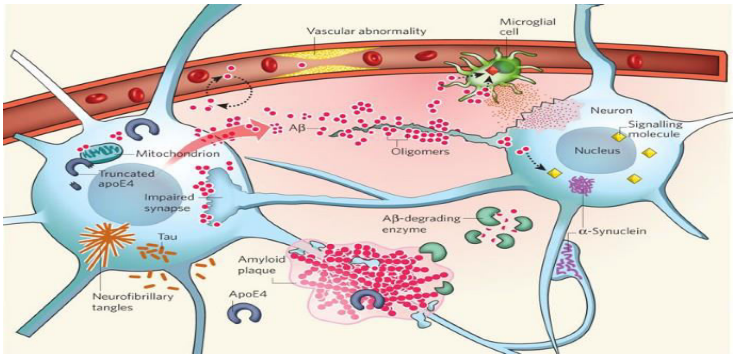


Fig. 1 - Prospects for defeating Alzheimer's disease

The etiology of AD is still not known and many factors such as reduced level of Cholinergic transmitters, accumulation of beta-amyloid peptide plaque, oxidative stress and hyper phosphorylation of microtubule-associated tau protein involved in the progression of the disease. The most common and important hypothesis about AD has been attributed to the levels of acetyl choline. There is two important types of catalytic enzyme namely acetyl cholinesterase (AChE) and Butyryl cholinesterase (BChE) which are able to hydrolysed cholinergic neuro transmitter.

1.4.1. STAGES OF ALZHEIMER'S DISEASE [22,23]

Alzheimer's disease is a progressive neurocognitive disorder that becomes worse over time. Although every person with Alzheimer's experiences the disease differently, it is possible to divide its typical progression into a series of stages.

In most people with Alzheimer's, symptoms first appear in their mild 60's . There are five stages in Alzheimer's disease

- **Stage 1:** Preclinical Alzheimer's disease
- **Stage 2:** Mild cognitive impairment due to Alzheimer's disease
- **Stage 3:** Mild dementia due to Alzheimer's disease
- **Stage 4:** Moderate dementia due to Alzheimer's disease
- **Stage 5:** Severe dementia due to Alzheimer's disease

1.4.2. DIAGNOSIS OF ALZHEIMER'S DISEASE [24]

The clinical manifestations of AD include disturbances in the areas of memory and language, visuospatial orientation, and higher executive function. Non cognitive changes include personality changes, decreased judgment ability, wandering, psychosis, mood disturbance, agitation, and sleep abnormalities.

The diagnostic evaluation of patients suspected of having AD comprises

- A history from a reliable informant (containing general medical history, neurological history, neuropsychiatric history, family history);
- Physical and neurological examination;
- Routine laboratory examinations (complete blood count, sequential multiple analysis, thyroid function tests, vitamin B12, folate, rapid plasma reagin); optional laboratory examinations (erythrocyte sedimentation rate, human immunodeficiency virus (HIV) serology, serology for Lyme's disease, urinalysis, urine drug screen, lumbar puncture, electroencephalography); and

- Neuroimaging (computed tomography or magnetic resonance imaging). Neuropathological examination (looking for the hallmark senile plaques and neurofibrillary tangles) from autopsy studies suggest a 90% accuracy rate in the clinical detection of AD.

These tests may be repeated to give information about how the persons memory and the other cognitive functions are changing over time .

1.4.3. ENZYMES [25-28]

Cholinesterase enzyme (ChE):

Cholinesterase is a family of enzymes present in central nervous system, particularly in nervous tissue, muscle and red cells, which catalyze the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid, a reaction necessary to allow a cholinergic neuron to return to its resting state after activation. It is one of many important enzymes needed for the proper functioning of nervous system of humans.

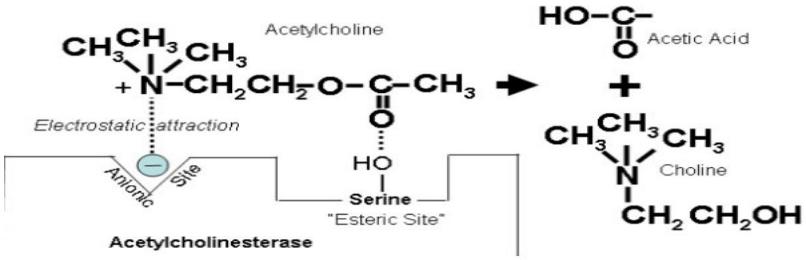


Fig. 2 - Scheme of acetylcholine hydrolysis

There are two cholinesterase types: Acetyl cholinesterase (AChE) and Butyryl cholinesterase (BChE). AChE was found primarily in the blood on red blood cell membranes, in neuromuscular junctions, in neural synapses while BChE is produced in the liver and found primarily in plasma. AChE hydrolyzes Acetylcholine more quickly while BChE hydrolysis Butyryl choline more quickly, making the difference between the enzymes. BChE levels may be reduced in patients with advanced liver disease and decrease must be greater than 75 percent before significant prolongation of neuromuscular blockade occurs with succinylcholine. Elevation of plasma BChE was observed in 90.5 percent cases of acute myocardial infarction and this can be used as a marker if substance toxicities.

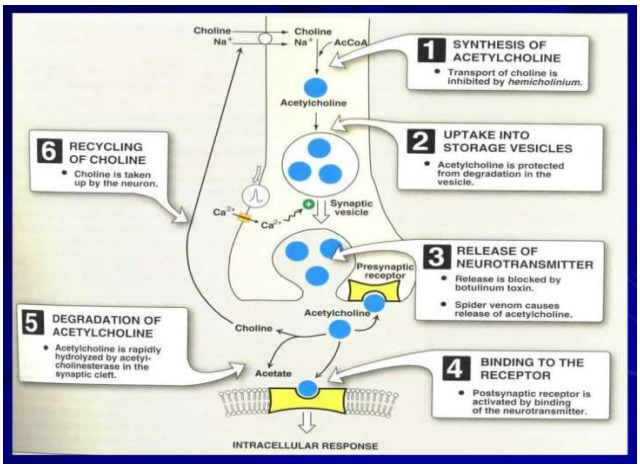


Fig. 3 - Cholinesterase Mechanism of action

According to a recent report on Alzheimer's patient, AChE activity in a specific region of the brain dramatically reduced, while BChE activity increase to compensate for the lack of AChE. Based on the important role of BChE in the hydrolysis of acetylcholine, simultaneous inhibition of both enzymes can be effective to the improvement of the symptoms in Alzheimer's patients

The X-ray crystallography studies have shown that AChE has two binding sites containing the catalytic active site (CAS) and the peripheral anionic site (PAS). AChE inhibitors act through the interaction with amino acid residues in the CAS and PAS and consequently inhibition of this two site increases the inhibitory activity.

Another most important cause of AD is the accumulation of β-amyloid (Aβ) plaques in the brain. One of the ways to prevent the accumulation of Aβ plaques is prevention the hydrolysis of amyloid precursor protein (APP) by inhibition of β-secretase. There are also reports that show accumulation of amyloid plaques and neurofibrillary tangles in the brain increased by oxidative damage. Therefore, preventing the formation of free radicals by antioxidant agents can be effective for treating AD.

1.4.4. TREATMENT OF ALZHEIMER'S DISEASE [29,30]

The goals of treatment are to achieve improvement in cognition and to minimize behavioral disturbances (depression, psychosis, agitation, and insomnia).

Psychosocial treatment

Environmental manipulation and prevention of other medical comorbidities can improve functioning of AD patients. Written daily reminders can be helpful in the performance of daily

activities (Prominent clocks, calendars, and windows) are important. Maintaining adequate hydration, nutrition, exercise is important.

Pharmacotherapy

Current pharmacological choices available to clinicians treating AD include cognitive enhancers for the treatment of the cognitive deficit and mood stabilizers, antipsychotics, antidepressants, and hypnotics for the treatment of behavioral disturbance.

Pharmacological treatment:

Pharmacologic medications are available to slow or stop the damage or destruction of neurons that cause Alzheimer's symptoms and make the disease fatal. Researchers hope to develop therapies targeting specific genetic, molecular, and cellular mechanisms so that the actual underlying cause of the disease can be stopped or prevented.

That future treatments to slow or stop the progression of Alzheimer's disease and preserve brain function will be most effective when administered early in the disease process, either at the MCI due to Alzheimer's or preclinical stage. Biomarker tests will be essential to identify which individuals are in these early stages and should receive treatments when they are available.

Marketed Cholinesterase inhibitors:

The US food and drug administration (FDA) approved AChE inhibitor drugs such as Tacrine, Donepezil, Galantamine, and Rivastigmine and the N-Methyl-D-aspartate (NMDA) receptor antagonist-memantine as palliative treatment for this devastating pathology.

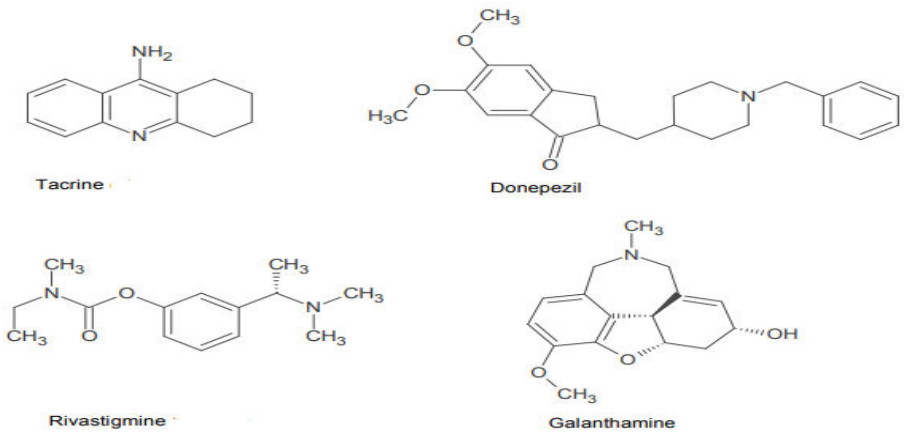
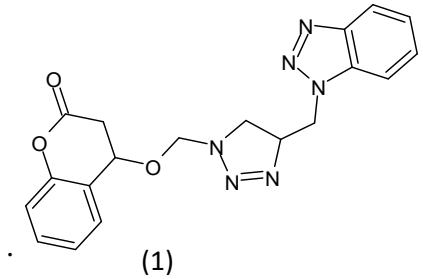


Fig. 4 - Cholinesterase Inhibitors

II. REVIEW OF LITERATURE

Anti-cholinesterase activity

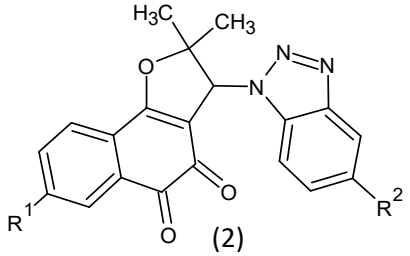
Atamjit Singh *et al.,* [31] (2020) synthesized novel series of triazole tethered coumarin Benzotriazole hybrids based on Donepezil skeleton as multifunctional agents for the treatment of Alzheimer's disease (AD). The inhibitory potential of all compounds against BChE, AChE and Aβ1-42 aggregation inhibition and chelating properties for metal ions along with DNA protective potential against degenerative actions was evaluated. Molecular modeling studies to confirm the potential of blocking both PAS and CAS of AChE and Aβ1-42 monomer were determined.



(1)

Anticancer activity

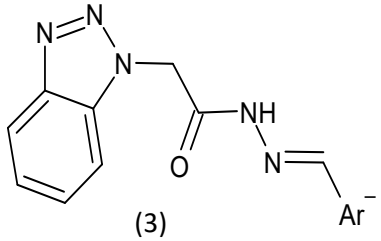
Li-Qiang Wu *et al.,* [32] (2021) reported a series of novel 3-(1-benzotriazole)-nor-β-lapachones as the NQO1-targeted anticancer agents and all the synthesized compounds shows good anti-proliferative activity against the breast cancer MCF-7, lung cancer A549 and hepatocellular carcinoma HepG2 cells in agreements with their NQO1 activity.



(2)

R^1=H, R^2 = n-butyl, 41 %

Asmaa E. Kassab *et al.,*[33] (2018) reported a series of Novel Benzotriazole N acylarylhydrazone hybrids and all the synthesized compounds were evaluated for their anticancer activity, effects on cell cycle profile, caspase-3 mediated apoptosis and FAK inhibition.

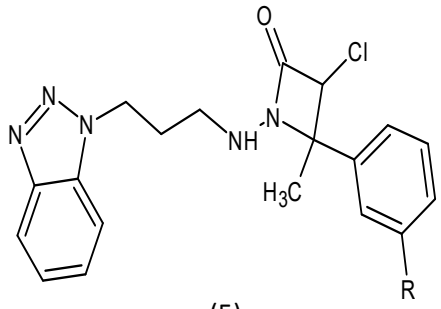


(3)

Ar = 2, 3 dihydroxy phenyl, 2, 4 dihydroxy phenyl, benzo thiophen-2-yl

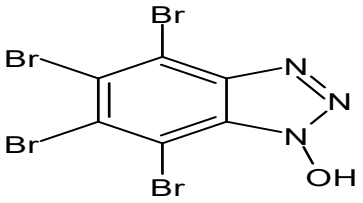
Anti-tubercular activity

Adesh Dubey *et al.,* [34] (2011), reported a Conventional and microwave assisted synthesis of 2-oxo-4-substituted aryl-azetidine derivatives of Benzotriazole and were evaluated for their anti-tubercular activity against Mycobacterium tuberculosis H37RV and antimicrobial activity.



(5)

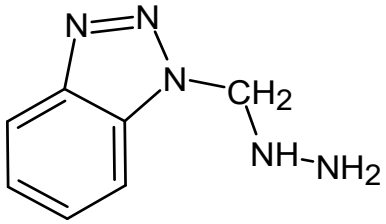
Ewa augustynowicz-kope *et al.,* [35] (2008), reported a series of 1-nitrobenzyloxybenzotriazoles was prepared by the benzylation of the respective halogen substituted 1-hydroxybenzotriazoles. The newly obtained compounds were tested against for Mycobacterium strains.



(4)

Anti-corrosive activity

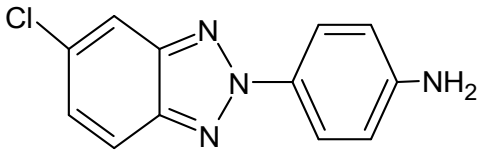
Kunika verma *et al.,* [36] (2020) reported a Microwave Assisted Synthesis of Benzotriazole Derivatives for Anti-Corrosive Study on Mild Steel in Acidic Medium.



(6)

Anti-viral activity

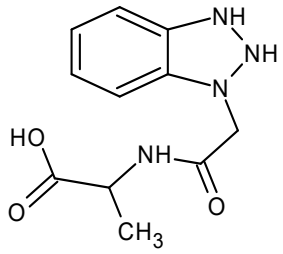
Roberta Ibba *et al.,* [37] (2018) synthesized a series of new 5-chlorobenzotriazole derivatives. Compounds were tested for cytotoxicity and antiviral activity in cell-based assays against several positive single-stranded RNA viruses: BVDV, YFV, CVB-5, and Sb-1; two negative single-stranded RNA viruses.



(7)

Anti-oxidant activity

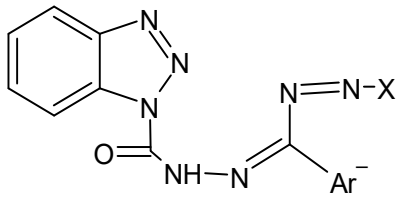
C. M. Jamkhandi *et al.,* [38] (2012) synthesized a derivatives of Benzotriazole substituted 2-(1H-1, 2, 3-benzotriazol-1-yl)-N-phenylacetamide and their antioxidant activity by adopting Griess reaction assay method.



(8)

Anti-fungal activity

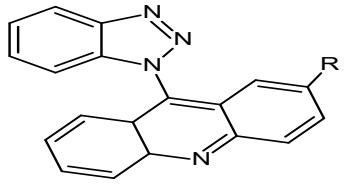
Muvvala S. Sudhir *et al.,* [39] (2013) were evaluated the antifungal activities of novel 1, 2, 3-benzotriazole derivatives synthesized by ultrasonic and solvent-free conditions.



(9)

Anti-bacterial activity

Narinder Pal Singh *et al.,* [40] (2011) synthesized Benzotriazole substituted acridine derivatives and those compounds were evaluated for anti-bacterial activity.



(10)

III. PURPOSE OF WORK

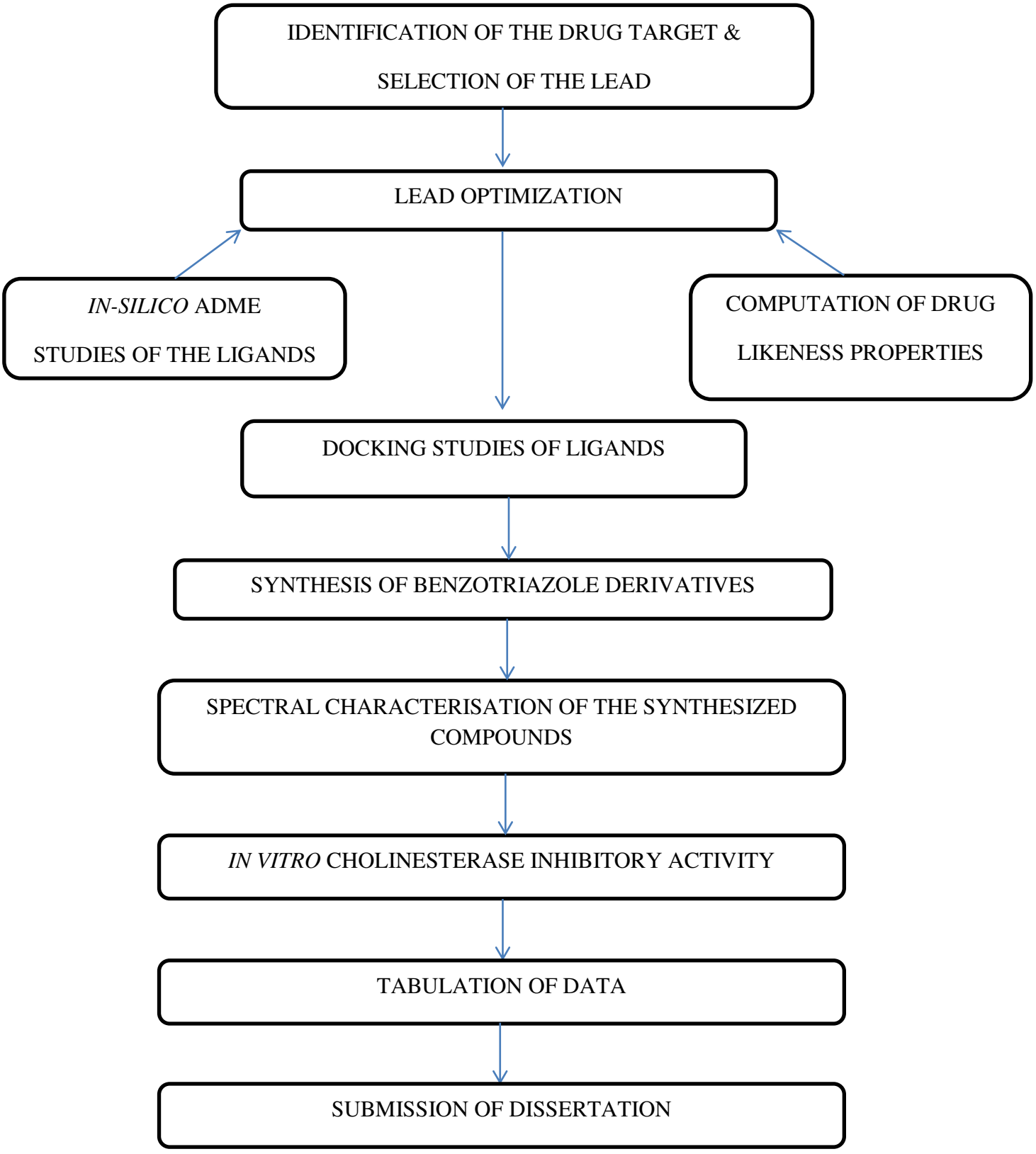
Alzheimer's disease is a chronic and progressive neurodegenerative disorder of the CNS which is associated with memory loss, cognitive impairment and ultimately death. It is estimated that more than 18 million people presently suffer from AD and the number of patients is expected to sharply increase to 70 million by 2050. The efforts to find cure for Alzheimer's have been disappointing. Neuropathological evidence has shown that the reduced levels of acetylcholine, amyloid senile plaques and neurofibrillary tangles formation within the brain of afflicted individuals play a crucial role in the pathogenesis of AD. Accordingly the enhancement of cholinergic neurotransmission and the inhibition of beta-amyloid peptide formation are considered as main approaches for effective treatment of Alzheimer's disease.

Cholinesterase inhibitors, which attempt to increase active levels of acetylcholine in the synaptic cleft by inhibiting the enzymes that degrade acetylcholine, are first line symptomatic therapy for AD. So, the choline esterase inhibitors are huge demand to develop a drug for treating Alzheimer's by which the risk of associated chronic symptoms can be reduced.

The main objective of the present study was to identify the design and synthesize novel active Benzotriazole derivatives displaying anti-Alzheimer activity through inhibition of cholinesterase enzyme based on structural modification of Benzotriazole.

The Rivastigmine was the standard drug used for the treatment of Alzheimer's disease. Where Benzotriazole structure was used to design some new compounds which can selected as good candidate for the treatment of neurodegenerative diseases.

IV. PLAN OF WORK



V. EXPERIMENTAL SECTION

5.1. PHASE I – *IN-SILICO* STUDY

Software and databases used

- Chemsketch (https://www.acdlabs.com/resources/freeware/chemsketch/)
- Molinspiration (https://www.molinspiration.com/cgi-bin/properties)
- Online Smiles Translator (https://cactus.nci.nih.gov/translate/)
- Qikprop
- Schrodinger Maestro v12.6, Glide 2020-3

5.1.1. Drug Design Approach

Drug designing for Cholinesterase inhibitor was done in following phases:

- Identification of the target enzymes
- Lead identification
- Lead Optimization
- Docking of the Ligand with enzymes (Acetyl cholinesterase – 4EY7, Butyryl cholinesterase – 4BDS)

Identification of the target enzymes [41-45]

The present study was focused on Anti-Alzheimer's activity. From various literature reviews, the cholinesterase enzymes were identified as the target enzyme which is involved in the neuro transmitter releases. The target enzymes were downloaded from RCSB-Protein Data Bank, AChE **(PDB ID: 4EY7)** and BChE **(PDB ID: 4BDS).**

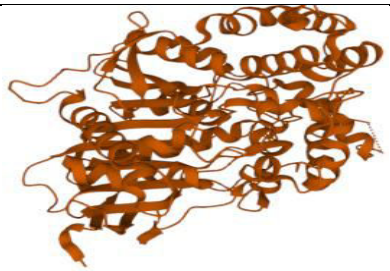
	**Method :** X-RAY DIFFRACTION **Resolution :** 2.35 Å **R – Value Free :** 0.211 **R – Value Work :** 0.175 **R – Value Observed :** 0.177

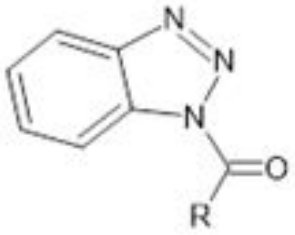
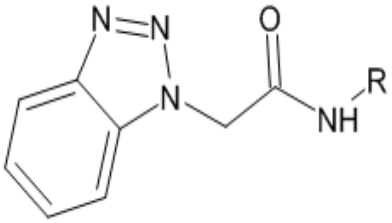
Fig. 5 - Acetyl Cholinesterase Enzyme (Without Ligand)

	**Method :** X-RAY DIFFRACTION **Resolution :** 2.10 Å **R – Value Free :** 0.209 **R – Value Work :** 0.175 **R – Value Observed :** 0.176

Fig. 6 - Butyryl Cholinesterase Enzyme(Without Ligand)

Lead Identification

From various literatures reviewed that derivatives of Benzotriazole, Benzotriazole hybrids have been reported to exhibit anti Alzheimer's activity.

Lead Molecule for Scheme I	Lead Molecule for Scheme II
	

Lead Optimization

Lead Optimization is a process in which lead compounds are altered to achieve maximum affinity towards the target. To make the lead moiety safer, effective and to possess good pharmacokinetic properties structural modifications were done.

ADME Calculation:

QikProp is a quick, accurate, easy to use absorption, distribution, metabolism and excretion (ADME) prediction program designed by Professor William L Jorgensen. QikProp predicts physically significant descriptors and pharmaceutically relevant properties of organic molecules, either individually or in batches. In addition to predicting molecular properties, QikProp provides ranges for comparing a particular molecules property with those of 95% of known drugs.

Docking with glide:

Required software's and servers

- Chemsketch or chemdraw - for drawing a structure and generate the smiles.
- Online smiles translator - to convert ligand to .sdf file
- Protein preparation wizard
- Ligand preparation
- Glide (receptor grid generation, ligand docking)

Procedure:

- Protein and ligand preparation for glide describes the preparation of the protein and

the ligands for use in glide.

- Receptor grid generation describes the use of the receptor grid generation panel to

Calculate the grids that represent the receptor and defining potential constraints to the receptor.

- Ligand docking describes the use of the ligand docking panel to set up and run

Docking jobs, and the use of glide constraints and distributed processing of multiple-

Ligand docking calculations.

- Visualizing glide docking results contains information on visualizing the results of

Glide docking runs, using the pose view mode in the project table and the glide xp visualizer.

I) PROTEIN PREPARATION WIZARD (PROTEIN PREPARATION AND REFINEMENT

To open protein preparation wizard

- Go to task → browse → protein preparation and refinement
    A. Import and process
    B. Review and modify
    C. Refine

A) Import and process

Enter PDB id and import the enzyme

- Click → convert selenomethionines to methionines
- Click → delete water beyond 5.00 Å from het groups
- Click → preprocess

B) Review and modify

- Click→ analyze workspace
- Delete unwanted chains

C) Refine

- Click → optimize
- After job completes
- Click→ minimize

II) RECEPTOR GRID GENERATION

Click task → open browse → click glide → click receptor grid generation

i. Receptor
ii. Site
iii. Constraints
iv. Rotatable groups
v. Excluded volumes

i) Receptor - Click show markers and pick to identify the ligand

ii) Site - Click → centroid of workspace ligand (selected in the receptor tab).

Go to → advance settings→ fix X,Y and Z bond length

iii) Constraints - (No change)

iv) Rotatable groups - (No change)

v) Excluded volumes - (No change)

Then click run job starts to run and the grid is obtained as output file.

E.g.: glide-grid_4ag8_zip.

After job completes, Click → file → import structures as .sd format

III) LIGPREP

Select structures from project table, workspace or file

- Click → neutralize
- Click → desalt, generate tautomer
- Click → generate all combinations (type 1 per ligand)
- Click → run.

After job completes the output file obtained as E.g. LigPrep ligand_out.maegz

IV) LIGAND DOCKING

i. Ligands
ii. Settings
iii. Core
iv. Constraints
v. Torsional Constraints
vi. Output

- Click → display receptor.
- Click browse in the tab and select the grid file of the enzyme (output of receptor grid generation)

i) Ligands

- Use ligands from workspace
- other settings are default

ii) Settings

- Select precision type,
- Select ligand sampling and
- other settings are default,
- Then, Go to → advance settings → click ok

iii) Core

(No change - default)

iv) Constraints

(No change - default)

v) Torsional constraints

(No change - default)

vi) Output

- Click pose viewer
- Write out at most 1 poses per ligand
- Number of poses per ligand to include 1
- Go to advance settings → click ok
- Change job name (if needed)
- Click → run and monitor the job

Output format: E.g.: glide-dock_sp_4ag8_pv.maegz

DOCKING RESULTS

View docked poses in the workspace

To view ligand interaction

Then go to → ligand interaction → to view interaction of each ligand with the binding sites of enzymes.

To view docking values

- Then go to → table (right side corner). Click → tree.
- Select options as needed (for glide select primary).
- Values were obtained and exported the values by selecting the option data → click → export. File was saved as spreadsheet format.

5.2. PHASE II – SYNTHETIC STUDY

Based on the results obtained from the docking results and the availability of the chemicals, compounds having good docking score and good ligand interaction where synthesized using conventional synthetic methods.

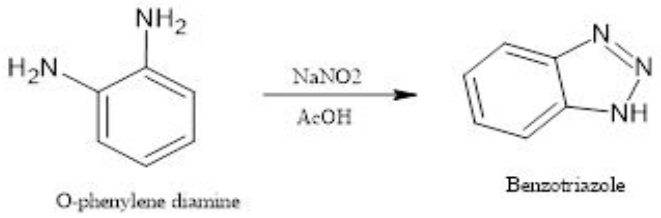
Chemicals and Reagents used:

- O-Phenylenediamine, Sodium nitrite, Acetic acid, Thionyl chloride, Dimethylformamide, Potassium carbonate, 2-Chlorobenzoic acid, 2-Bromobenzoic acid, 2-Nitrobenzoic acid, Phenoxyacetic acid.
- Ethylchloroacetate, Potassium carbonate, Acetone, Ethanol, Acetonitrile, n-Hexane (mobile phase), 4-Chloroaniline, 4-Bromoaniline, 4-Aminopyridine, p-Nitro aniline, 2-Nitro aniline.

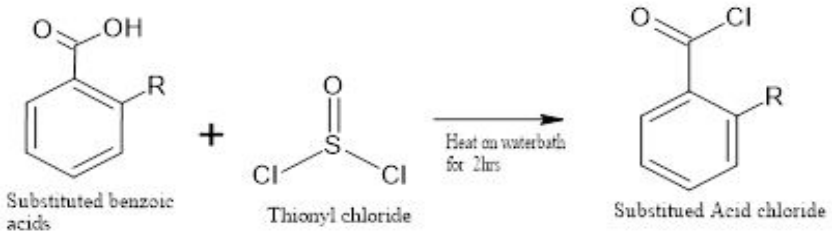
All the reagents and chemicals were procured from sigma Aldrich, high Media, Loba Chem, and Sd fine chemicals. The procured compounds were used for synthetic procedure.

5.2.1. SYNTHETIC SCHEME I

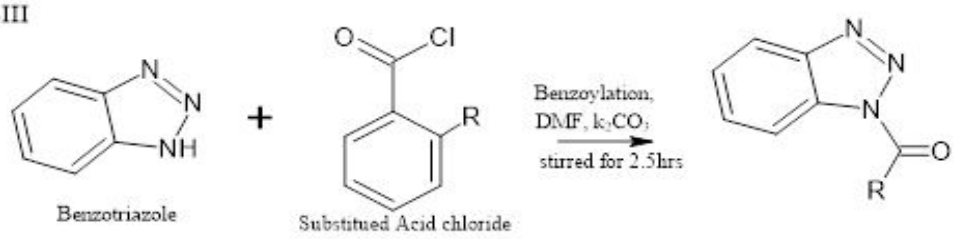
STEP-I

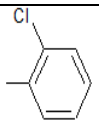
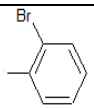
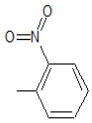
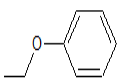


STEP-II



STEP-III



Compound	Substitution
BA-1A	
BA-1B	
BA-1C	
BA-1D	

Procedure (scheme I) [46]

I) Synthesis of Benzotriazole:

10.8gm of o-phenylenediamine is added to mixture of 12g (11.5 ml) of and glacial acetic acid and 30 ml of water, which is cooled to 15°C, stir. Then solution of 7.5g of sodium nitrite in 15 ml water is added in portion. The temperature rises slowly to 85°C and then cools slowly. When temperature is 45°C the mixture is chilled at ice bath for 30 min. Pale brown solid separated by the filtration. The recrystallization is done using benzene as solvent.

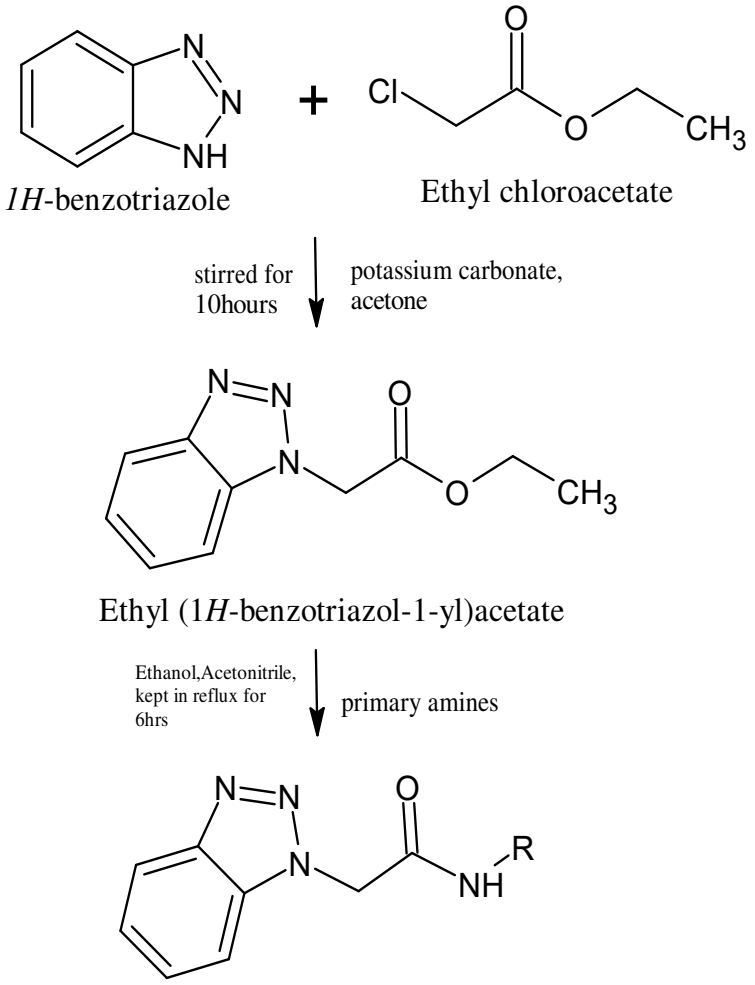
II) Preparation of acid chloride:

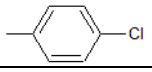
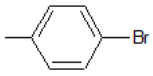
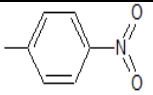
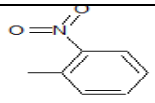
In a clean and dry 100 ml round bottom flask substituted-benzoic acid (0.025 mol) was taken then thionyl chloride (0.05 mol) was added then the mixture was heated on a water bath for 2 hours. After that the unreacted thionyl chloride was removed by heating in water bath such a way that water vapor should not came indirect contact with acid chloride as it prone to hydrolysis. Then the acid chloride so formed is free from thionyl chloride and kept it in air tight container.

III) Preparation of (1H-benzotriazol-1-yl) (2-substituted) methanone:

A solution of 150 mg (2.5 mmol) Benzotriazole, DMF, and potassium carbonate 2.0 g (7.5 mmol) were added, the mixture was stirred for 2.5 hours at room temperature, and acid chloride was added. After stirring for 2.5 hours the mixture was poured into distilled water (150 ml) and extracted with diethyl ether (3-50 ml.). The combined extracts were dried with $MgSO_2$, and filtered. The ether was removed by vacuum distillation. The product formed was collected and dried

5.2.2. SYNTHETIC SCHEME II



Compound	Substitution
BE-2A	
BE-2B	
BE-2C	
BE-2D	

Procedure (scheme II)[47]

I) Synthesis of ethyl 1H-benzotriazol-1.ylacetate

A mixture of Benzotriazole (0.1M), ethylchloroacetate (0.1M) and 0.3g of potassium carbonate in 60 ml of acetone was stirred for 10 hours. The solvent was removed under reduced pressure. A solid mass was produced and then needle shaped brown crystals were obtained after recrystallization from the mixture of chloroform and ether (8:2%V /V). The yield obtained was 60% and MP was 40˚C.

II) Synthesis of 2-(*1H*-1, 2, 3-benzotriazol-1-yl) - N-phenylacetamide derivatives:

In a round bottom flask, ethyl *1H*-benzotriazol-1-ylacetate and substituted aromatic primary amines was treated in equimolar concentration and refluxed for six hours in ethanol and acetonitrile as solvent. After cooling, forms precipitates as solid and the reaction mixture is collected via the filtration using Buchner funnel, and dried in a vacuum desiccator.

5.3. PHASE III – ANALYTICAL STUDY

INSTRUMENTS:

- Mechanical stirrer (REMI Elektrotechnik Ltd) in the Department of Pharmaceutical Chemistry, College of Pharmacy, SRIPMS, Coimbatore.
- Melting point was determined using Visible Range melting point apparatus (LABINDIA) in the Department of Pharmaceutical Chemistry, College of Pharmacy, SRIPMS, Coimbatore.
- UV Spectra was recorded using (JASCO V 530) - UV Visible Spectrophotometer in the Department of Pharmaceutical Analysis, College of Pharmacy, SRIPMS, Coimbatore.
- IR Spectra was recorded using (SHIMADZU) - IR spectrophotometer in the Department of Pharmaceutical Analysis, College of Pharmacy, PSG Institutions, Coimbatore.
- Mass Spectroscopy in Interdisciplinary Institute of Indian System of Medicine, SRM Institute of Science and Technology, Kattankulathur, Chennai.
- H1-NMR Spectroscopy-(BRUKER)NMR 300MHz in Department of Applied Chemistry, Karunya Institute of Technology and Sciences, Coimbatore.

5.4. PHASE IV – *IN-VITRO* ENZYME INHIBITORY STUDY

REAGENTS AND CHEMICALS USED:

Tris HCL buffer, Acetyl Cholinesterase enzyme solution, Acetyl Thiocholine Iodide, Butyryl Cholinesterase enzyme solution, Butyryl Thiocholine Iodide, DTNB(5,5' – dithiobis – (2-nitrobenzoic acid)) [Ellman's Reagent], and Water.

PROCEDURE:

5.4.1. Ellman's Reagent Method [49]

The inhibitory effects of synthesized Benzotriazole derivatives on AChE and BuChE activities were measured by slightly modifying the spectrophotometric Ellman's method.

Acetylthiocholine iodide (AChE) and Butyrylthiocholine iodide (BChE) was used as a substrate of the reaction. 5, 5'-Dithiobis (2-nitro-benzoic acid) (DTNB) was used for the determination of the AChE/BuChE activities.

The final volume 4 ml of the reaction mixture consist of 1.3ml of tris-HCI buffer (ImM, pH 8.0) treated with the 0.4ml of different concentration (10 to 320 µg/ml) of the drug solution and to it 0.1 ml of AChE/BuChE (0.28 U/ml) is added. The mixture was incubated for 15 min and to it 0.3 ml of Acetylthiocholine iodide (0.024 mg/ml) and Butyrylthiocholine iodide (0.0238 mg/ml) and 1.9ml DTNB (3mM).The final mixture (4ml) was further incubated for 30mins at room temperature and the absorbance of the reaction mixture was taken at 412nm.

The control was prepared by replacing the drug with the suitable solvent.

The blank was prepared by replacing all the reagent with the solvent (water) to nullify the effect of color of the test drug. All determinations of the assay were done in triplicate and the results were expressed as standard error of mean.

The percentage inhibition was calculated using the formula

$$\% \text{ Inhibition} = (A0-A1)/A0 \times 100$$

A0 = Absorbance of Control A1 = Absorbance of Sample

VI. RESULTS AND DISCUSSION

6.1. *IN SILICO* STUDIES

6.1.1. LEAD OPTIMIZATION

After the selection of lead was modified with various substituents. Since Benzotriazole are reported to possess anti-Alzheimer's activity and it was incorporated with the substituents. It has to be subjected to optimization in order to evaluate their ADME properties.

Table 1 - ADME Properties of Benzotriazole derivatives using QIKPROP

QIKPROP Parameters Agreeable Ranges:

S.NO	DESCRIPTORS	RANGE
1	#stars	0-5
2	#amine	0-1
3	#amidine	0
4	#acid	0-1
5	#amide	0-1
6	#rotor	0-15
7	#rtvFG	0-2
8	CNS	-2 to +2
9	MW	130.0 – 725.0
10	Dipole	1.0 – 12.5
11	SASA	300.0 – 1000.0
12	FOSA	0.02 – 750.0
13	FISA	7.0 – 330.0
14	PISA	0.0 – 450.0
15	WPSA	0.0 – 175.0
16	Volume	500.0 – 2000.0
17	Donor HB	0.0 – 6.0
18	Acceptor HB	2.0 – 20.0
19	Dip^2/V	0.0 – 0.13
20	ACxDN^.5/SA	0.0 – 0.05
21	Glob	0.75 – 0.95

22	Qppolrz	13.0 – 70.0
23	QplogPC16	4.0 – 18.0
24	QplogPoct	8.0 – 35.0
25	QplogPw	4.0 – 45.0
26	QplogPo/w	-2.0 to 6.5
27	QplogS	-6.5 to 0.5
28	CIQPlogS	-6.5 to 0.5
29	QplogHERG	Below -5
30	QPPCaco	<25 Poor, >500 great
31	QplogBB	-3.0 to 1.2
32	QPPMDCK	<25 Poor, >500 great
33	QplogKp	-8.0 to -1.0
34	IP(ev)	7.9 – 10.5
35	EA(eV)	-0.9 to 1.7
36	#metab	1 – 8
37	QplogKhsa	-1.5 to 1.5
38	Human Oral Absorption	1,2 or 3 for low, medium or high
39	Percent Human Oral Absorption	>80% is high, <25% is poor
40	SA Fluorine	0.0 – 100.0
41	SaamideO	0.0 - 35.0
42	PSA	7.0 – 200.0
43	#NandO	2 – 15
44	Rule of Five	mol_MW < 500, QplogPo/w<5, donorHB≤5,accptHB≤10
45	Rule of Three	QplogS> -5.7, QPPCaco > 22nm/s, #Primary Metabolites < 7
46	#ring atoms	Number of atoms in rings
47	#in34	Number of atoms in 3- or 4-membered rings
48	#in56	Number of atoms in 5- or 6-membered rings
49	#noncon	Number of ring atom not able to form conjugated aromatic systems
50	#nonHatm	Number of heavy atoms(nonhydrogen atoms)

Molecular and ADME Properties predicted by QIKPROP

Table 2- Molecular and ADME properties of Benzotriazole Derivatives (Scheme-I)

Title	#stars	#amine	#amidine	#acid	#amide	#rotor	#rtvFG	CNS	mol MW	dipole
BA-1A	1	0	0	0	0	1	0	0	257.679	2.04
BA-1B	1	0	0	0	0	1	0	0	302.13	1.56
BA-1C	0	0	0	0	0	2	0	-1	268.231	5.246
BA-1D	1	0	0	0	0	3	0	0	253.26	0.808
BA-1E	0	0	0	0	0	1	0	0	237.26	1.622
BA-1F	0	0	0	0	0	2	0	-1	239.233	2.225
BA-1G	1	0	0	0	0	1	0	1	381.026	1.148
BA-1H	1	0	0	0	0	1	0	0	241.224	2.756
BA-1I	1	0	0	0	0	1	0	0	349.13	2.753
BA-1J	1	0	0	0	0	1	0	0	257.679	2.635
BA-1K	1	0	0	0	0	2	1	-1	281.27	3.421
BA-1L	1	0	0	0	0	1	0	1	292.124	2.572
BA-1M	0	0	0	0	0	2	0	-1	238.248	3.518
BA-1N	0	0	0	0	0	2	0	0	266.302	2.694
BA-1O	1	0	0	0	0	2	0	-2	282.258	6.991
BA-1P	0	0	0	0	0	2	0	0	253.26	2.194
BA-1Q	0	0	0	0	0	1	0	0	251.287	1.677
BA-1R	0	0	0	0	0	3	0	-1	269.259	3.066
BA-1S	1	0	0	1	0	3	0	-2	303.292	4.737
BA-1T	0	0	0	0	0	1	0	0	224.221	3.115

Table 3- Molecular and ADME properties of Benzotriazole Derivatives (Scheme-I)

Title	SASA	FOSA	FISA	PISA	WPSA	volume	donorHB	accptHB	dip^2/V	ACxDN^.5/SA
BA-1A	474.591	0	81.279	336.353	56.959	789.078	0	4.5	0.005275	0
BA-1B	478.039	0	80.765	335.567	61.707	796.471	0	4.5	0.003054	0
BA-1C	478.212	0	150.28	327.931	0	810.762	0	5.5	0.03395	0
BA-1D	508.915	27.37	99.85	381.696	0	842.61	0	5.25	0.000775	0
BA-1E	486.418	88.212	88	310.205	0	806.94	0	4.5	0.00326	0
BA-1F	465.397	0	140.65	324.747	0	767.319	1	5.25	0.00645	0.011
BA-1G	508.403	0	81.026	286.427	140.95	851.489	0	4.5	0.001549	0
BA-1H	463.589	0	88.075	328.695	46.82	763.328	0	4.5	0.009952	0
BA-1I	489.052	0	88.001	317.549	83.502	809.956	0	4.5	0.009355	0
BA-1J	478.327	0	88	318.8	71.528	791.007	0	4.5	0.008779	0
BA-1K	547.825	98.434	146.51	302.884	0	913.038	0	6.5	0.012821	0
BA-1L	499.286	0	81.433	287.911	129.94	834.402	0	4.5	0.007926	0
BA-1M	470.372	0	149.64	320.73	0	776.812	1.5	5.5	0.015933	0.014
BA-1N	533.5	153.87	90.719	288.915	0	899.845	0	5.5	0.008065	0
BA-1O	520.233	75.277	175	269.96	0	875.269	0	5.5	0.055845	0
BA-1P	492.539	93.288	87.985	311.266	0	823.901	0	5.25	0.005844	0
BA-1Q	519.059	176.35	88.079	254.627	0	867.524	0	4.5	0.003241	0
BA-1R	500.727	88.685	138.99	273.057	0	844.819	1	6	0.011129	0.012
BA-1S	517.284	0	215.49	299.343	2.449	870.412	1	8.5	0.025775	0.016
BA-1T	447.707	0	116.49	331.219	0	734.545	0	6	0.013209	0

Table 4- Molecular And ADME Properties of Benzotriazole Derivatives (Scheme-I)

Title	Glob	QPpolrz	QPlogPC16	QPlogPoct	QPlogPw	QPlogPo/w	QPlogS	CIQPlogS	QPlogHERG	QPPCaco
BA-1A	0.87015	27.995	8.857	11.836	7.934	2.3	-2.94	-3.357	-5.237	1679.304
BA-1B	0.86926	28.283	8.952	11.925	7.935	2.369	-3.026	-4.301	-5.248	1698.278
BA-1C	0.87931	28.14	9.159	12.861	9.099	1.25	-2.074	-3.117	-5.049	372.211
BA-1D	0.84776	29.286	9.329	12.392	8.729	1.991	-2.624	-2.915	-5.816	1119.497
BA-1E	0.86175	28.46	8.451	11.659	7.81	2.122	-2.916	-2.923	-5.248	1450.085
BA-1F	0.87095	26.371	8.802	13.1	10.199	1.352	-2.688	-2.95	-5.169	459.318
BA-1G	0.85456	30.016	9.642	12.794	7.703	2.971	-3.947	-5.976	-5.251	1688.612
BA-1H	0.87131	26.891	7.89	11.483	7.898	2.038	-2.686	-3.007	-5.182	1447.735
BA-1I	0.85925	28.651	9.108	12.229	7.896	2.469	-3.33	-5.299	-5.324	1450.075
BA-1J	0.86476	27.905	8.871	11.915	7.874	2.301	-3.074	-3.357	-5.241	1450.105
BA-1K	0.83084	31.994	9.751	14.276	10.056	1.427	-2.869	-2.93	-5.714	404.178
BA-1L	0.85849	29.346	9.425	12.605	7.69	2.817	-3.726	-4.088	-5.195	1673.676
BA-1M	0.86883	26.713	9.035	14.246	11.224	1.21	-2.754	-2.865	-5.184	377.438
BA-1N	0.84491	31.333	9.178	13.182	8.562	2.198	-3.122	-3.076	-5.419	1366.508
BA-1O	0.8506	30.168	9.416	13.83	8.974	1.432	-2.87	-3.415	-5.237	216.974
BA-1P	0.86293	28.507	8.637	12.09	8.35	1.869	-2.487	-2.915	-5.234	1450.555
BA-1Q	0.84749	30.354	8.603	12.201	7.511	2.452	-3.505	-3.221	-5.214	1447.592
BA-1R	0.86312	28.337	9.167	14.124	10.44	1.474	-2.872	-3.248	-5.049	476.33
BA-1S	0.85229	29.612	10.112	16.528	13.642	0.647	-2.332	-2.952	-3.542	22.698
BA-1T	0.87939	25.764	8.171	11.872	9.531	0.747	-1.397	-1.951	-5.063	778.461

Table 5- Molecular And ADME Properties of Benzotriazole Derivatives (Scheme-I)

Title	QPlogBB	QPPMDCK	QPlogKp	IP(eV)	EA(eV)	#metab	QPlogKhsa	HumanOralAbsorption	PercentHumanOralAbsorption	SAfluorine
BA-1A	-0.073	1777.09	-1.737	9.358	1.409	0	-0.338	3	100	0
BA-1B	-0.058	1909.833	-1.73	9.339	1.39	0	-0.316	3	100	0
BA-1C	-0.853	169.99	-2.942	9.438	1.656	1	-0.585	3	80.276	0
BA-1D	-0.544	558.905	-1.727	9.351	1.529	2	-0.468	3	93.178	0
BA-1E	-0.287	739.26	-1.953	9.305	1.381	1	-0.286	3	95.955	0
BA-1F	-0.793	213.371	-2.776	9.263	1.332	1	-0.339	3	82.507	0
BA-1G	0.109	5157.095	-1.908	9.404	1.465	0	-0.156	3	100	0
BA-1H	-0.155	1332.031	-1.889	9.42	1.524	0	-0.412	3	95.446	46.82
BA-1I	-0.087	2119.429	-1.927	9.349	1.505	0	-0.277	3	100	0
BA-1J	-0.106	1822.353	-1.922	9.385	1.492	0	-0.332	3	100	0
BA-1K	-0.972	185.824	-2.961	9.426	1.57	0	-0.555	3	81.953	0
BA-1L	0.085	4445.625	-1.91	9.417	1.476	0	-0.206	3	100	0
BA-1M	-0.879	172.572	-2.956	8.534	1.238	1	-0.339	3	80.15	0
BA-1N	-0.404	693.315	-1.982	8.412	1.256	1	-0.326	3	95.935	0
BA-1O	-1.17	94.861	-3.602	9.597	1.861	2	-0.397	3	77.146	0
BA-1P	-0.345	739.519	-1.853	9.296	1.373	1	-0.48	3	94.475	0
BA-1Q	-0.314	737.886	-2.15	9.302	1.373	2	-0.11	3	100	0
BA-1R	-0.861	221.925	-2.831	9.387	1.48	2	-0.327	3	83.507	0
BA-1S	-1.589	10.845	-4.148	9.356	1.488	0	-0.889	2	55.005	0
BA-1T	-0.501	377.39	-2.404	9.469	1.6	2	-0.897	3	83.067	0

Table 6- Molecular And ADME Properties of Benzotriazole Derivatives (Scheme-I)

Title	SAamideO	PSA	#NandO	#ringatoms	#in34	#in56	#noncon	#nonHatm	Jm
BA-1A	0	57.725	4	15	0	15	0	18	5.426
BA-1B	0	57.487	4	15	0	15	0	18	5.3
BA-1C	0	97.944	7	15	0	15	0	20	2.586
BA-1D	0	68.746	5	15	0	15	0	19	11.288
BA-1E	0	58.213	4	15	0	15	0	18	3.212
BA-1F	0	79.948	5	15	0	15	0	18	0.823
BA-1G	0	57.918	4	15	0	15	0	19	0.533
BA-1H	0	58.31	4	15	0	15	0	18	6.402
BA-1I	0	58.211	4	15	0	15	0	18	1.934
BA-1J	0	58.209	4	15	0	15	0	18	2.597
BA-1K	0	94.664	6	15	0	15	0	21	0.416
BA-1L	0	58.027	4	15	0	15	0	19	0.675
BA-1M	0	84.597	5	15	0	15	0	18	0.465
BA-1N	0	62.021	5	15	0	15	0	20	2.099
BA-1O	0	101.813	7	15	0	15	0	21	0.095
BA-1P	0	66.338	5	15	0	15	0	19	11.877
BA-1Q	0	58.255	4	15	0	15	0	19	0.52
BA-1R	0	89	6	15	0	15	0	20	0.533
BA-1S	0	118.391	7	15	0	15	0	21	0.1
BA-1T	0	71.335	5	15	0	15	0	17	35.5

Table 7- Molecular And ADME Properties of Benzotriazole Derivatives (Scheme-II)

Title	#stars	#amine	#amidine	#acid	#amide	#rotor	#rtvFG	CNS	mol MW	Dipole
BE-2A	0	0	0	0	0	3	0	0	286.72	4.938
BE-2B	0	0	0	0	0	3	0	0	331.171	4.929
BE-2C	0	0	0	0	0	4	0	-2	297.273	8.882
BE-2D	0	0	0	0	0	4	0	-2	297.273	4.741
BE-2E	0	0	0	0	0	4	0	-2	268.274	9.099
BE-2F	0	0	0	0	0	3	0	-1	253.263	5.602
BE-2G	0	0	0	0	0	3	0	-1	253.263	4.498
BE-2H	0	0	0	0	0	3	0	0	270.265	5.034
BE-2I	0	0	0	0	0	3	0	0	378.172	4.941
BE-2J	0	0	0	0	0	3	0	0	266.302	8.222
BE-2K	0	0	0	0	0	3	0	0	280.329	8.2
BE-2L	0	0	0	0	0	4	0	0	282.301	7.892
BE-2M	0	0	0	1	0	5	0	-2	332.333	11.687
BE-2N	0	0	0	0	0	5	1	-2	324.338	7.261
BE-2O	0	0	0	0	0	4	0	-2	267.29	8.518
BE-2P	0	0	0	0	0	4	1	-2	310.312	5.912
BE-2Q	0	0	0	0	0	6	0	-2	357.414	7.387
BE-2R	0	0	0	0	0	6	0	-2	407.446	12.187
BE-2S	0	0	0	0	1	4	0	0	266.302	8.369
BE-2T	0	2	0	0	1	5	0	1	288.352	9.703

Table 8- Molecular And ADME Properties of Benzotriazole Derivatives (Scheme-II)

Title	SASA	FOSA	FISA	PISA	WPSA	volume	donorHB	accptHB	dip^2/V	ACxDN^.5/SA
BE-2A	522.458	43.53	105.354	302.063	71.51	885.919	1	4.5	0.027527	0.008613
BE-2B	527.763	43.559	105.518	301.43	77.256	894.999	1	4.5	0.027141	0.008527
BE-2C	553.758	41.245	197.64	314.873	0	925.13	1	5.5	0.085278	0.009932
BE-2D	586.887	36.279	204.683	345.925	0	964.019	1	5.5	0.023315	0.009372
BE-2E	527.542	41.847	156.91	328.786	0	874.996	2	5.25	0.094624	0.014074
BE-2F	489.491	43.337	134.051	312.103	0	827.658	1	6	0.037916	0.012258
BE-2G	492.402	43.652	128.76	319.989	0	831.802	1	6	0.024328	0.012185
BE-2H	507.205	43.519	105.46	311.411	46.815	857.583	1	4.5	0.029552	0.008872
BE-2I	533.725	43.563	105.583	301.091	83.488	905.338	1	4.5	0.026971	0.008431
BE-2J	548.103	129.288	101.625	317.19	0	912.684	1	4.5	0.074068	0.00821
BE-2K	580.342	217.519	101.685	261.138	0	972.869	1	4.5	0.06911	0.007754
BE-2L	551.879	134.65	101.712	315.518	0	927.689	1	5.25	0.067142	0.009513
BE-2M	581.517	41.568	234.927	302.523	2.499	976.82	2	8.5	0.139815	0.020672
BE-2N	626.169	184.233	161.59	280.346	0	1071.709	1	6.5	0.04919	0.010381
BE-2O	523.417	41.203	154.215	327.999	0	877.078	2.5	5.5	0.082731	0.016614
BE-2P	609.879	139.897	160.534	309.449	0	1018.996	1	6.5	0.0343	0.010658
BE-2Q	652.842	80.865	167.232	404.745	0	1157.483	2.5	5.5	0.047139	0.013321
BE-2R	680.147	43.213	237.43	398.224	1.28	1204.428	2.5	9.5	0.123309	0.022085
BE-2S	542.97	83.339	94.278	365.352	0	911.047	1	4.5	0.07688	0.008288
BE-2T	554.062	269.154	136.936	147.972	0	965.507	2	8	0.097502	0.02042

Table 9- Molecular And ADME Properties of Benzotriazole Derivatives (Scheme-II)

Title	Glob	QPpolrz	QPlogPC16	QPlogPoct	QPlogPw	QPlogPo/w	QPlogS	ClQPlogS	QPlogHERG	QPPCaco
BE-2A	0.853841	30.259	10.004	14.428	9.008	2.86	-4.082	-4.153	-5.338	992.723
BE-2B	0.851023	30.616	10.119	14.569	9.02	2.938	-4.161	-5.045	-5.38	989.171
BE-2C	0.829176	31.308	10.541	16.146	10.538	1.814	-3.771	-3.982	-5.798	132.338
BE-2D	0.804145	33.161	11.148	15.95	10.876	2.01	-4.343	-3.982	-6.351	113.473
BE-2E	0.838646	29.435	10.103	16.78	11.51	1.77	-3.418	-3.501	-5.711	322.052
BE-2F	0.870939	28.023	9.279	14.439	10.645	1.454	-2.687	-2.943	-5.109	530.516
BE-2G	0.868677	28.264	9.325	14.318	10.669	1.524	-2.735	-2.943	-5.183	595.476
BE-2H	0.860662	29.214	9.012	13.997	9.027	2.606	-3.673	-3.827	-5.272	990.431
BE-2I	0.847985	31.027	10.252	14.734	9.034	3.024	-4.292	-6.002	-5.428	987.772
BE-2J	0.830201	31.474	9.757	15.073	9.122	2.787	-4.314	-3.749	-5.792	1076.946
BE-2K	0.818184	33.348	9.94	15.54	8.819	3.097	-4.793	-4.026	-5.719	1075.527
BE-2L	0.833533	31.417	9.909	15.34	9.639	2.598	-3.972	-3.782	-5.73	1074.896
BE-2M	0.81874	32.617	11.457	20.543	14.974	1.099	-3.246	-3.563	-4.1	14.849
BE-2N	0.808832	36.203	11.474	17.593	10.996	2.478	-4.482	-4.131	-5.962	290.765
BE-2O	0.846596	29.511	10.247	17.516	12.455	1.643	-3.262	-3.38	-5.578	341.572
BE-2P	0.802976	35.013	11.141	17.015	11.368	2.148	-4.384	-3.855	-6.196	297.55
BE-2Q	0.816645	40.181	13.566	20.848	12.936	3.326	-5.059	-5.453	-6.429	257.063
BE-2R	0.804914	41.998	14.482	24.998	17.376	1.584	-4.364	-4.968	-6.588	55.507
BE-2S	0.837047	31.227	10.052	15.055	10.691	2.265	-3.244	-3.108	-4.537	866.908
BE-2T	0.852662	30.688	9.642	18.381	14.456	-0.45	0.274	0.06	-4.7	20.041

Table 10- Molecular And ADME Properties of Benzotriazole Derivatives (Scheme-II)

Title	QPlogBB	QPPMDCK	QPlogKp	IP(eV)	EA(eV)	#metab	QPlogKhsa	HumanOralAbsorption	PercentHumanOralAbsorption	SAfluorine
BE-2A	-0.405	1209.637	-2.109	9.102	1.277	1	0.048	3	100	0
BE-2B	-0.399	1295.528	-2.114	9.269	1.289	1	0.07	3	100	0
BE-2C	-1.594	55.59	-3.669	9.201	1.533	2	-0.091	3	75.543	0
BE-2D	-1.788	47.076	-3.689	9.055	1.583	3	-0.003	3	75.494	0
BE-2E	-1.165	145.37	-2.869	8.935	0.822	2	-0.175	3	82.199	0
BE-2F	-0.796	249.334	-2.603	9.508	1.343	3	-0.365	3	84.228	0
BE-2G	-0.755	282.491	-2.477	9.42	1.276	4	-0.356	3	85.534	0
BE-2H	-0.452	883.669	-2.078	9.275	1.302	1	-0.024	3	95.821	46.815
BE-2I	-0.391	1399.328	-2.117	9.025	1.285	1	0.096	3	100	0
BE-2J	-0.6	535.979	-1.987	8.967	0.821	2	0.108	3	100	0
BE-2K	-0.63	535.216	-2.186	8.963	0.816	4	0.263	3	100	0
BE-2L	-0.654	534.877	-1.899	8.882	0.825	2	-0.035	3	96.414	0
BE-2M	-2.068	6.86	-4.303	8.955	0.815	2	-0.666	2	54.352	0
BE-2N	-1.385	130.167	-3.03	9.379	1.316	1	0.044	3	85.549	0
BE-2O	-1.112	154.917	-2.822	8.681	0.808	4	-0.218	3	81.908	0
BE-2P	-1.337	133.454	-3.004	9.057	0.911	1	-0.055	3	83.797	0
BE-2Q	-1.47	113.939	-2.6	8.12	1.189	3	0.368	3	89.553	0
BE-2R	-2.238	22.088	-3.916	8.755	1.338	2	-0.186	3	67.441	0
BE-2S	-0.575	637.452	-1.586	8.969	0.775	2	-0.258	3	92.79	0
BE-2T	-0.197	14.165	-7.167	8.962	0.802	2	-0.737	2	47.613	0

Table 11- Molecular and ADME Properties of Benzotriazole Derivatives (Scheme-II)

Title	SAamideO	PSA	#NandO	#ringatoms	#in34	#in56	#noncon	#nonHatm	Jm
BE-2A	0	70.078	5	15	0	15	0	20	0.201
BE-2B	0	70.053	5	15	0	15	0	20	0.176
BE-2C	0	116.045	8	15	0	15	0	22	0.011
BE-2D	0	112.676	8	15	0	15	0	22	0.003
BE-2E	0	93.4	6	15	0	15	0	20	0.139
BE-2F	0	82.937	6	15	0	15	0	19	1.301
BE-2G	0	83.167	6	15	0	15	0	19	1.555
BE-2H	0	70.052	5	15	0	15	0	20	0.479
BE-2I	0	70.063	5	15	0	15	0	20	0.148
BE-2J	0	71.021	5	15	0	15	0	20	0.182
BE-2K	0	70.983	5	15	0	15	0	21	0.033
BE-2L	0	79.03	6	15	0	15	0	21	0.534
BE-2M	0	130.924	8	15	0	15	0	23	0.009
BE-2N	0	105.624	7	15	0	15	0	24	0.01
BE-2O	0	95.698	6	15	0	15	0	20	0.22
BE-2P	0	107.177	7	15	0	15	0	23	0.013
BE-2Q	0	96.444	6	21	0	21	0	27	0.008
BE-2R	0	131.434	8	21	0	21	0	29	0.002
BE-2S	20.589	71.221	5	15	0	15	0	20	3.935
BE-2T	23.769	91.82	7	15	0	15	4	21	0.037

6.1.2. DOCKING STUDIES

The docking results of Acetylcholinesterase (4EY7) and Butyrylcholinesterase (4BDS) with ligands of scheme-I BA-1A to BA-1T and scheme-II BE-2A to BE-2T and the standard Rivastigmine was carried out. The ligands showing best binding affinity towards the selected enzymes and standard Rivastigmine was reported. The best docked structures should have binding energy lower to the standard.

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Table 12 - Binding Energies of BA-1A-1D and BE-2A-2D with AChE(4EY7)

Compound Code	glide rotatable bonds	Docking score	glide Ligand Efficiency	Glide gscore	glide evdw	glide energy	glide emodel
BA-1A	2	-7.414	-0.412	-7.414	-33.838	-36.629	-55.43
BA-1B	2	-7.309	-0.406	-7.309	-35.514	-37.483	-55.903
BA-1C	3	-7.768	-0.338	-7.768	-32.17	-33.794	-55.648
BA-1D	4	-8.179	-0.43	-8.179	-34.912	-39.766	-61.855
BE-2A	4	-7.864	-0.343	-7.864	-39.108	-44.243	-67.137
BE-2B	4	-7.783	-0.389	-7.783	-39.16	-45.545	-68.65
BE-2C	5	-7.453	-0.339	-7.453	-43.054	-49.619	-75.774
BE-2D	5	-7.513	-0.342	-7.513	-39.225	-46.676	-70.606
RIVASTIGMINE	6	-12.152	-0.675	-12.202	-32.76	-41.51	-57.004

The ligands BA-1A to 1D and BE-2A to 2D was showing the best docked pose to 4EY7. Though, some derivatives showed inferior binding interactions, all the selected derivatives were planned to synthesize and screen for Acetylcholinesterase inhibitory activity.

The binding sites and active sites are been showed in the snapshots.

Binding Interaction of Rivastigmine with AChE

Rivastigmine interacts with Acetylcholinesterase enzyme at PHE 295, ARG 296, TYR 341, TYR 337, TRP 286. Binding energy was found to be -12.152 kcal/mol

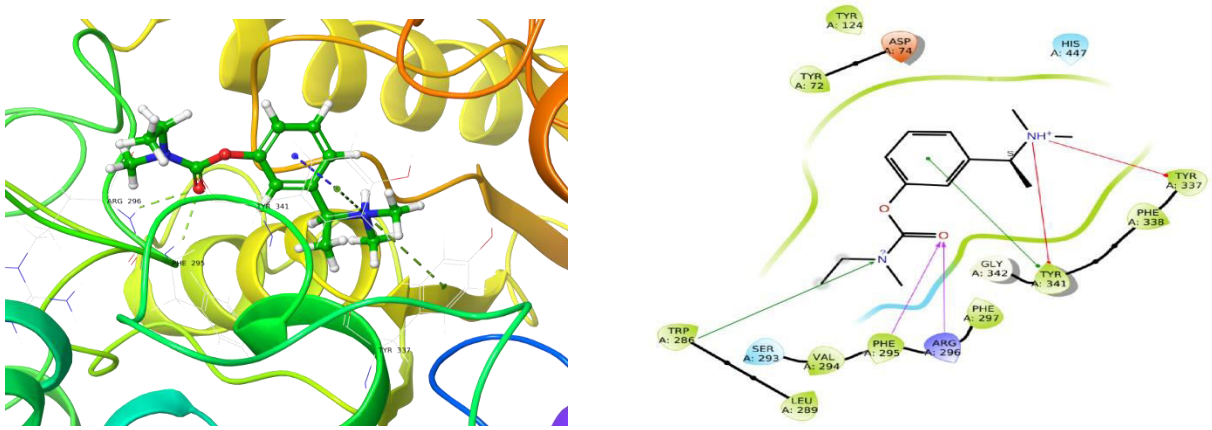


Fig. 7 – Snapshots of Rivastigmine binding with 4EY7

Binding Interaction of BA-1A-1D and BE-2A-2D with AChE

BA-1A

BA-1A interacts with Acetylcholinesterase enzyme at PHE 295, TYR 341. Binding energy was found to be -7.414 kcal/mol

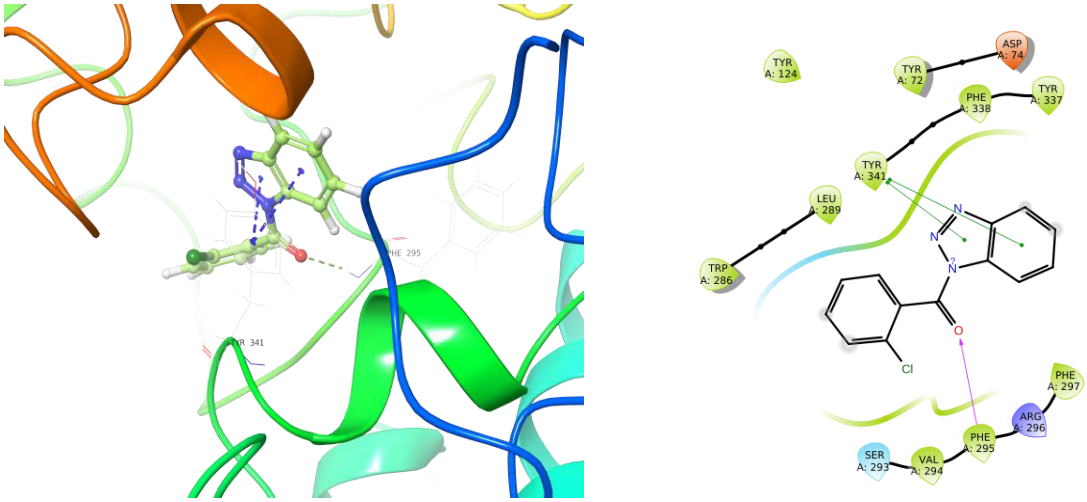


Fig. 8 – Snapshots of BA-1A binding with 4EY7

BA-1B

BA-1B interacts with Acetylcholinesterase enzyme at TYR 341, TYR 337, TRP 86. Binding energy was found to be –7.309 kcal/mol

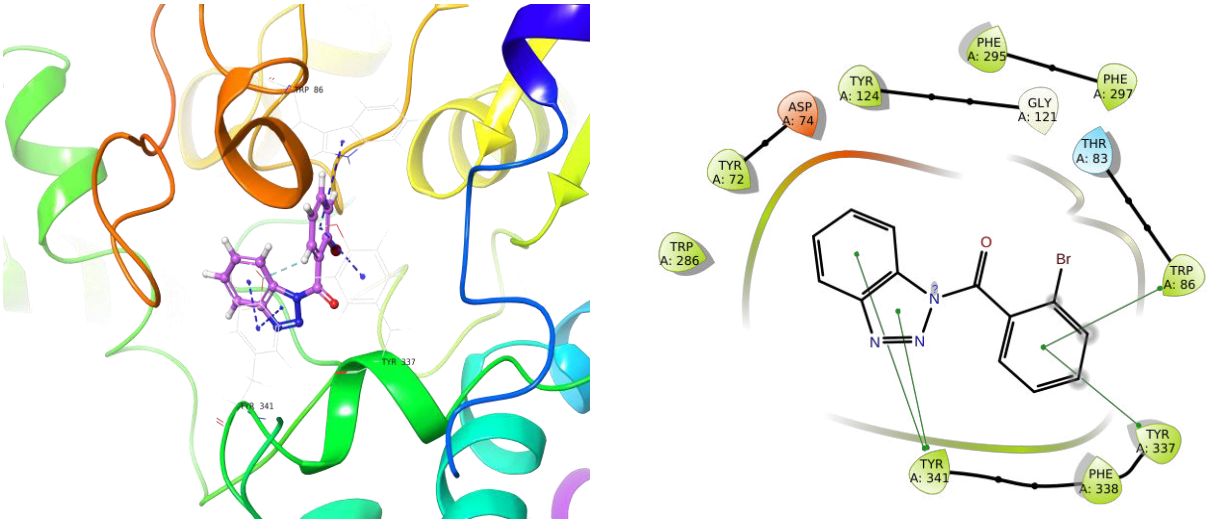


Fig. 9 – Snapshots of BA-1B binding with 4EY7

BA-1C

BA-1C interacts with Acetylcholinesterase enzyme at PHE 338, TYR 337, TYR 124, TRP 86. Binding energy was found to be -7.768 kcal/mol

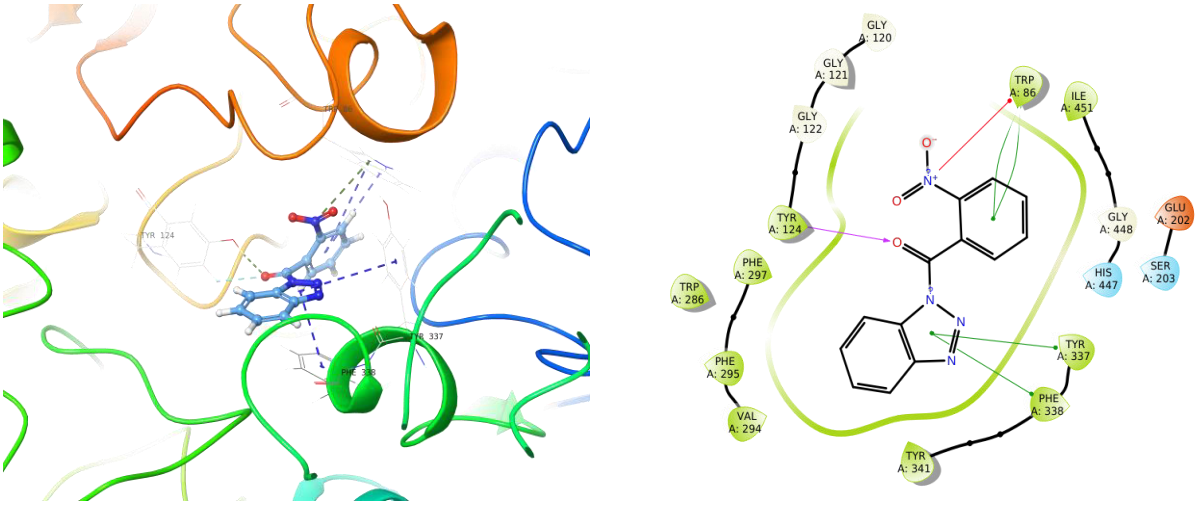


Fig. 10 – Snapshots of BA-1C binding with 4EY7

BA-1D

BA-1D interacts with Acetylcholinesterase enzyme at PHE 338, TYR 337, TYR 124, TRP 86. Binding energy was found to be -8.179 kcal/mol

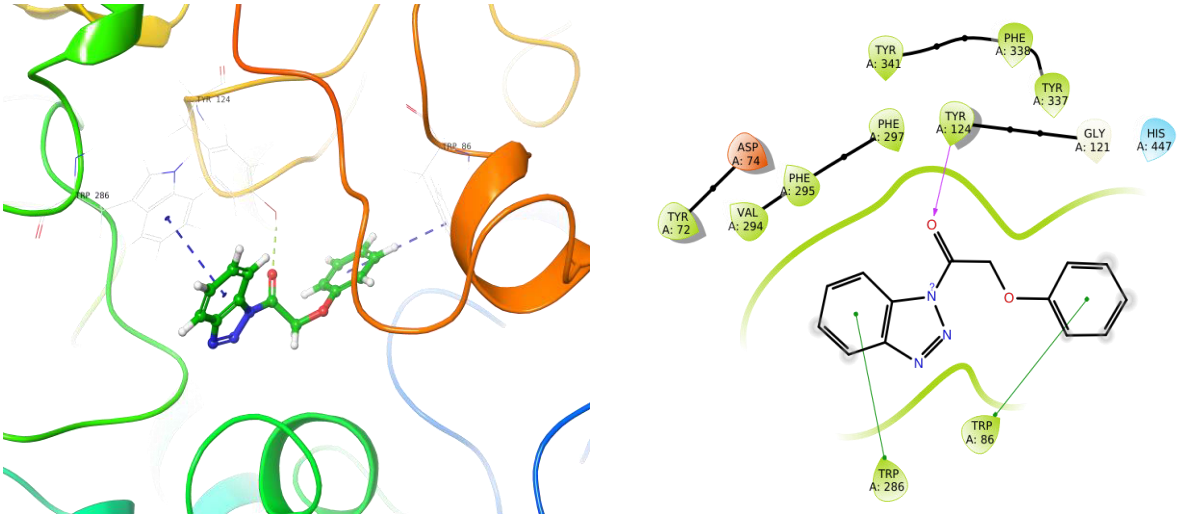


Fig. 11 – Snapshots of BA-1D binding with 4EY7

BE-2A

BE-2A interacts with Acetylcholinesterase enzyme at TRP 386, TYR 341, TYR 124, TYR 133, TRP 86. Binding energy was found to be -7.864 kcal/mol

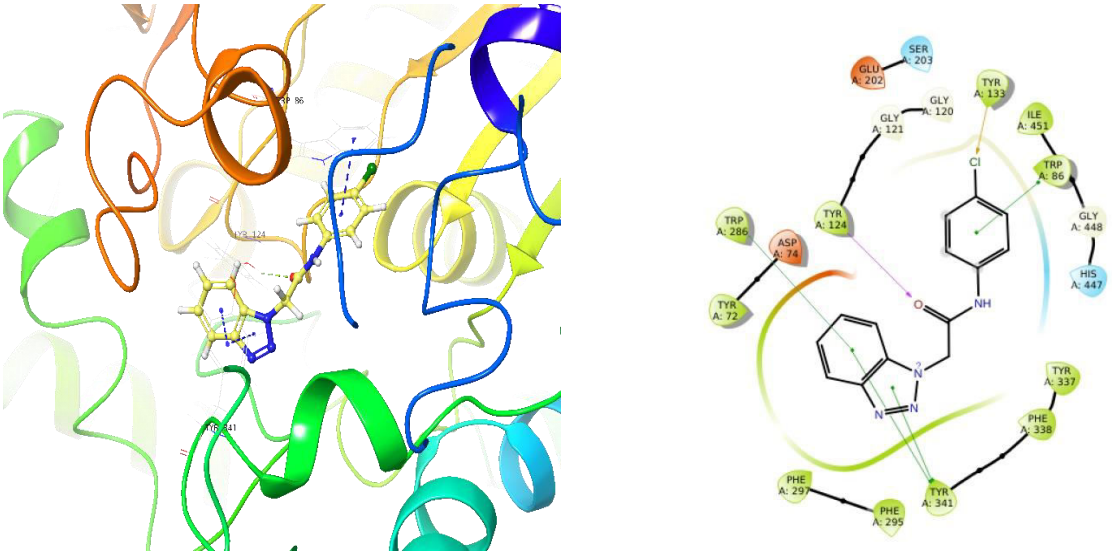


Fig. 12 – Snapshots of BE-2A binding with 4EY7

BE-2B

BE-2B interacts with Acetylcholinesterase enzyme at TYR 341, TYR 124, TYR 133, TRP 86, HIS 447. Binding energy was found to be -7.783 kcal/mol

Fig. 13 – Snapshots of BE-2B binding with 4EY7

BE-2C

BE-2C interacts with Acetylcholinesterase enzyme at TYR 341, TYR 124, GLY 202, TRP 86, HIS 447. Binding energy was found to be -7.453 kcal/mol

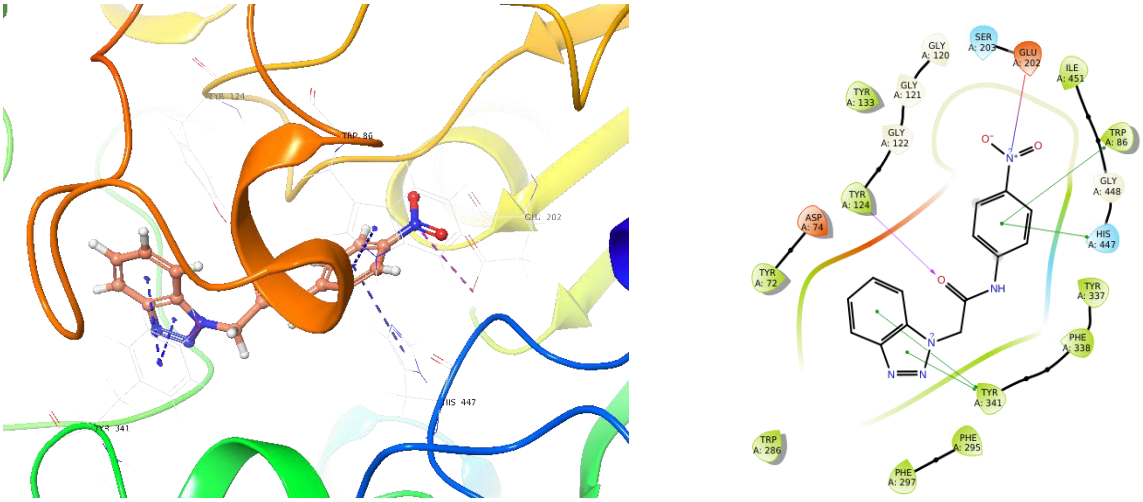


Fig. 14 – Snapshots of BE-2C binding with 4EY7

BE-2D

BE-2D interacts with Acetylcholinesterase enzyme at TYR 124, TYR 34, TRP 86, TYR 124. Binding energy was found to be -7.513 kcal/mol

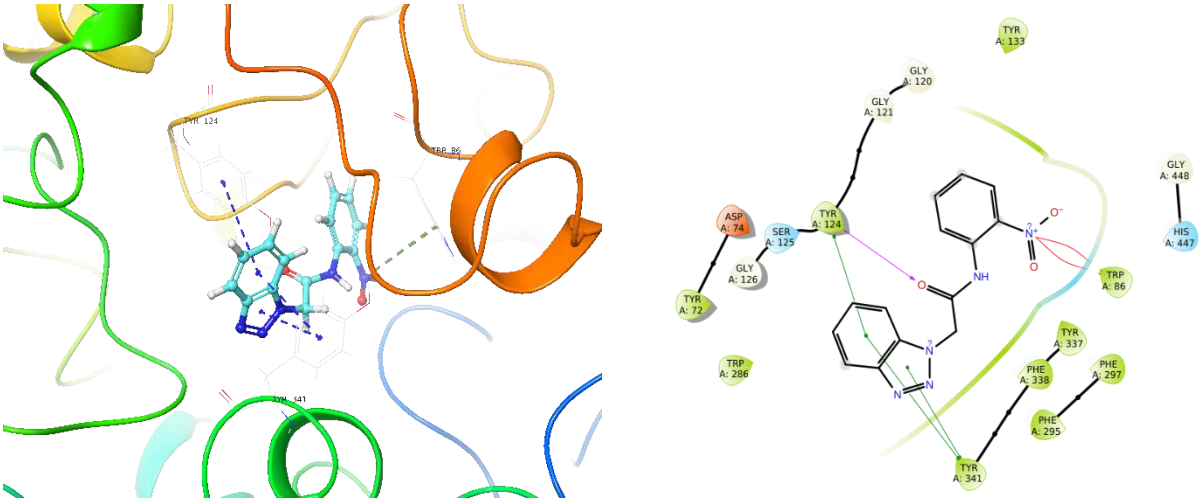


Fig. 15 – Snapshots of BE-2D binding with 4EY7

Table 13- Binding Energies of BA-1A-1D and BE-2A-2D with BuChE(4BDS)

Compound Code	glide rotatable bonds	Docking score	glide Ligand Efficiency	Glide gscore	glide evdw	glide energy	glide emodel
BA-1A	2	-5.305	-0.295	-5.305	-35.412	-37.621	-54.665
BA-1B	2	-5.321	-0.296	-5.321	-38.479	-40.176	-55.668
BA-1C	3	-5.773	-0.289	-5.773	-39.417	-39.018	-55.618
BA-1D	4	-5.938	-0.26	-5.938	-36.851	-37.739	-50.305
BE-2A	4	-6.247	-0.312	-6.247	-37.027	-41.755	-58.752
BE-2B	4	-6.826	-0.341	-6.826	-39.017	-41.753	-60.749
BE-2C	5	-5.269	-0.24	-5.269	-43.408	-44.799	-61.724
BE-2D	5	-5.504	-0.25	-5.504	-36.934	-44.83	-60.872
RIVASTIGMINE	6	-5.974	-0.332	-6.023	-34.31	-38.368	-51.754

The ligands BA-1A to 1D and BE-2A to 2D was showing the best docked pose to 4BDS. Though, some derivatives showed inferior binding interactions, all the selected derivatives were planned to synthesize and screen for Butyrylcholinesterase activity.

The binding sites and active sites are been showed in the snapshots.

Binding Interaction of Rivastigmine with 4BDS

Rivastigmine interacts with Butyrylcholinesterase enzyme at ASP 70, TYR 332, TRP 82. Binding energy was found to be -5.974 kcal/mol

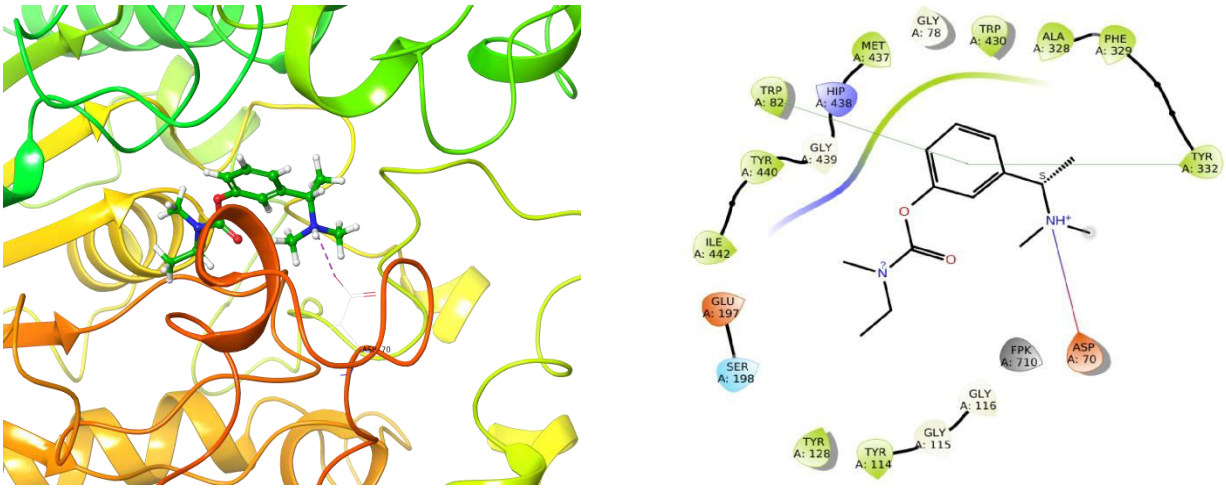


Fig. 16 – Snapshots of Rivastigmine binding with 4BDS

Binding Interaction of BA-1A-1D and BE-2A-2D with BuChE

BA-1A

BA-1A interacts with Butyrylcholinesterase enzyme at GLU 197, TYR 332, HIP 438. Binding energy was found to be -5.305 kcal/mol

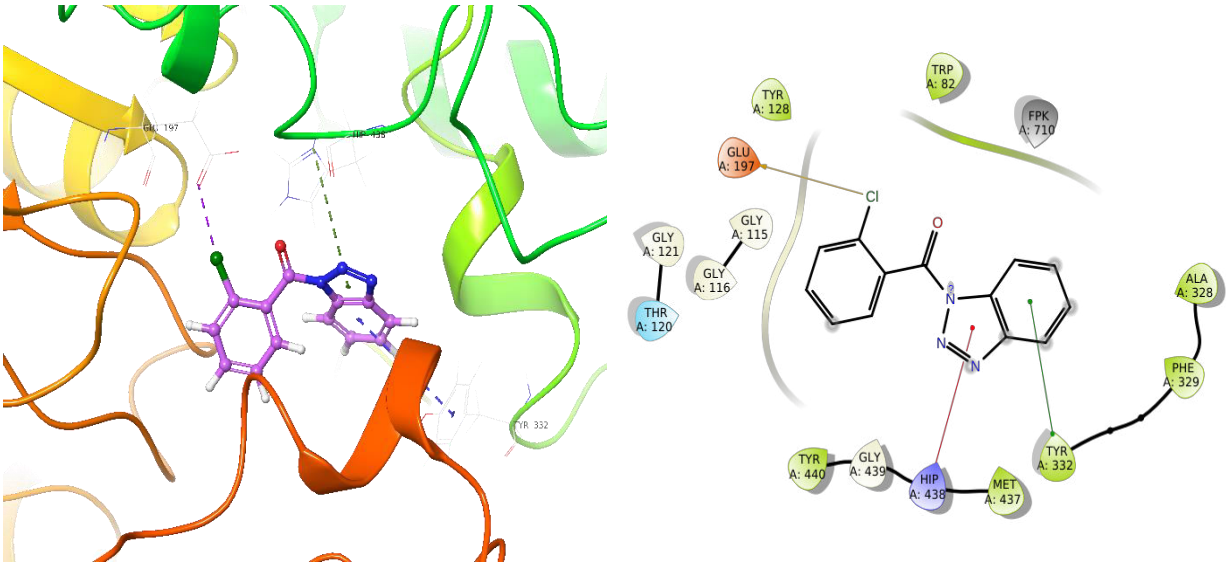


Fig. 17 – Snapshots of BA-1A binding with 4BDS

BA-1B

BA-1B interacts with Butyrylcholinesterase enzyme at GLU 197, GLY 115, HIP 438. Binding energy was found to be -5.321 kcal/mol

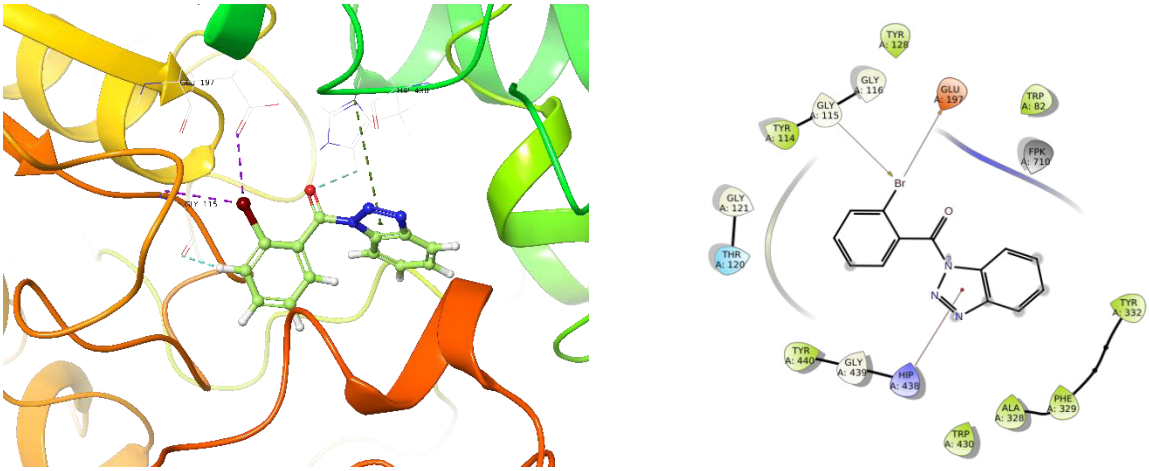


Fig. 18 – Snapshots of BA-1Bbinding with 4BDS

BA-1C

BA-1C interacts with Butyrylcholinesterase enzyme at ASP 70, TRP 82, HIP 438. Binding energy was found to be -5.773 kcal/mol

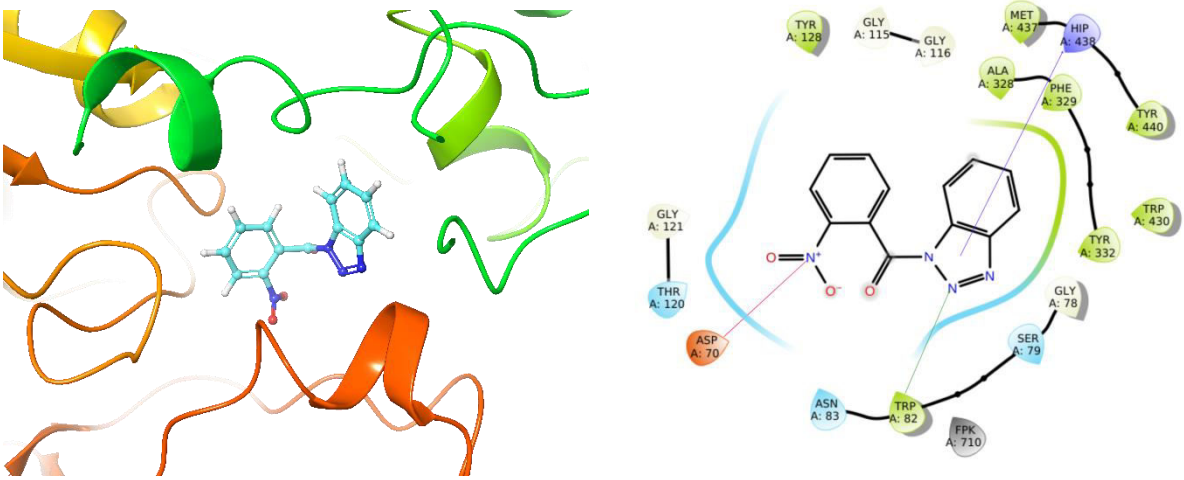


Fig. 19 – Snapshots of BA-1C binding with 4BDS

BA-1D

BA-1D interacts with Butyrylcholinesterase enzyme at TYR 332, TRP 82. Binding energy was found to be -5.938 kcal/mol

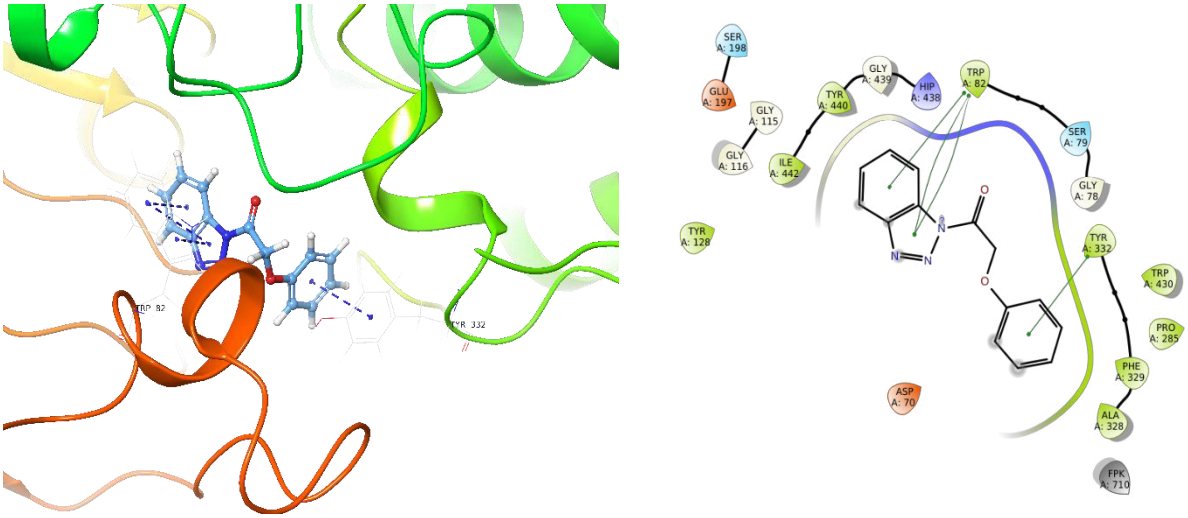


Fig. 20 – Snapshots of BA-1D binding with 4BDS

BE-2A

BE-2A interacts with Butyrylcholinesterase enzyme at ASP 70, TYR 128. Binding energy was found to be -6.247 kcal/mol

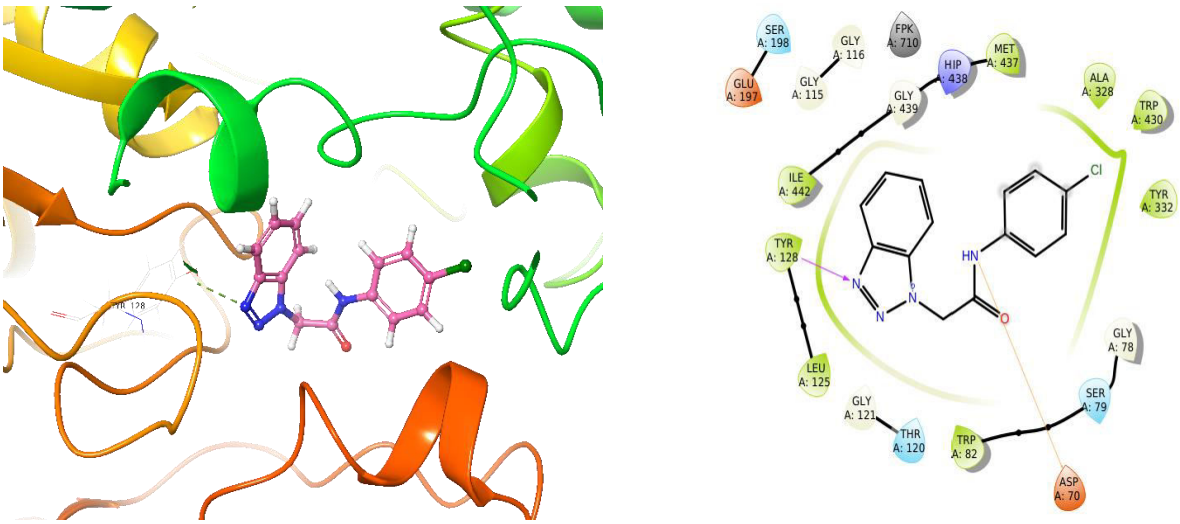


Fig. 21 – Snapshots of BE-2A binding with 4BDS

BE-2B

BE-2B interacts with Butyrylcholinesterase enzyme at TRP 82. Binding energy was found to be -6.826 kcal/mol.

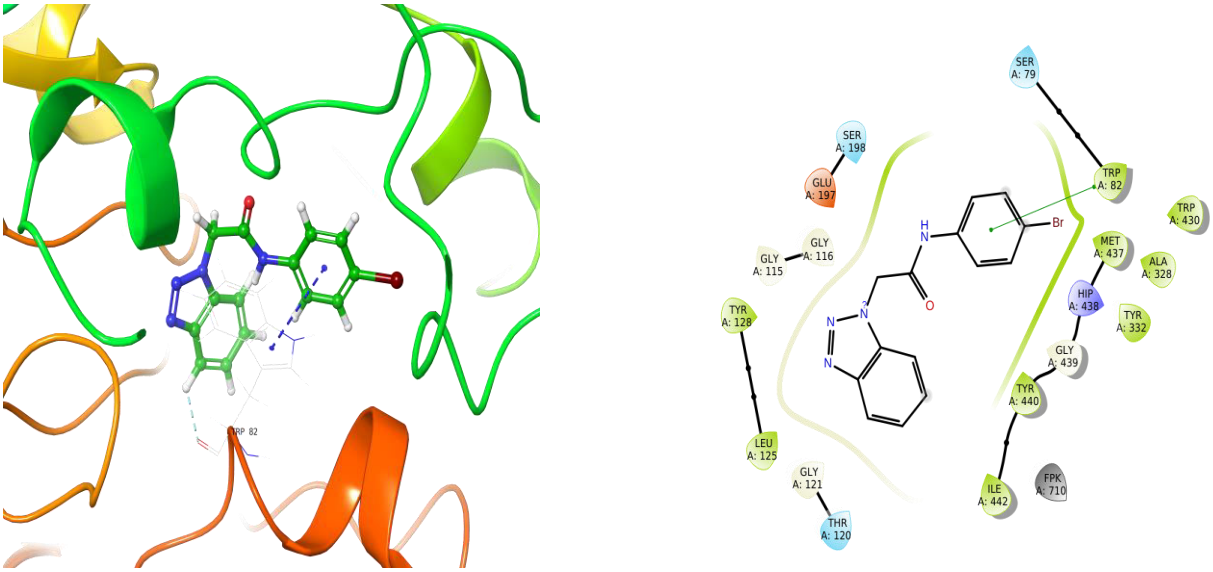


Fig. 22 – Snapshots of BE-2B binding with 4BDS

BE-2C

BE-2C interacts with Butyrylcholinesterase enzyme at ASP 70, TYR 332, TRP 82. Binding energy was found to be -5.269 kcal/mol

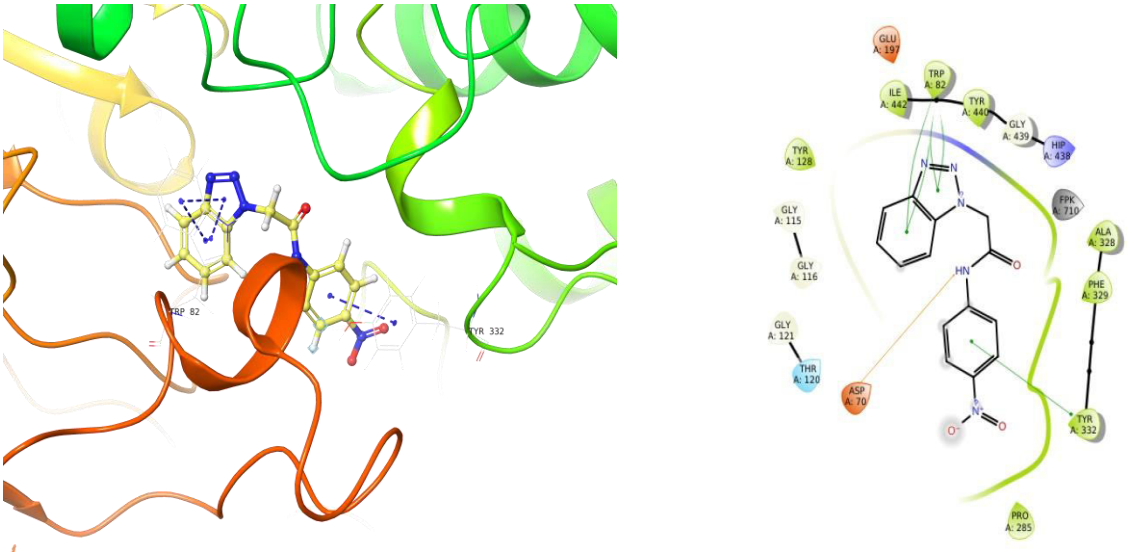


Fig. 23 – Snapshots of BE-2Cbinding with 4BDS

BE-2D

BE-2D interacts with Butyrylcholinesterase enzyme at TYR 332, FPK 710. Binding energy was found to be -5.504 kcal/mol

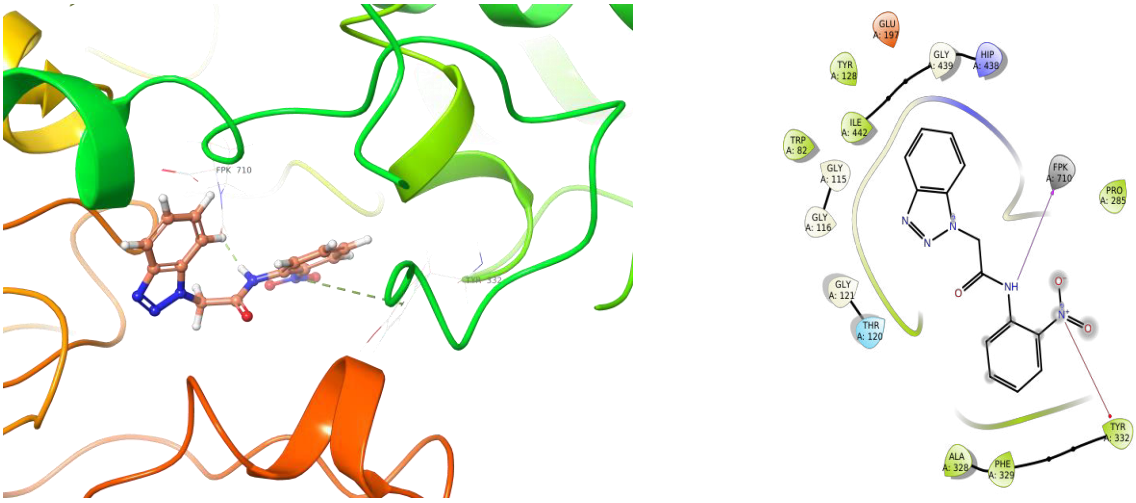


Fig. 24 – Snapshots of BE-2D binding with 4BDS

All the ligands BA-1A to 1T and BE-2A – 2T showed binding interactions with Acetylcholinesterase enzyme (PDB: 4EY7) and Butyrylcholinesterase enzyme (PDB:4BDS)

Among all the derivatives, only eight Benzotriazole derivatives have shown the highest binding energies with both the enzymes, when compared to the standard Rivastigmine. The highest binding energy shown by the new derivatives on interactions with 4EY7 and 4BDS was found to be best for BA-1A to BA-1D and BE-2A to BE-2D series with their corresponding binding energies respectively. Thus, BA-1A to BA-1D series the 2-chloro, 2-bromo, 2- Nitro, Phenoxy-substituted derivatives showed excellent interactions. Thus, BE-2A to BE-2D series the 4-chloro, 4-bromo, 2- Nitro, p- Nitro - substituted derivatives showed good binding interactions. All the selected derivatives were planned to synthesize and screen for *In vitro*-enzyme inhibitory activity.

6.2. PHYSICAL CHARACTERIZATION DATA

All the eight new compounds BA-1A to 1D and BE-2A to 2D obtained from two different schemes were prepared in good yield and evaluated by their physical characterization (Melting point and TLC) and spectral characterization (UV, IR, MASS and NMR) data.

Recrystallization Solvent: Ethanol

Solvent system: Acetone: Benzene (3:7)

Visualizing agent: Iodine Vapor

Table 14 - Physical characterization of Benzotriazole derivatives

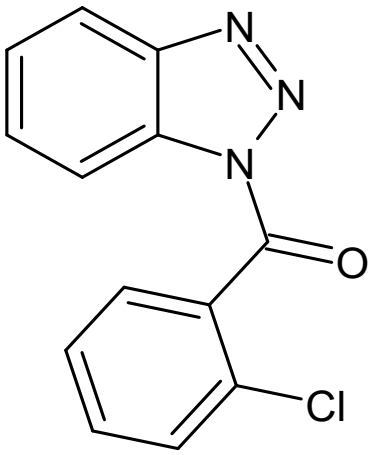
(BA-1A to 1D and BE-2A to 2D)

S. NO	COMPOUND CODE	R	MOLECULAR FORMULA	MOLECULAR WEIGHT	% YIELD	MELTING POINT	Rf VALUE	SOLUBILITY
1	BA-1A	$-C_6H_4Cl$	$C_{13}H_8ClN_3O$	257.67	71	147.5	0.82	Ethanol
2	BA-1B	$-C_6H_4Br$	$C_{13}H_8BrN_3O$	302.12	68	135.2	0.71	Ethanol
3	BA-1C	$-C_6H_4NO_2$	$C_{13}H_8N_4O_3$	268.22	60	149.6	0.69	Ethanol
4	BA-1D	$-C_7H_4O$	$C_{14}H_{11}N_3O_2$	253.25	66	128.7	0.66	Ethanol
5	BE-2A	$-C_6H_4Cl$	$C_{14}H_{11}ClN_4O$	286.71	79	115.6	0.68	$CHCl_3$
6	BE-2B	$-C_6H_4Br$	$C_{14}H_{11}BrN_4O$	331.16	65	108.3	0.59	$CHCl_3$
7	BE-2C	$-C_6H_4NO_2$	$C_{14}H_{11}N_5O_3$	297.26	53	128.1	0.61	$CHCl_3$
8	BE-2D	$-C_6H_4NO_2$	$C_{14}H_{11}N_5O_3$	297.26	69	111.8	0.45	$CHCl_3$

6.3. SPECTRAL STUDIES OF COMPOUNDS

The structures of compounds synthesized during the present study were established on the basis of physical data, UV, IR, MASS and NMR Spectral data. The purity of the compounds was established by single spot-on TLC plates.

COMPOUND CODE: BA-1A



Chemical name: (1*H*-benzotriazol-1-yl) (2-chlorophenyl) methanone

UV SPECTRUM

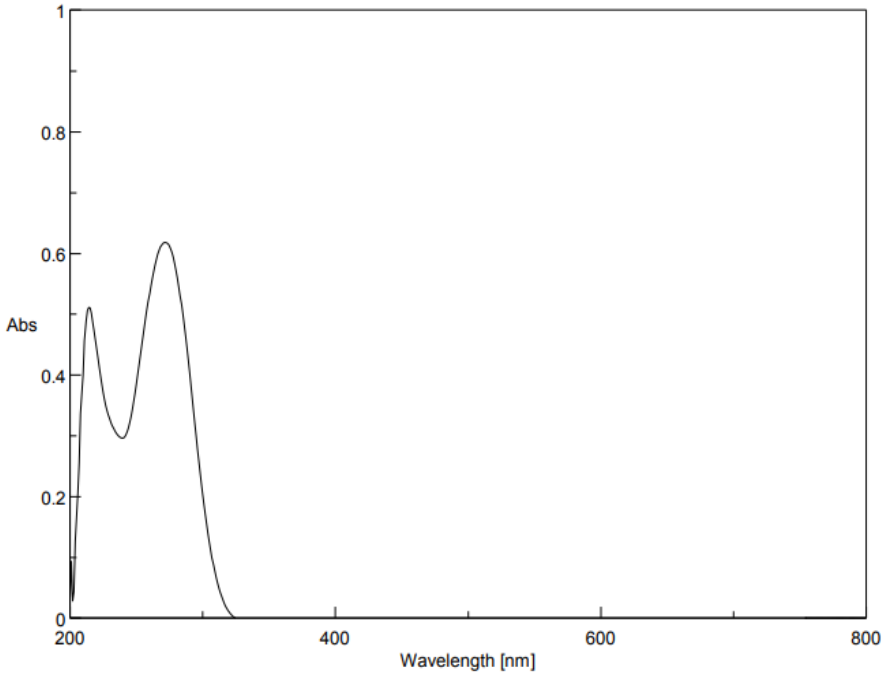


Fig. 25 – UV Spectrum of Compound BA-1A

Solvent Used: Ethanol

$\lambda_{max.}$ 272nm

IR SPECTRUM OF BA-1A

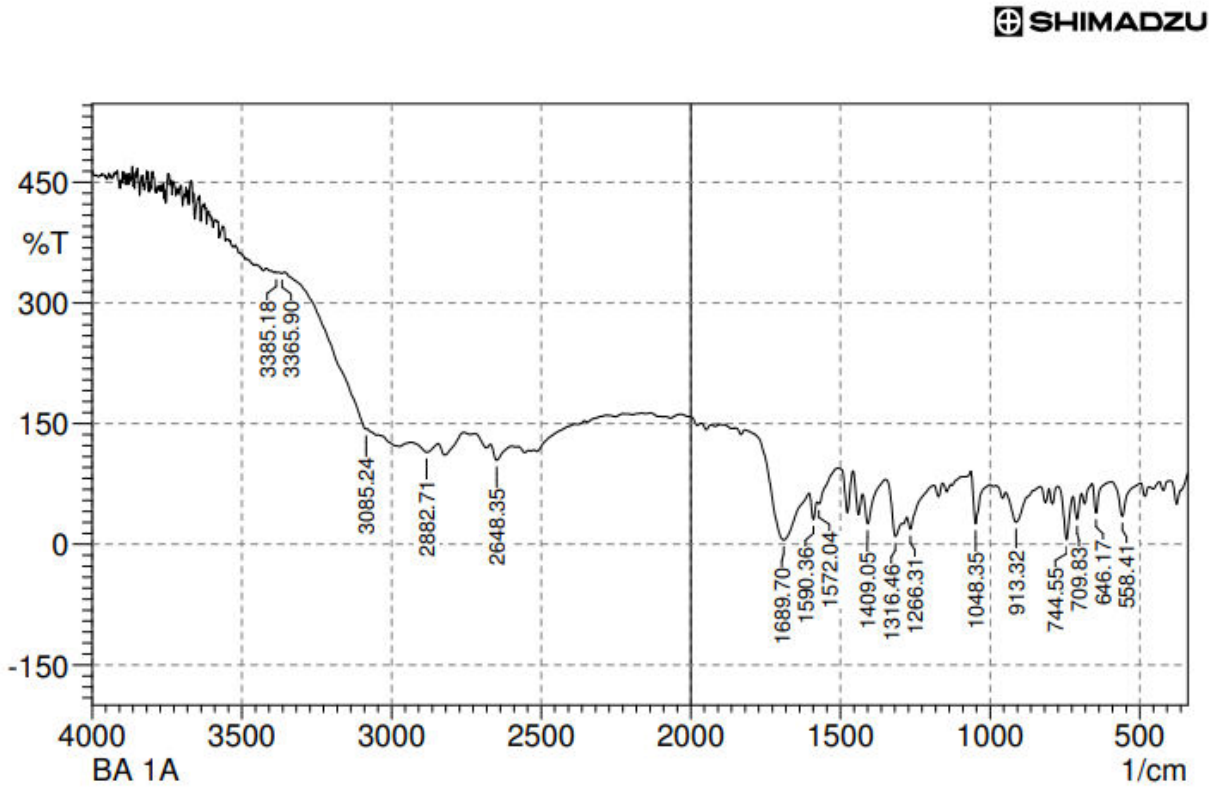


Fig. 26 – IR Spectrum of Compound BA-1A

IR (KBr, vmax in cm-1)

Table 15 – IR Spectral Values of Compound BA-1A

S.NO	TYPES OF VIBRATION	FREQUENCY(cm-1)
1	C = O stretching	1689.70
2	Mono substituted aromatic C-H Bending	744.55
3	Aromatic C-H stretching	3085.24
4	Aromatic C = C Stretching	1590.36,1572.04
5	N-C Stretching	1266.31

MASS SPECTRUM OF BA-1A

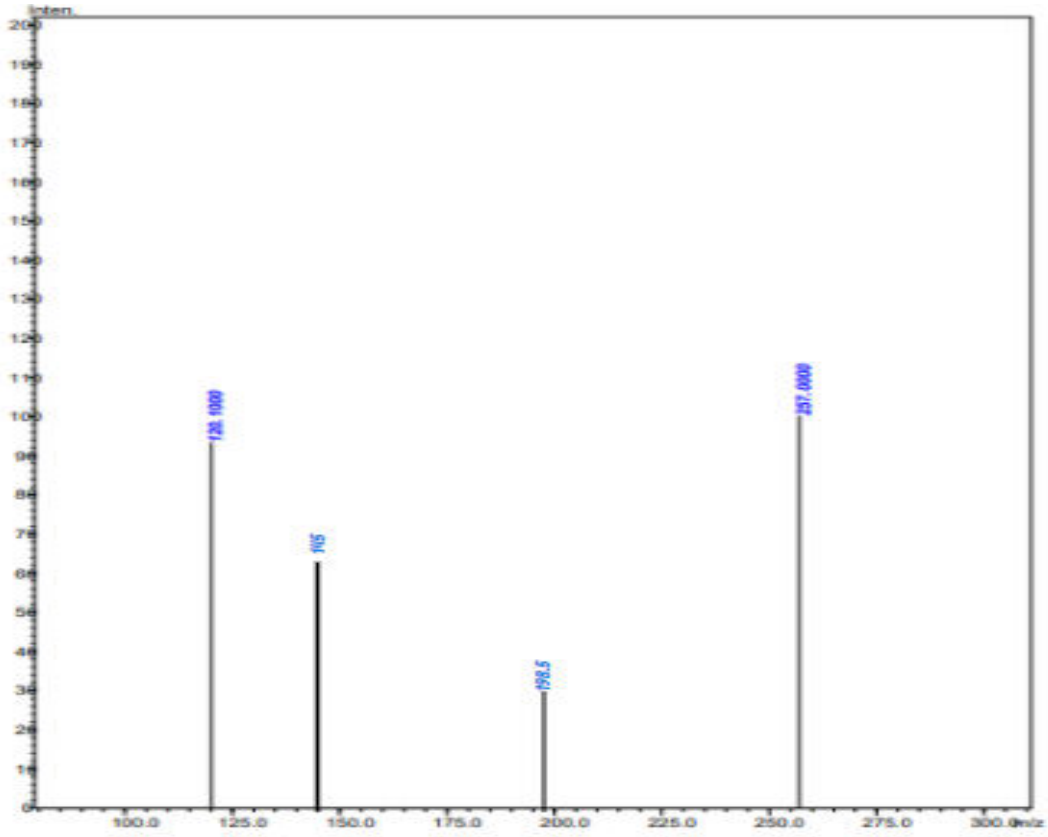
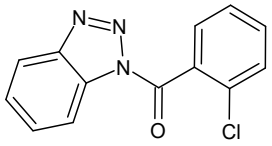
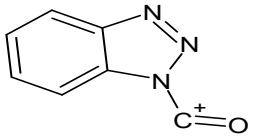
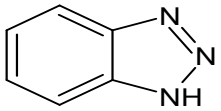


Fig. 27 – Mass Spectrum of Compound BA-1A

Table 16 – Mass Spectral Values of Compound BA-1A

COMPOUND	m/z value
	257.00
	145
	120.10

^{1}H NMR SPECTRUM OF BA-1A

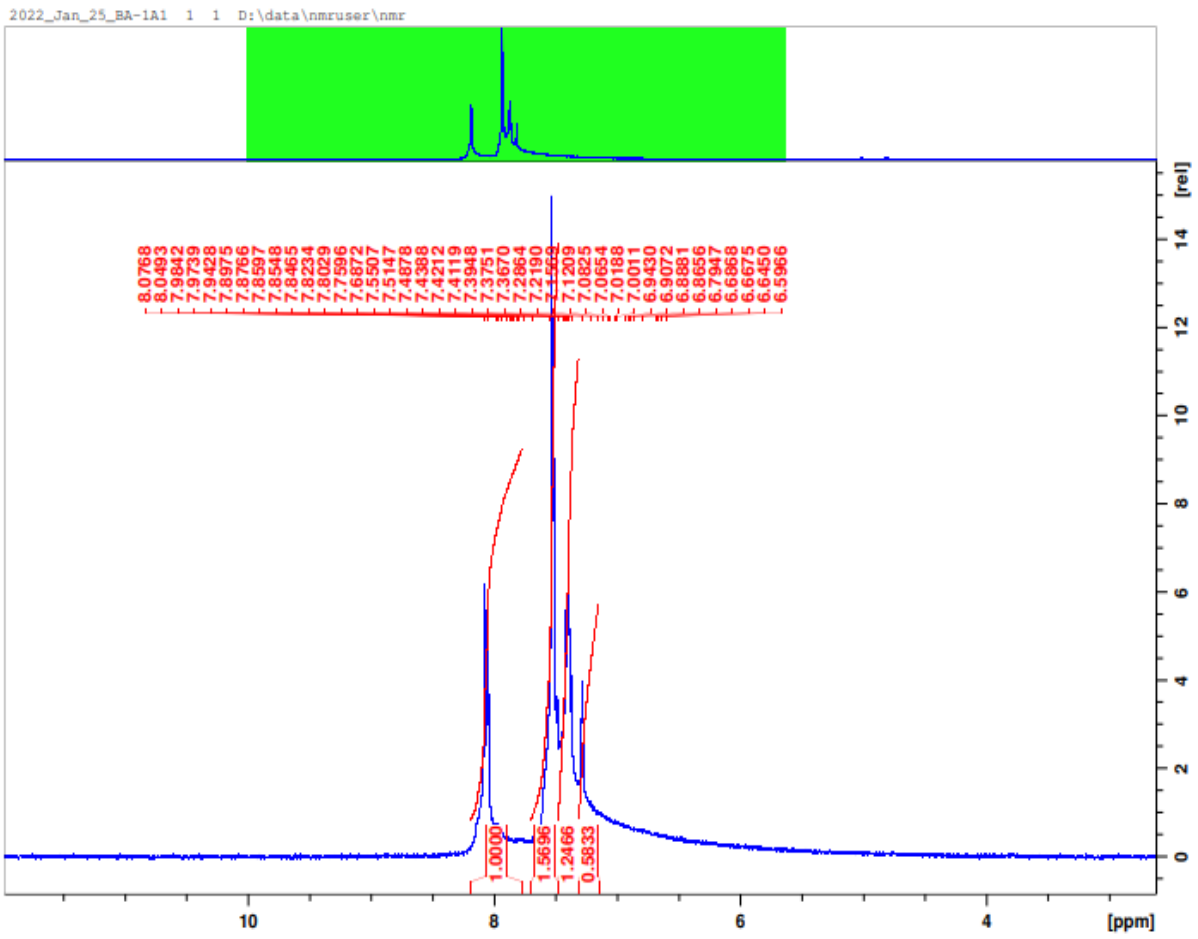
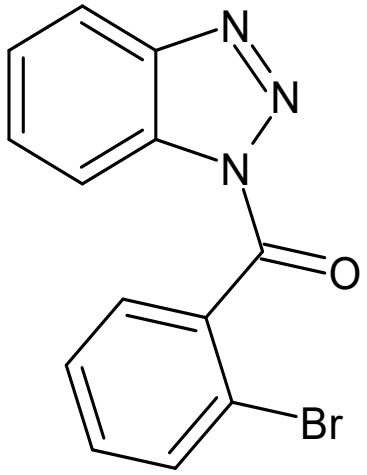


Fig. 28 – ^{1}H NMR Spectrum of Compound BA-1A

Table 17 – ^{1}H NMR Spectral Values of Compound BA-1A

S. No	Chemical Shift	Multiplicity	No. of Protons	Type of Protons
1	7.2 – 7.8	Triplet	4	Aromatic - H
2	7.9- 8.2	Doublet	4	Aromatic - H

COMPOUND CODE: BA-1B



Chemical Name: (1*H*-benzotriazol-1-yl) (2-bromophenyl) methanone

UV SPECTRUM

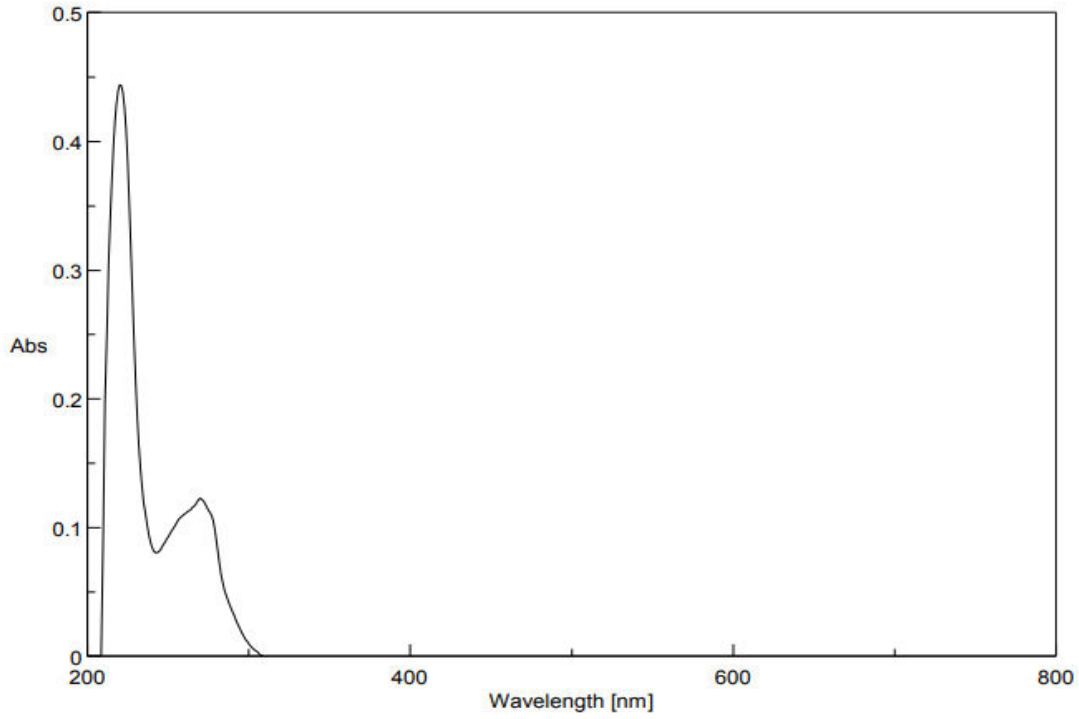


Fig. 29 – UV Spectrum of Compound BA-1B

Solvent Used: Ethanol

λ_{max} : 269nm

IR SPECTRUM OF BA-1B

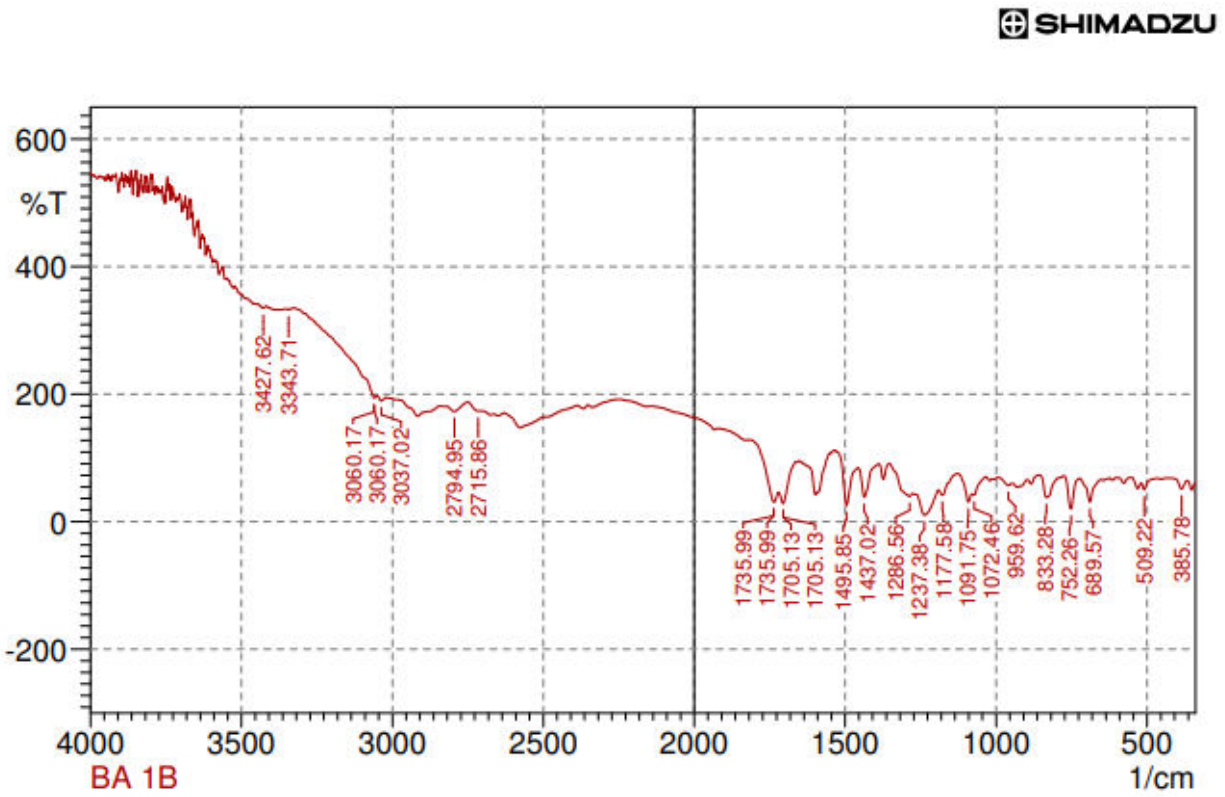


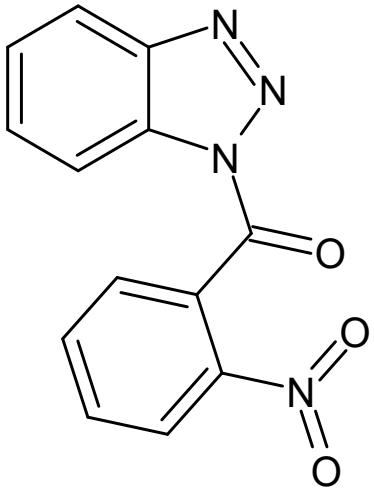
Fig. 30 – IR Spectrum of Compound BA-1B

IR (KBr, vmax in cm-1)

Table 18 – IR Spectral Values of Compound BA-1B

S.NO	TYPES OF VIBRATION	FREQUENCY(cm-1)
1	C = O stretching	1705.13
2	Mono substituted aromatic C-H Bending	752.26
3	Aromatic C-H stretching	3060.17
4	Aromatic C = C Stretching	1495.85
5	N-C Stretching	1237.38

COMPOUND CODE: BA-1C



Chemical Name: (1*H*-benzotriazol-1-yl) (2-nitrophenyl) methanone

UV SPECTRUM

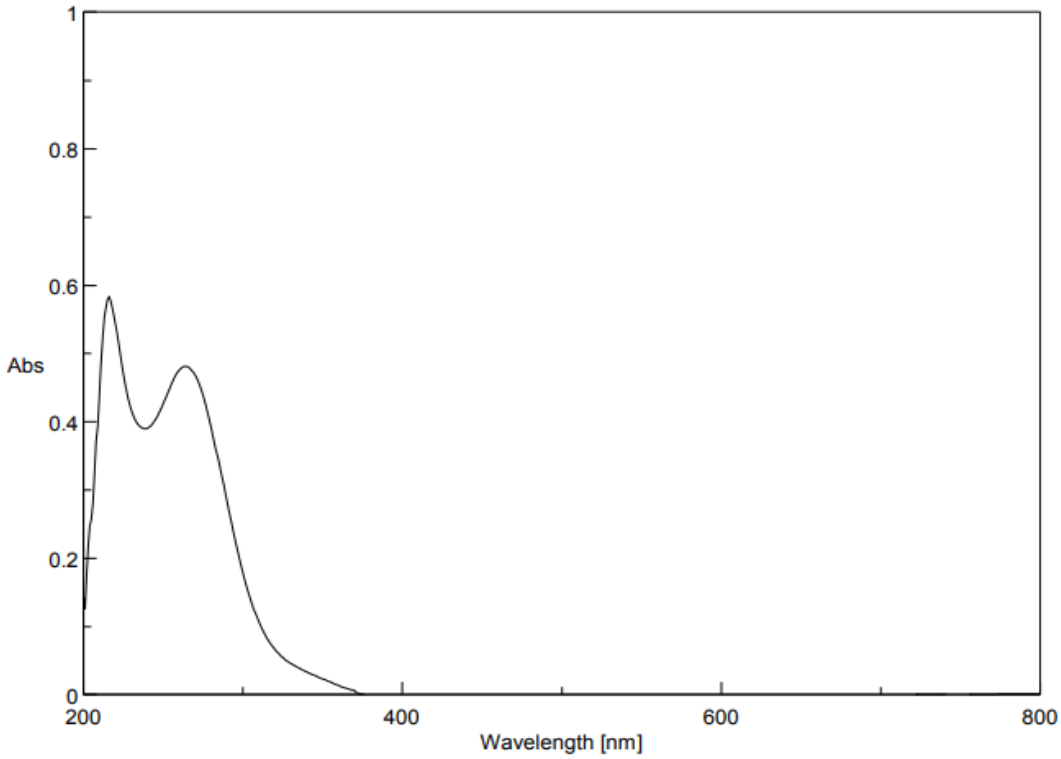


Fig. 31 – UV Spectrum of Compound BA-1C

Solvent Used: Ethanol

λ_{max} : 264nm

IR SPECTRUM OF BA-1C

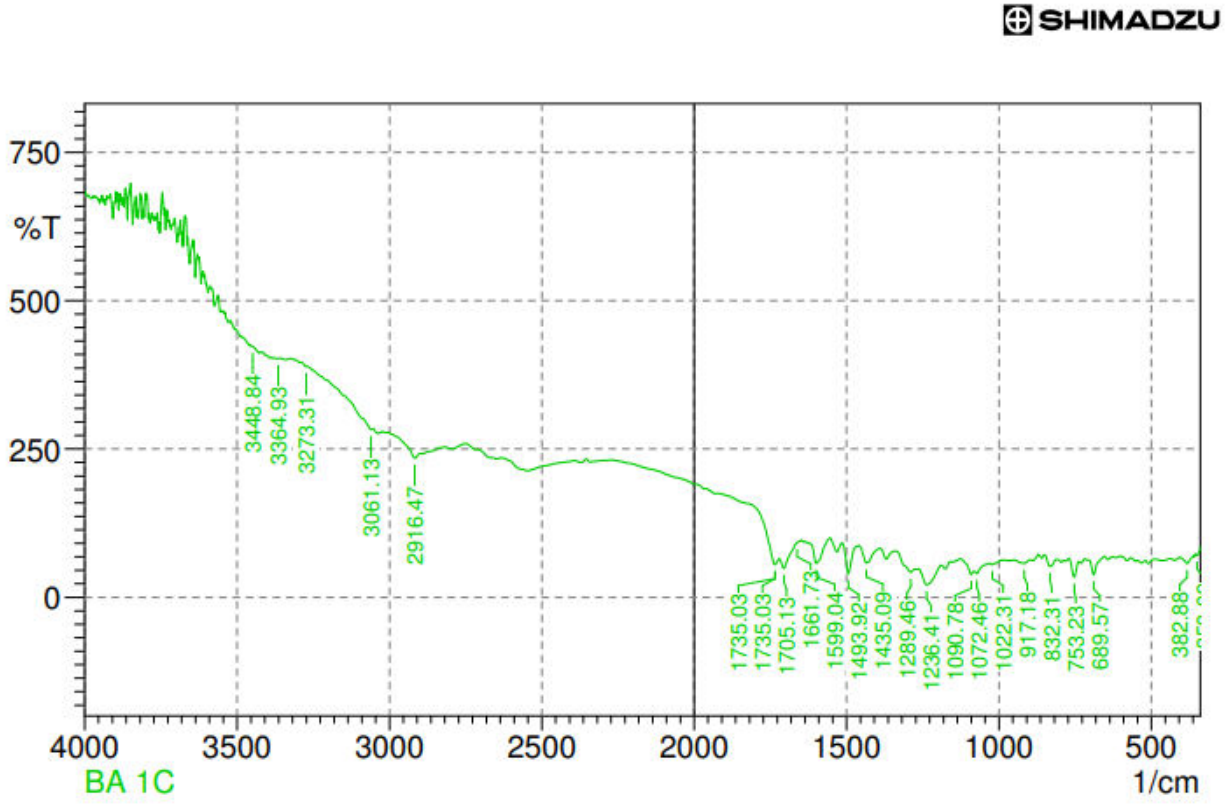


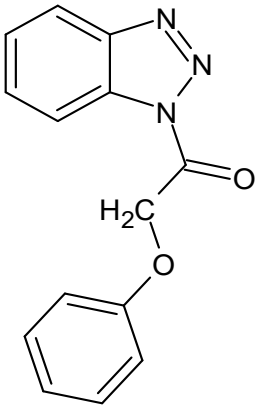
Fig. 32 – IR Spectrum of Compound BA-1C

IR (KBr, vmax in cm-1)

Table 19 – IR Spectral Values of Compound BA-1C

S.NO	TYPES OF VIBRATION	FREQUENCY(cm-1)
1	C = O stretching	1705.13
2	Mono substituted aromatic C-H bending	753.23
3	Aromatic C-H stretching	3061.13
4	Aromatic C = C Stretching	1493.92
5	Aromatic -NO_2	1599.04

COMPOUND CODE: BA-1D



Chemical Name: 1-(1*H*-benzotriazol-1-yl)-2-phenoxyethan-1-one

UV SPECTRUM

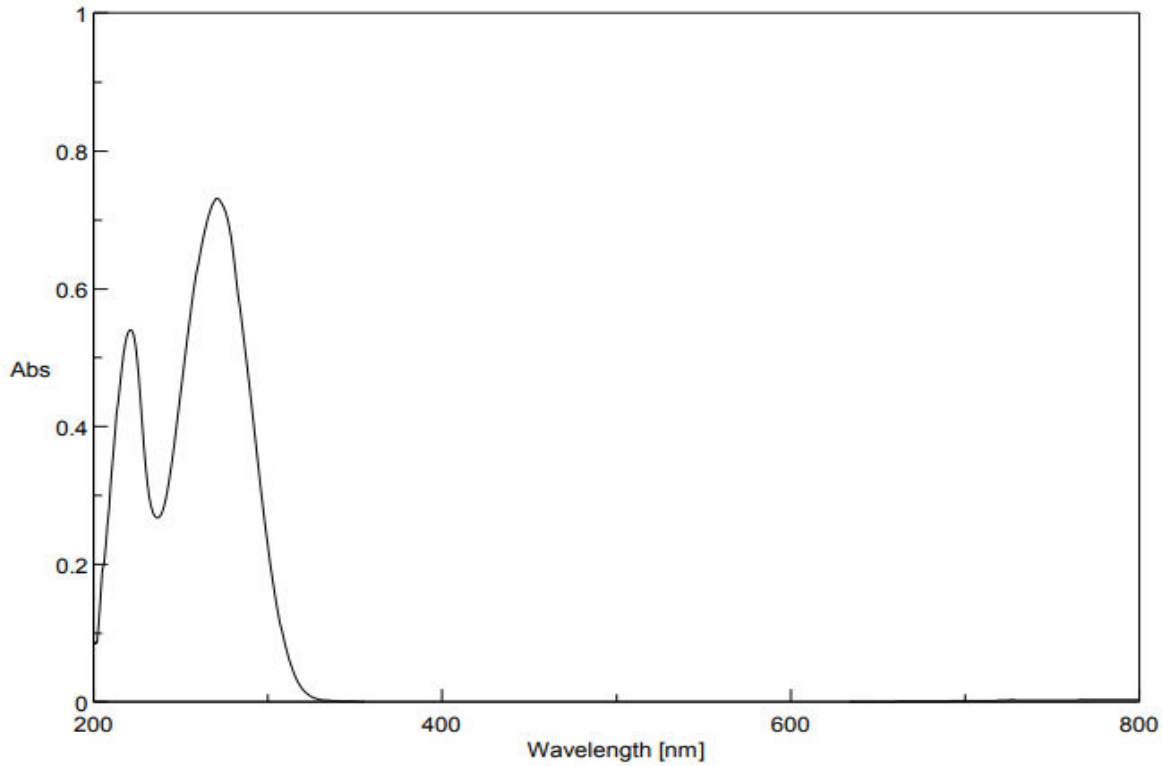


Fig. 33– UV Spectrum of Compound BA-1D

Solvent Used: Ethanol

λ_{max} : 270nm

IR SPECTRUM OF BA-1D

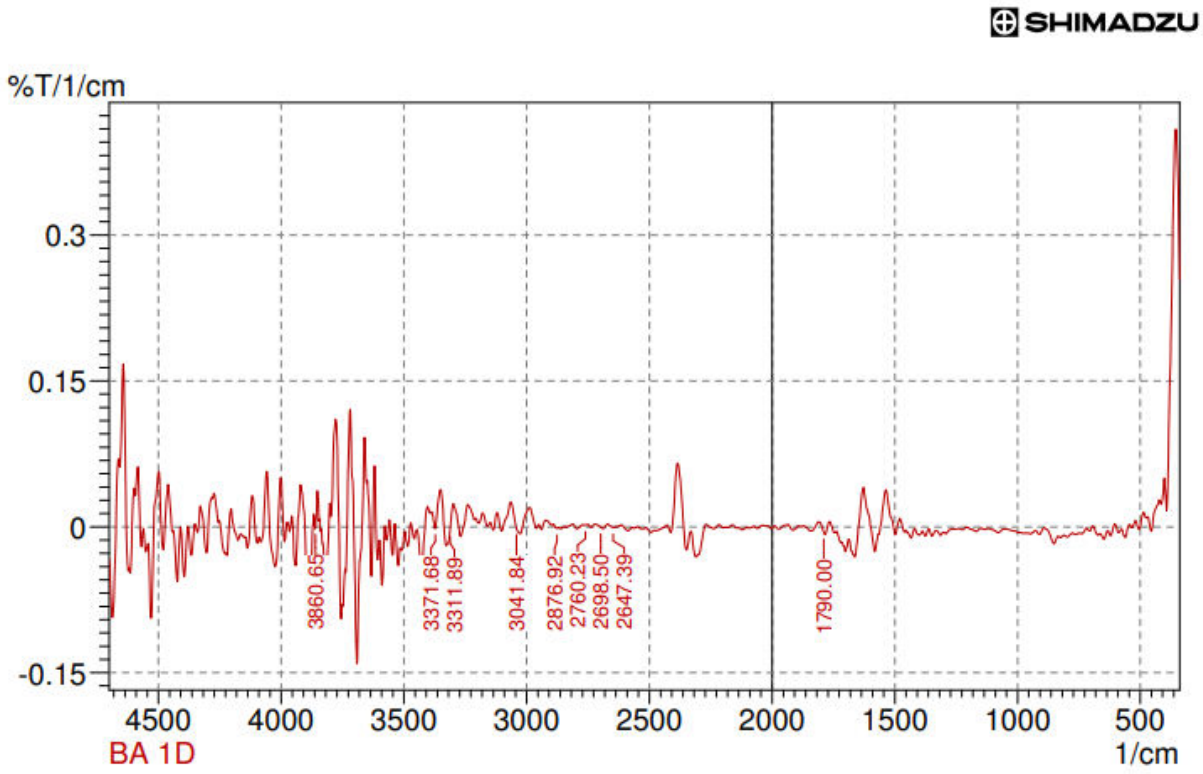


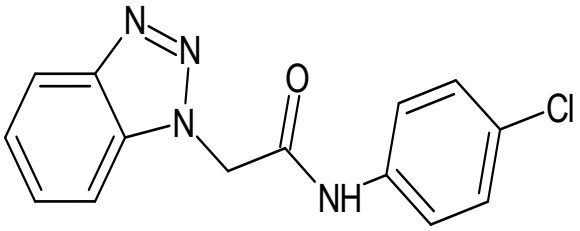
Fig. 34 – IR Spectrum of Compound BA-1D

IR (KBr, vmax in cm-1)

Table 20 – IR Spectral Values of Compound BA-1D

S.NO	TYPES OF VIBRATION	FREQUENCY(cm-1)
1	C = O stretching	1790.00
2	Methylene(-CH_2) stretching	2876.92
3	Aromatic C-H stretching	3041.84

COMPOUND CODE: BE-2A



Chemical Name: 2-(1*H*-benzotriazol-1-yl)-*N*-(4-chlorophenyl) acetamide

UV SPECTRUM

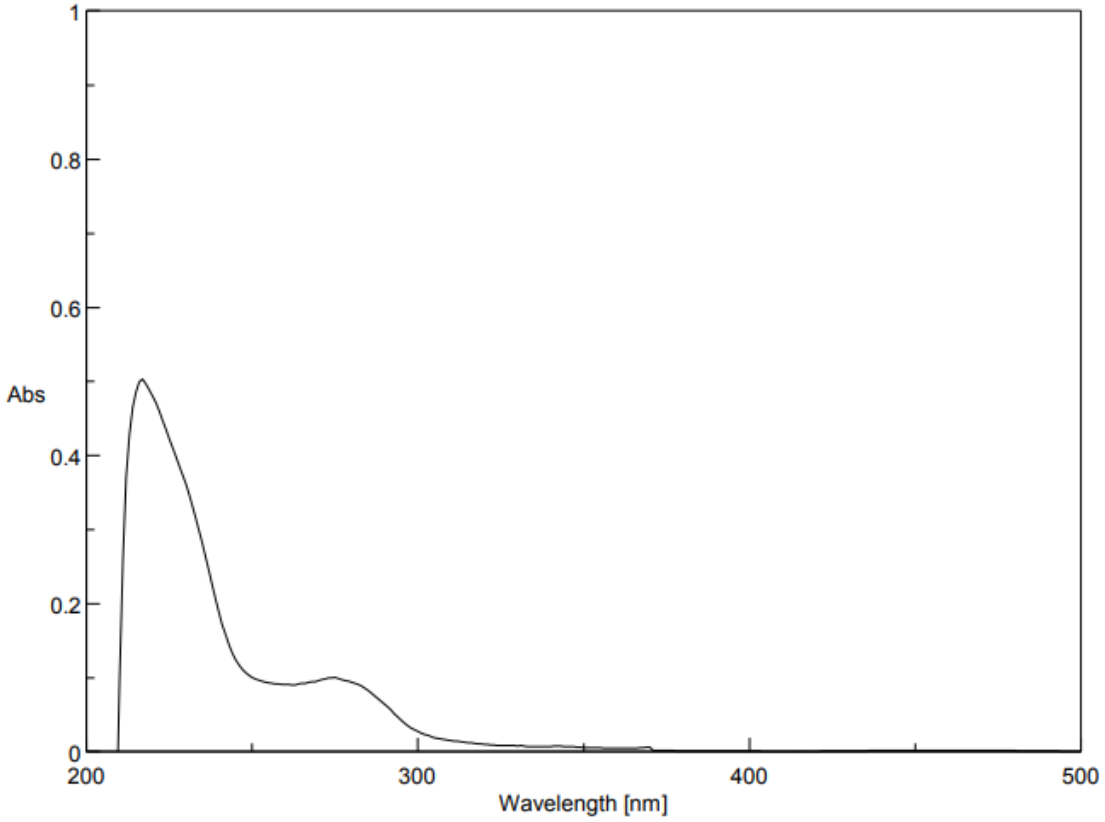


Fig. 35 – UV Spectrum of Compound BE-2A

Solvent Used: Ethanol

$\lambda_{max:}$ 272nm

IR SPECTRUM OF BE-2A

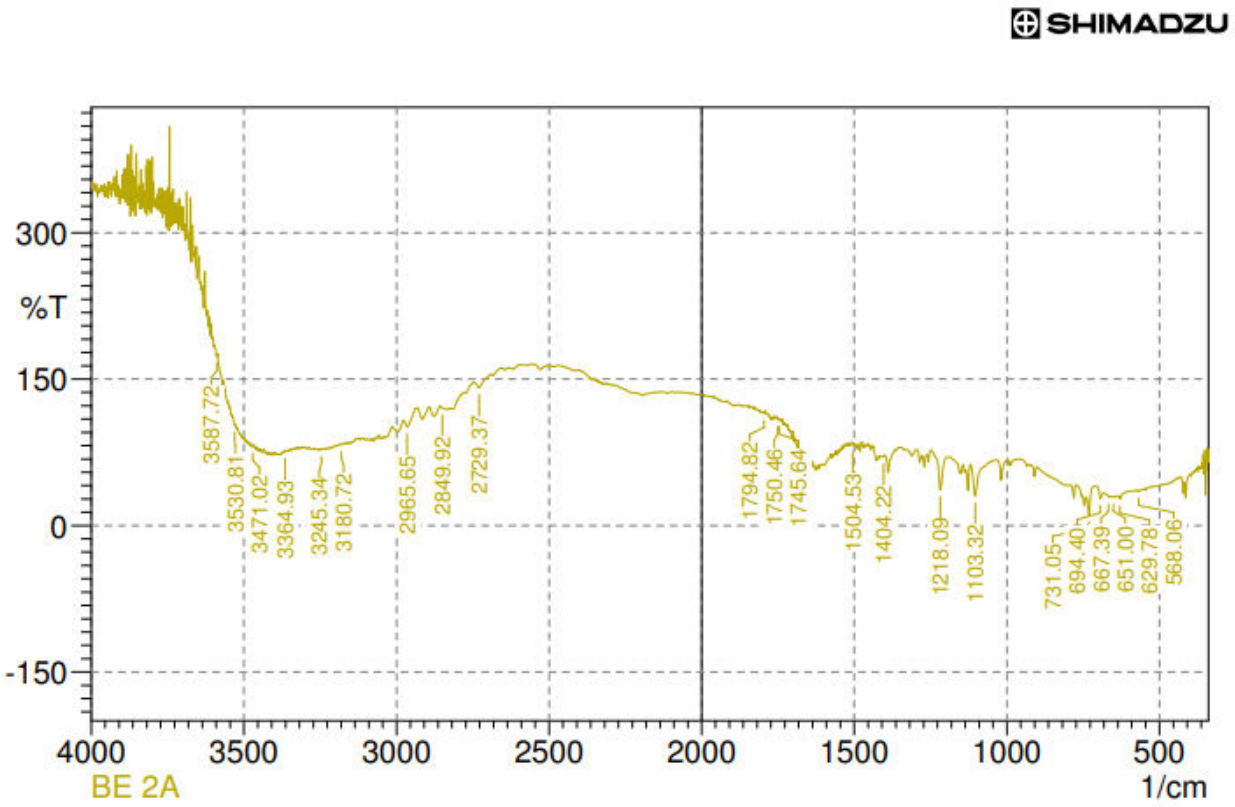


Fig. 36 – IR Spectrum of Compound BE-2A

IR (KBr, vmax in cm-1)

Table 21 – IR Spectral Values of Compound BE-2A

S.NO	TYPES OF VIBRATION	FREQUENCY(cm-1)
1	Aromatic (O = C-NH)	1745.64
2	Methylene(-CH_2)stretching	2965.65
3	Aromatic C-H stretching	3180.72
4	Aromatic C = C Stretching	1504.53
5	N-C stretching	1218.09
6	P-substituted benzene	694.40

MASS SPECTRUM OF BE-2A

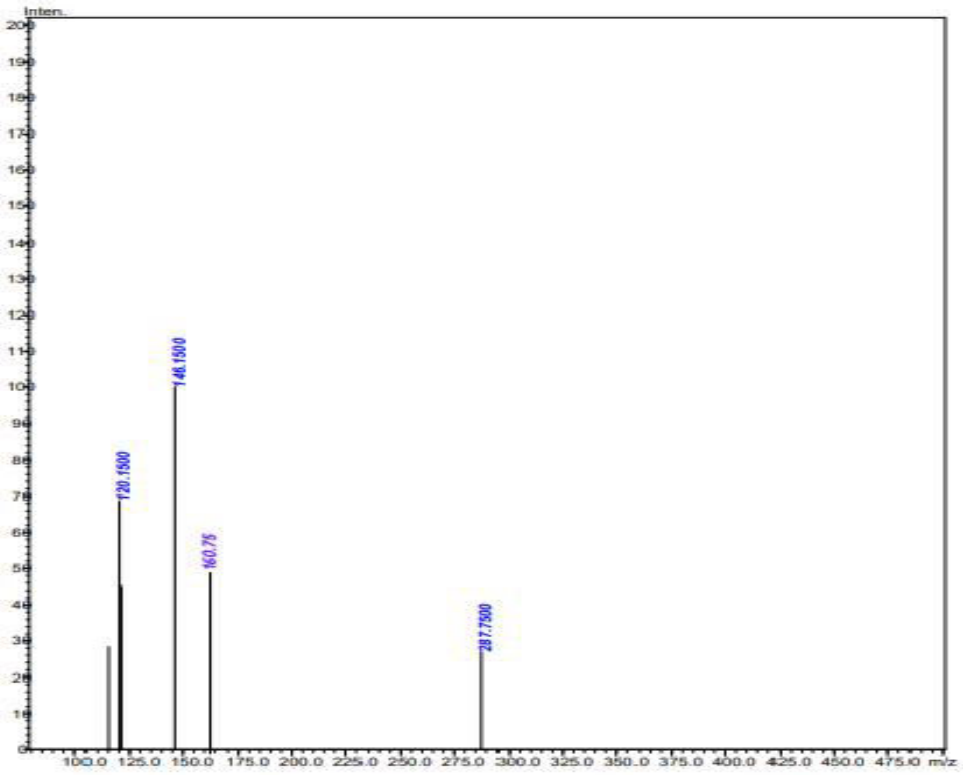
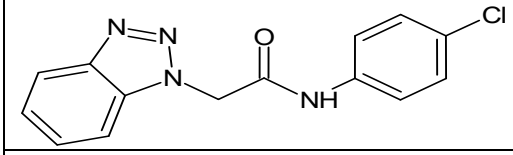
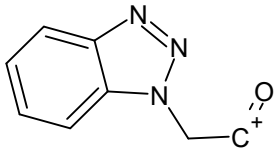
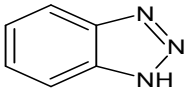


Fig. 37 – Mass Spectrum of Compound BE-2A

Table 22 – Mass Spectral Values of Compound BE-2A

COMPOUND	m/z value
	287.75
	160.75
	120.15

^{1}H NMR SPECTRUM OF BE-2A

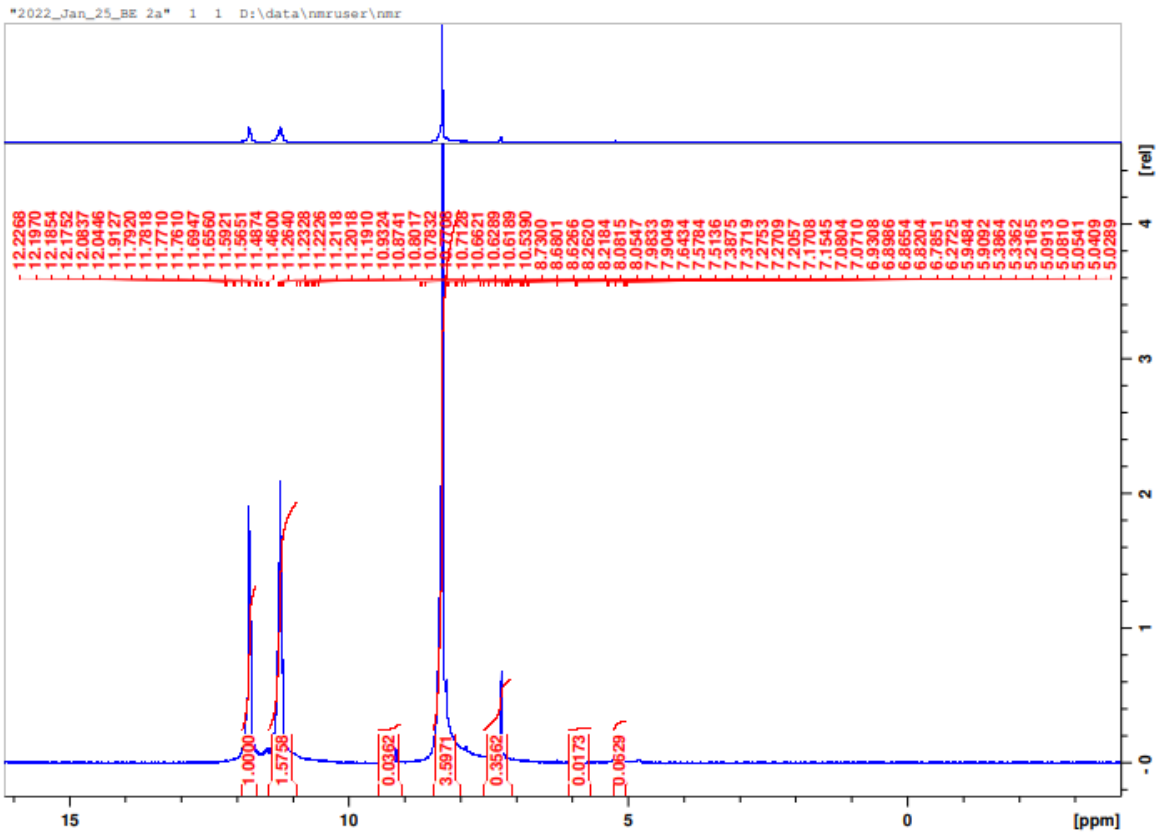
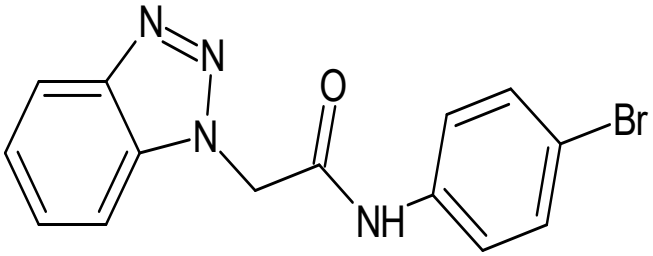


Fig. 38 – ^{1}H NMR Spectrum of Compound BE-1A

Table 23 – ^{1}H NMR Spectral Values of Compound BE-2A

S. No	Chemical Shift	Multiplicity	No. of Protons	Type of Protons
1	7.2 – 7.8	Triplet	4	Aromatic - H
2	7.9- 8.4	Doublet	4	Aromatic - H
3	10.8 – 11.2	Singlet	1	-CH_2 (deshielded)
4	11.4 – 11.6	Singlet	1	-NH

COMPOUND CODE: BE-2B



Chemical Name: 2-(1*H*-benzotriazol-1-yl)-*N*-(4-bromophenyl) acetamide

UV Spectrum

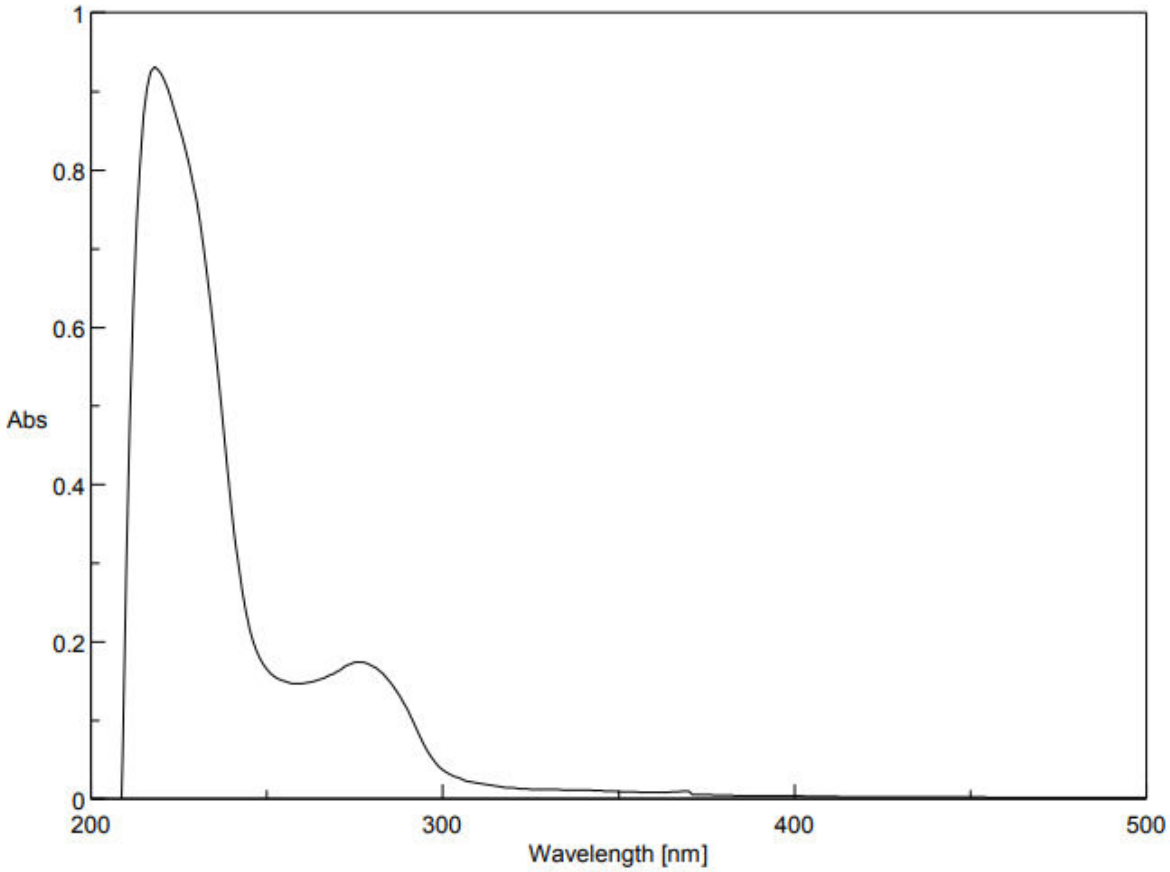


Fig. 39 – UV Spectrum of Compound BE-2B

Solvent Used: Ethanol

λ_{max} : 271nm

IR SPECTRUM OF BE-2B

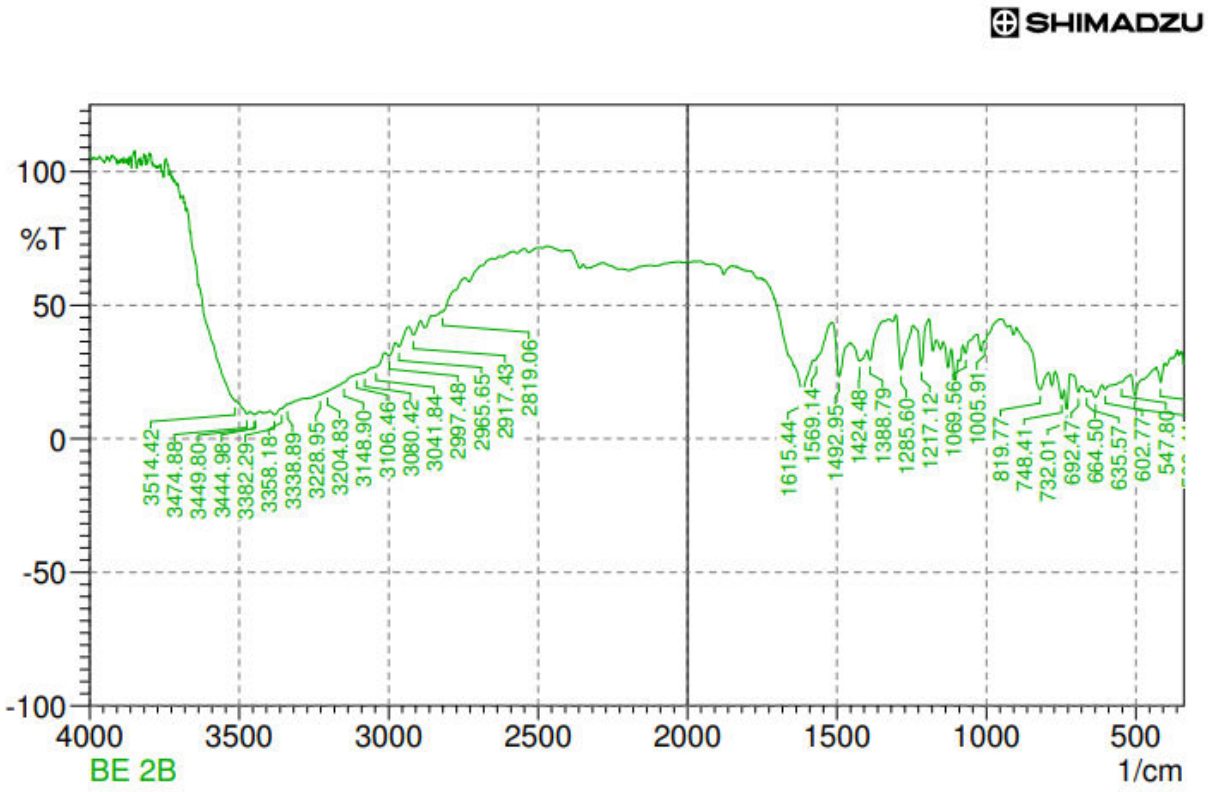


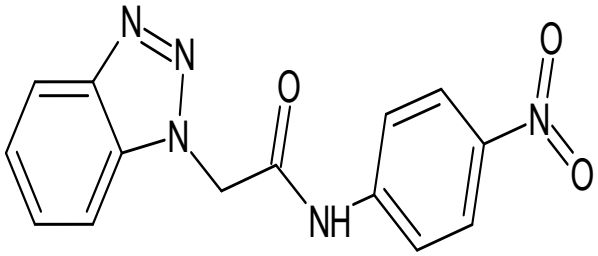
Fig. 40 – IR Spectrum of Compound BE-2B

IR (KBr, vmax in cm-1)

Table 24 – IR Spectral Values of Compound BE-2B

S.NO	TYPES OF VIBRATION	FREQUENCY(cm-1)
1	Aromatic (O = C-NH)	1615.44
2	Methylene(-CH_2)stretching	2965.65
3	Aromatic C-H stretching	3106.46
4	Aromatic C = C Stretching	1492.95
5	N-C stretching	1285.60
6	p-substituted benzene	692.47

COMPOUND CODE: BE-2C



Chemical Name: 2-(1*H*-benzotriazol-1-yl)-*N*-(4-nitrophenyl) acetamide

UV SPECTRUM

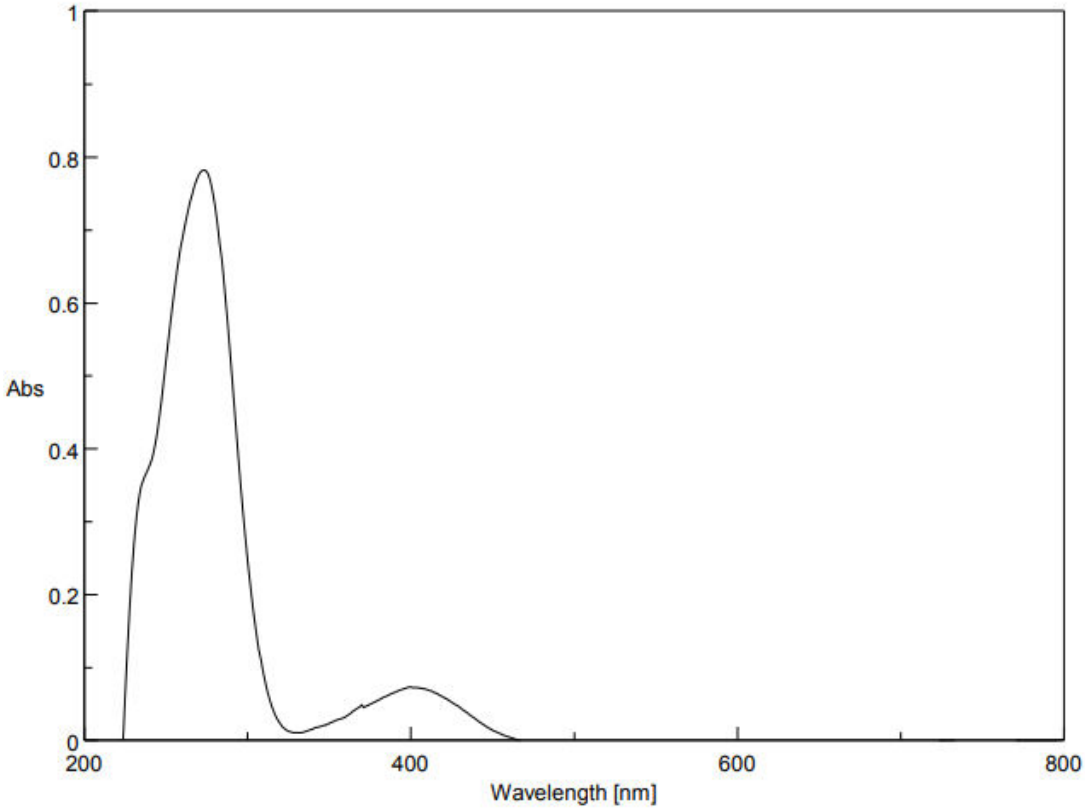


Fig. 41 – UV Spectrum of Compound BE-2C

Solvent Used: Ethanol

$\lambda_{max:}$ 274nm

IR SPECTRUM OF BE-2C

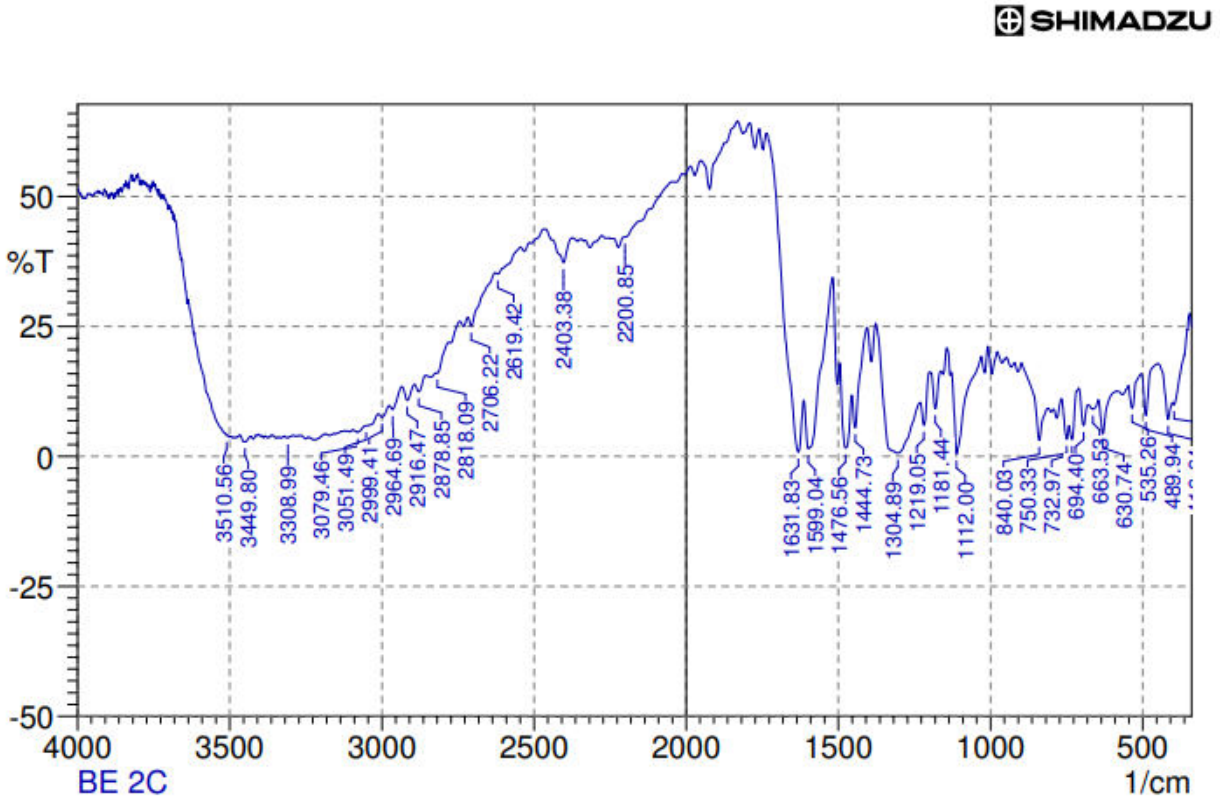


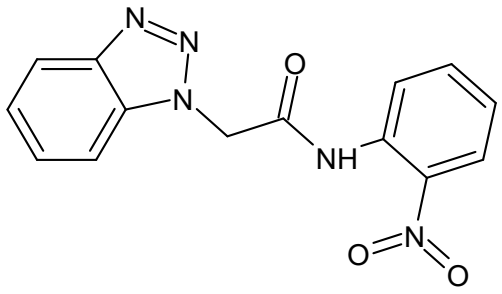
Fig. 42 – IR Spectrum of Compound BE-2C

IR (KBr, vmax in cm-1)

Table 25 – IR Spectral Values of Compound BE-2C

S.NO	TYPES OF VIBRATION	FREQUENCY(cm-1)
1	Aromatic (O = C-NH)	1631.83
2	Methylene(-CH_2)stretching	2964.69
3	Aromatic C = C Stretching	1599.04
4	N-C stretching	1219.05
5	p-substituted benzene	694.40

COMPOUND CODE: BE-2D



Chemical Name: 2-(1*H*-benzotriazol-1-yl)-*N*-(2-nitrophenyl) acetamide

UV SPECTRUM

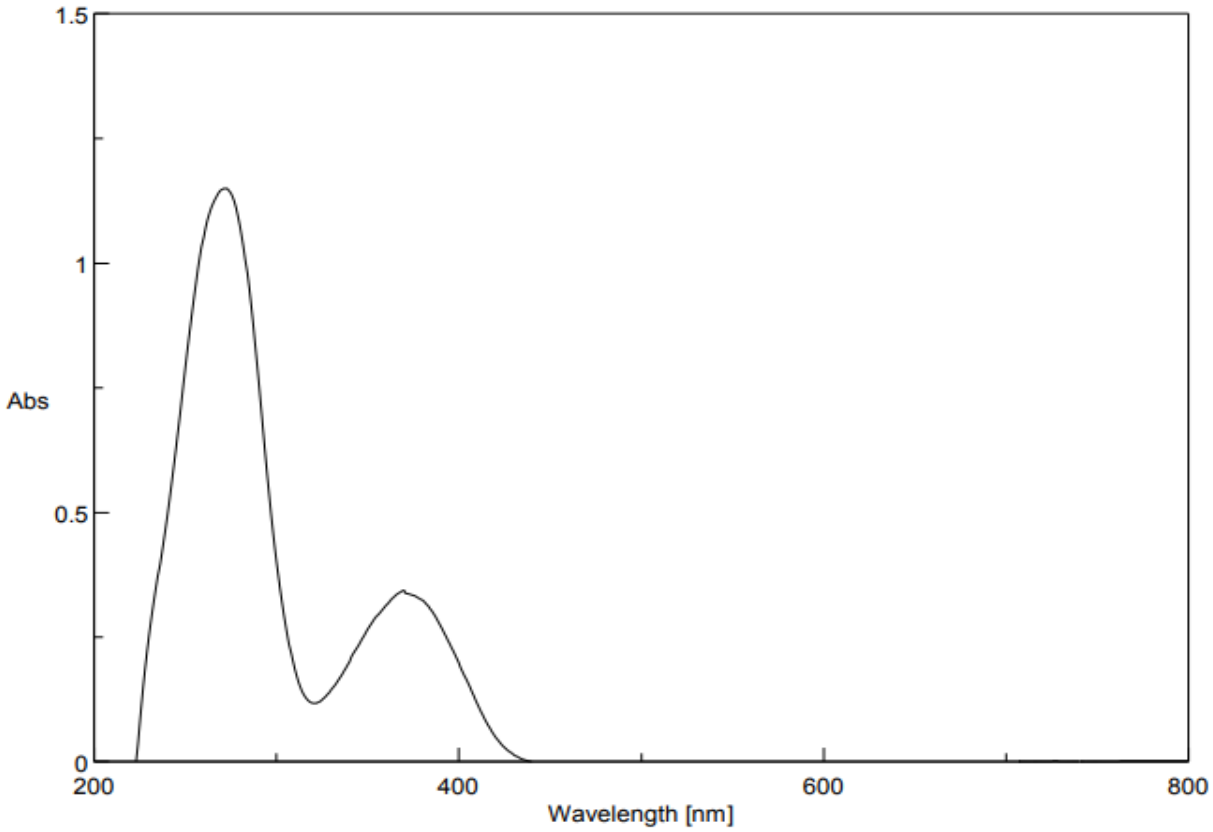


Fig. 43 – UV Spectrum of Compound BE-2D

Solvent Used: Ethanol

$\lambda_{max.}$ 272nm

IR SPECTRUM OF BE-2D

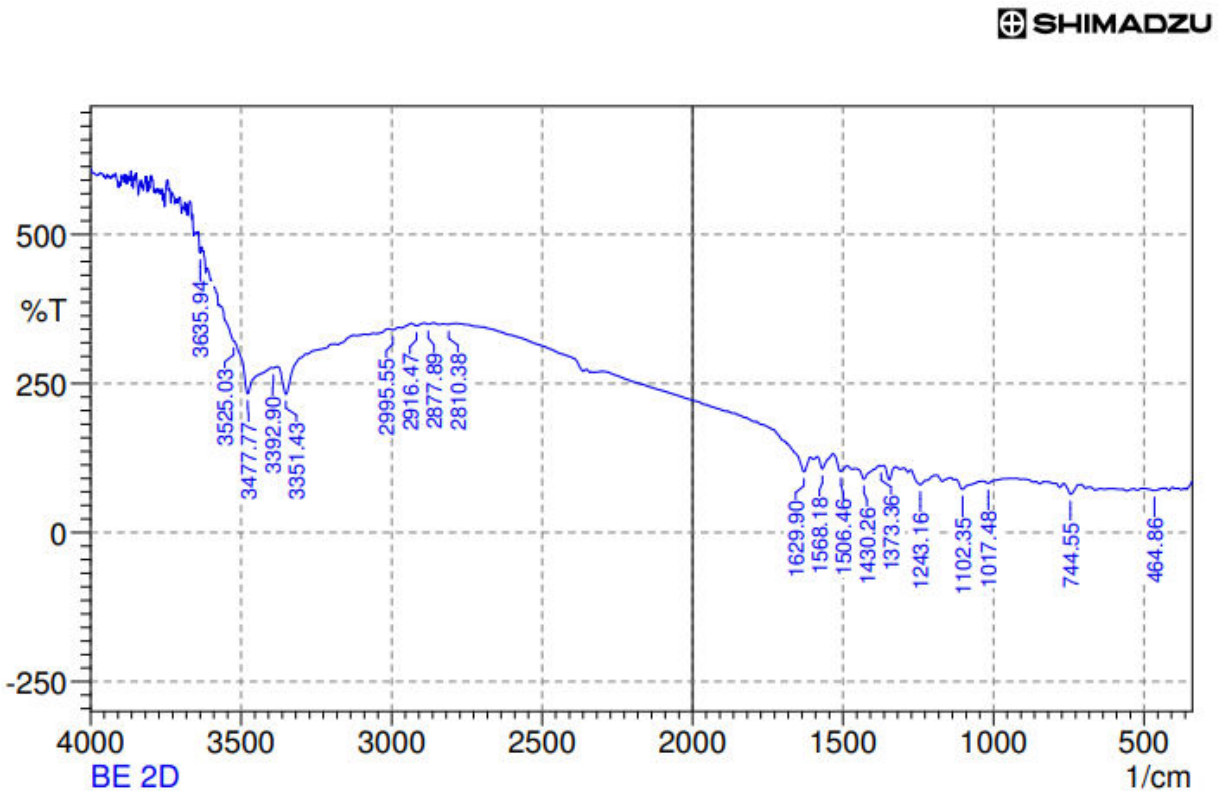


Fig. 44 – IR Spectrum of Compound BE-2D

IR (KBr, vmax in cm-1)

Table 26 – IR Spectral Values of Compound BE-2D

S.NO	TYPES OF VIBRATION	FREQUENCY(cm-1)
1	Aromatic (O = C-NH)	1629.90
2	Methylene(-CH_2)stretching	2995.55
3	Aromatic C = C Stretching	1568.18
4	N-C stretching	1243.16
5	p-substituted benzene	744.55

6.4. *IN-VITRO* CHOLINESTERASE INHIBITORY STUDY:

An increase in AChE leads to an increase in breakdown of ACh to acetate and choline and BuCh to butyrate and choline leading to the development of neurodegenerative disease. Usually these inhibitors are used in the treatment of neurodegenerative disease. When these inhibitions occur, it results in breakdown of acetylcholine into acetate and choline and butyrylcholine into butyrate and choline and it is measured spectroscopically. The inhibitory activity of the Benzotriazole derivatives against AChE and BuChE was determined by Ellman's method to this method AChE is estimated by using Acetylthiocholine Iodide (substrate) and BuChE is estimated by butyrylthiocholine Iodide and DTNB. A yellow color compound is produced by Thiocholine when it reacts with dithiobisnitro benzoate ion measures the enzymatic activity *invitro* AChE and BuChE inhibition studies revealed that, the all compounds were showed good inhibition constant values when compared to standard.

The absorbance of the selected Benzotriazole derivatives (BA-1A to 1D and BE-2A to 2D) was increased upon increasing the concentration in a dose dependent manner.

The percentage inhibition was calculated for synthesized compounds and standard Rivastigmine.

Table 27 - AChE inhibitory activity of different concentration of Benzotriazole derivatives and standard Rivastigmine

Compound code	Concentration (µg/ml)						IC_{50}(µg/ml)
	10	20	40	80	160	320	
BA-1A	19.87±0.34	21.50±0.68	32.67±0.56	45.42±0.69	59.25±0.42	65.68±0.76	105.0±2.88
BA-1B	22.85±0.97	27.10±0.50	40.15±1.41	46.18±1.18	57.18±1.40	61.97±1.92	101.3±1.85
BA-1C	21.38±0.59	27.20±0.65	34.84±0.62	49.91±0.31	65.90±0.33	71.69±0.20	80.3±0.88
BA-1D	40.52±0.34	45.22±0.61	48.84±0.55	53.47±0.41	56.31±0.47	60.52±0.24	43.3±0.88
BE-2A	27.10±0.50	32.67±0.56	43.54±0.28	57.04±0.68	59.66±0.62	65.90±0.33	42.3±0.33
BE-2B	35.07±0.91	40.46±0.95	44.76±1.10	53.77±1.13	55.75±0.73	55.50±2.68	51.6±0.88
BE-2C	35.84±0.71	41.85±1.12	44.52±0.54	49.22±0.82	57.85±0.082	59.81±0.57	80.0±1.15
BE-2D	38.52±0.38	39.04±0.32	45.43±0.38	51.06±0.36	59.55±0.41	63.53±0.45	65.5±1.45
RIVASTIGMINE	47.26±0.27	49.54±0.85	53.74±0.63	57.04±0.68	59.49±0.38	62.45±0.55	25.0±0.57

All the determinations were carried out in triplicate and the values are expressed as Mean ± SEM

Table 28 - BuChE inhibitory activity of different concentration of Benzotriazole derivatives and standard Rivastigmine

Compound code	Concentration (µg/ml)						IC_{50}(µg/ml)
	10	20	40	80	160	320	
BA-1A	38.73±0.13	39.16±0.01	43.71±0.03	45.62±0.34	56.75±0.74	59.77±0.10	97.0±0.57
BA-1B	32.84±0.44	33.52±0.32	36.83±1.03	39.98±1.11	47.28±1.75	51.86±0.25	243.3±3.33
BA-1C	42.99±0.16	44.16±0.35	47.15±0.78	52.11±0.46	54.93±0.30	57.44±0.34	69.0±2.88
BA-1D	46.25±0.05	48.56±0.24	49.76±0.61	52.38±0.16	54.98±0.35	57.87±0.15	75.6±2.33
BE-2A	37.97±0.80	41.34±0.09	45.58±0.17	51.20±0.19	54.37±0.50	58.69±0.08	75.3±0.33
BE-2B	37.44±0.16	40.41±0.04	41.15±0.79	44.78±0.72	51.00±0.02	53.69±0.29	125.0±2.88
BE-2C	38.44±0.13	39.99±0.28	43.17±0.08	49.42±1.08	53.88±0.09	56.22±0.31	82.3±1.45
BE-2D	41.20±0.09	43.03±0.02	43.38±0.05	44.69±0.03	56.11±1.73	58.39±0.57	97.6±1.45
RIVASTIGMINE	35.41±0.28	38.24±0.48	43.05±1.25	52.98±0.44	55.29±0.20	57.96±0.51	60.0±1.15

All the determinations were carried out in triplicate and the values are expressed as Mean ± SEM

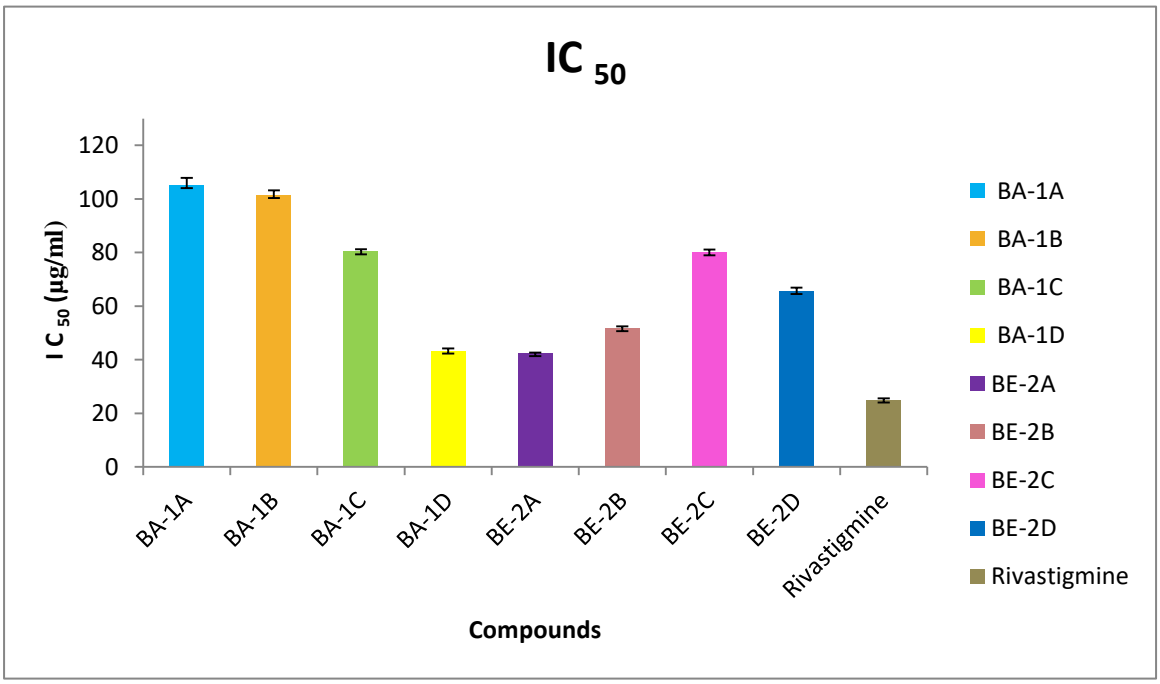


Fig. 45 – IC $_{50}$ values of Acetylcholinesterase (4EY7) Inhibitory Compounds

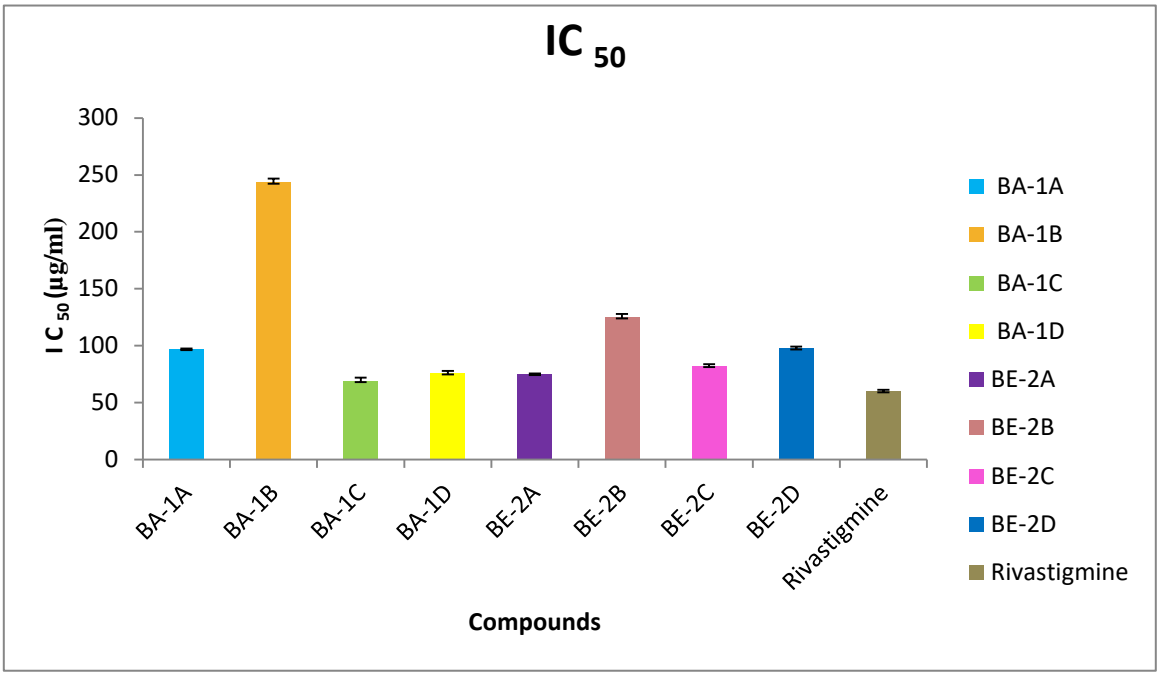


Fig. 46 – IC $_{50}$ values of Butyrylcholinesterase (4BDS) Inhibitory Compounds

All the newly synthesized compounds were tested for their Anti-Alzheimer's activity against AChE and BuChE. Almost all the synthesized analogs were active and showed that AChE and BuChE inhibitory activity at a various concentration (10, 20, 40, 80, 160, and 320 µg/ml) by Ellman's method and there was dose dependent increase in the percentage inhibition for all the concentrations.

In vitro Acetylcholinesterase inhibitory activity was determined for the synthesized compounds, BA-1A – 1D and BE-2A – 2D at a concentration ranging from 10 – 320 µg/ml. An increase in the percentage of inhibition to AChE was observed for increase in concentration.

- Among the BA-1A – 1D derivatives, BA-1D was found to be more active and the IC_{50} was found to be 43.3 µg/ml (Table 27 & Fig. 45).
- Among the BE-2A – 2D derivatives, BE-2A was found to be more active and the IC_{50} was found to be 42.3 µg/ml (Table 27 & Fig. 45).
- The standard drug Rivastigmine at concentration of 10 µg/ml has shown percentage inhibition 47.26±0.27 and for 320 µg/ml the percentage inhibition was 62.45±0.55 with IC_{50} of 25.0 µg/ml (Table 27 & Fig. 45).

Similarly, *In vitro* Butyrylcholinesterase inhibitory activity was determined for the synthesized compounds, BA-1A – 1D and BE-2A – 2D at a concentration ranging from 10 – 320 µg/ml. An increase in the percentage of inhibition to BuChE was observed for increase in concentration.

- Among the BA-1A – 1D derivative, BA-2C was found to be more active and the IC_{50} was found to be 69.0 µg/ml (Table 28 & Fig. 46).
- Among the BE-2A – 2D derivatives, BE-2A was found to be more active and the IC_{50} was found to be 75.3 µg/ml (Table 28 & Fig. 46).
- The standard drug Rivastigmine at concentration of 10 µg/ml has shown percentage inhibition 35.41±0.28 and for 320 µg/ml the percentage inhibition was 57.96±0.51 with IC_{50} of 60.0 µg/ml (Table 28 & Fig. 46).

Among all the synthesized compounds, for AChE the compounds BA-1D (43.3 ± 0.88) & BE-2A (42.3 ± 0.33), shows good percentage inhibition and the concentration required to inhibit 50 percentage of Acetylcholinesterase activity was moderate when compared to the standard Rivastigmine (25.0 ± 0.57).

For, BuChE the compounds BA-2C (69.0 ± 2.88) & BE-2A (75.3 ± 0.33) shows good percentage inhibition and concentration required to inhibit 50 percentage of butyrylcholinesterase activity was moderate when compared to the standard Rivastigmine (60.0 ± 1.15). This proves moderate activity of the synthesized compounds.

VII. SUMMARY AND CONCLUSION

SUMMARY

The present Study was focused on predicting the protein-ligand interactions, design, synthesis and evaluation of substituted Benzotriazole derivatives as possible anticholinesterase inhibitors.

IN SILICO STUDIES

- **Selection of target:**

Cholinesterase inhibitors was selected as the target for Anti-Alzheimer's activity. The corresponding target was downloaded from RCSB protein databank (**PDB: 4EY7, & 4BDS**).

- **Selection of lead:**

The lead Benzotriazole was selected based on several literature reviews. Derivatives of Benzotriazole, Benzotriazole hybrids were reported to have anticholinesterase activity.

- **Lead optimization:**

Lead optimization was done by observing in-silico ADMET studies and computation of molecular and ADME properties. All the selected ligands had good ADMET properties and hence were eligible for the further study.

- **Docking**

Molecular docking studies were performed using glide software. The ligands were docked with the target (4EY7 & 4BDS). The ligands BA-1A to 1D and BE-2A to 2D was showing best docked pose.

SYNTHESIS:

In this present work two schemes were developed for the compounds to be synthesized. Eight new compounds were synthesized. Benzotriazole was the starting compounds for both the schemes.

In the first scheme, O-phenylenediamine was reacted with sodium nitrite and acetic acid to form Benzotriazole. By treating substituted benzoic acid with thionyl chloride to form a

substituted acid chloride and further Benzotriazole and substituted acid chloride reacts to obtain desired products.

In second scheme, synthesized Benzotriazole was treated with Ethyl chloroacetate to form intermediate ethyl-1*H*-Benzotriazoyl-acetate and further treated with various primary amines to obtain desired products.

PHYSICAL CHARACTERIZATION:

Melting point of newly synthesized compounds were determined. Rf values were determined by fixing various suitable solvent system on precoated silica gel G plates. The structure was finally characterized by UV, IR, Mass, and ^{1}HNMR Spectra.

***IN-VITRO* ENZYME INHIBITORY ACTIVITY**

Neurodegenerative disease is characterized by decrease in the level of neurotransmitters, oxidative stress and neuro inflammation in brain, mostly the treatments are based on enhancing the Cholinergic function in brain there by improve the level of neuro transmitter from break down.

Cholinesterase inhibitors were developed based on cholinergic hypothesis of Alzheimer's disease, where Cholinesterase inhibitors reduce the degradation of the synaptic acetylcholine; improve level of acetylcholine in a dose-dependent manner. AChE and BuChE are two different enzymes located in brain that responsible for hydrolysis. Rivastigmine; dual AChE and BuChE inhibitors where it was used as standard.

All the newly synthesized compounds were screened for anticholinesterase activity using Ellman's method and all the compounds showed moderate activity. It was observed that the nature and size of the substituents play a role in influencing the activity of the compounds. The compounds BA-D and BE-2A showed good percentage inhibition for AChE activity when compared to the standard Rivastigmine. Similarly, the compounds BA-2C and BE-2A shows good percentage inhibition for BuChE activity when compared with the standard Rivastigmine.

This clearly demonstrate that our compounds have potential to increase the level of acetyl choline and it could also be used in prevention and control of Alzheimer's disease.

CONCLUSION

After analyzing the results of the present work, following conclusions were made,

The present work basically aims to identify the correct conformations of ligands in the active site protein and to predict the affinity of the ligand towards the protein. Structural based drug design approach proved to be a tool in minimizing the tedious drug discovery process.

The *In silico* molecular and ADME properties was established the compounds to be pharmacokinetically active. QIKPROP was used for filtering the compounds and selecting the lead compounds.

Docking results confirmed the possibility of Benzotriazole moiety to possess anticholinesterase activity. The binding energy obtained from docking study further confirmed the possibility of the affinity of the selected leads towards the enzyme Cholinesterase.

The compounds were synthesized based on the developed scheme and good yields obtained. Synthesized compounds structures were confirmed by Melting point, Rf value, UV, IR, Mass and NMR spectra. All compounds were screened for anti-Alzheimer's activity all the showed the better activity.

Derivatives of Benzotriazole were proven as a potent anti-Alzheimer's agents via anti-cholinesterase inhibition. Novel structure based drug design process helped to screen several compounds for specific activity. Present work could be considered as preliminary study of the titled moiety towards AChE & BuChE activity and further confirmation can be done by several site specific inhibitory actions.

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