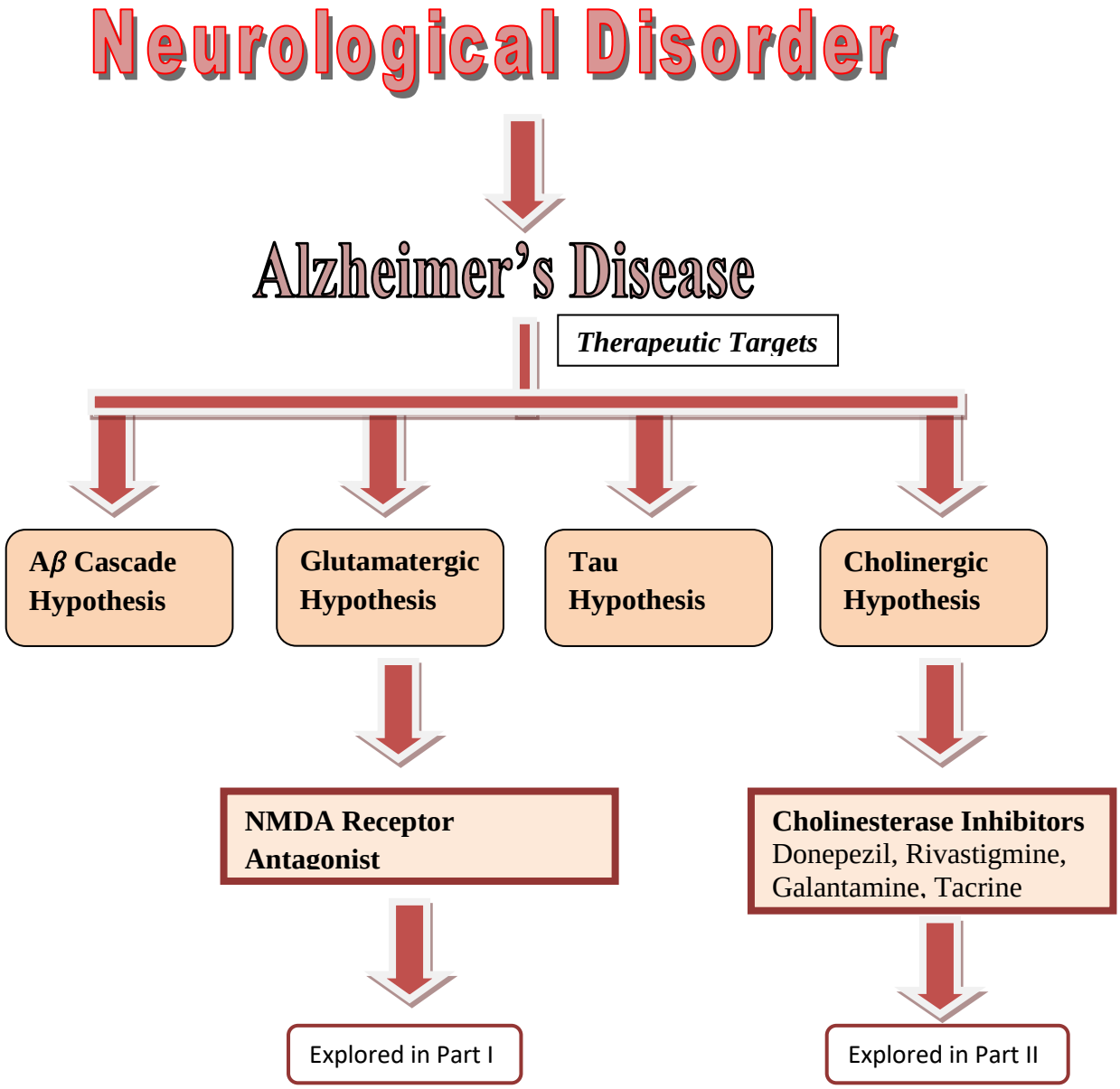
Summary

SUMMARY

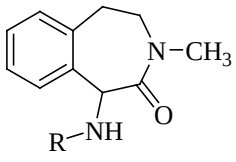
A neurological disorder is a disorder of the nervous system of the body that causes structural, biochemical or electrical abnormalities in the brain, spinal cord or other nerves and results in a range of symptoms. Neurological disorder like Parkinson's disease, Alzheimer's disease, Huntington's disease and Amyotrophic lateral sclerosis occur as a result of neurodegenerative processes. Alzheimer's is a most common type of dementia that causes problems with memory, thinking and behavior.



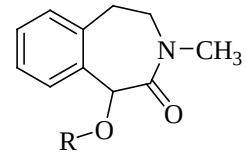
PART I

Potential NMDA Receptor Antagonist

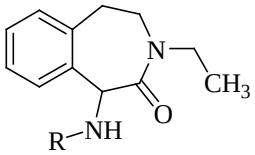
Overstimulation of the NMDA subtype by excessive release of glutamate is responsible for excitotoxic cell death within the nervous system causing neurological disorders. Benzazepine derivatives reported for their affinity to the PCP-binding site of the NMDA receptor and exhibits considerable interaction with the NMDA receptor. Hence, our aim was to synthesize a series of 3-benzazepine derivatives and screening them for NMDA receptor antagonistic activity.



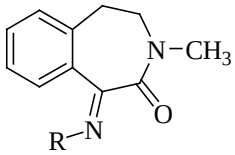
7 R = n-Propyl
8 R = Iso-Propyl
9 R = Butyl
10 R = Cyclopropyl
11 R = Phenyl
12 R = 2-Pydinyl
13 R = Benzyl
14 R = 4-Methyl benzyl
15 R = 4-Methoxy benzyl
16 R = 4-(Trifluoromethyl) benzyl
17 R = 4-Fluoro benzyl
18 R = 4-Chloro benzyl
19 R = 3-Chloro benzyl
20 R = 2-Chloro benzyl
21 R = 3,4-Dimethoxy benzyl
22 R = 2-Pydinylmethyl
23 R = Phenylethyl
24 R = 4-Chlorophenylethyl



25 R = Methyl
26 R = Ethyl
27 R = Phenyl



34 R = n-Propyl
35 R = Isobutyl
36 R = Butyl
37 R = Phenyl
38 R = Furfuryl
39 R = Benzyl
40 R = 4-Methyl benzyl
41 R = 4-Methoxy benzyl
42 R = 4-Fluoro benzyl
43 R = 3-Fluoro benzyl
44 R = 3-Chloro benzyl
45 R = 2-Methyl benzyl
46 R = Phenylethyl
47 R = 4-Chlorophenylethyl
48 R = 4-Methoxyphenylethyl



49 R = n-Propyl
50 R = Isobutyl
51 R = Butyl
52 R = Cyclopropyl
53 R = Cyclohexyl
54 R = Phenyl
55 R = Benzyl
56 R = 4-Methyl benzyl
57 R = 4-Methoxy benzyl
58 R = 4-Fluoro benzyl
59 R = 4-Chloro benzyl
60 R = 4-Bromo benzyl
61 R = 3-Chloro benzyl
62 R = Phenylethyl
63 R = 4-Methoxyphenylethyl

Fig. 1: Benzazepine-2-ones with substituents at 1 and 3 position

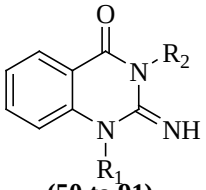
The newly synthesized benzazepine derivatives were assessed as potential NMDAR antagonists using cell-based assay. From the primery *in-vitro* screening some of the test compounds (**9, 14, 19, 25, 26, 27, 37, 39** and **44**) were identified as potent NMDAR antagonists. From the initially synthesized 15 compounds, the most effective compounds (**9** and **14**) were selected for further activities and showed significant result compared to NMDA receptor antagonist memantine.

	Test	**Observation (Compound 9 and 14)**
1	NMDA-induced excitotoxicity on SH-SY5Y cells	83.55 ± 3.74 and 90.42 ± 3.51 % neuroprotection
2	$A\beta_{1-42}$ aggregation: ThT assay	23.80 ± 2.74 and 34.86 ± 1.54 % inhibition
3	$A\beta_{1-42}$ aggregation: CR binding assay	28.79 ± 1.33 and 38.10 ± 1.54 % inhibition
4	$A\beta_{1-42}$-induced excitotoxicity on rat hippocampal neurons	Significant protection
5	Morris water maze test and Y maze test	Significantly reduced ELT and increased the platform area crossings
6	ROS scavenging activity	Significantly reduced $A\beta_{1-42}$-induced ROS generation.

PART II

Novel Cholinesterase Inhibitors

As per the cholinergic hypothesis, level of acetylcholine decreases below normal level in Alzheimer's disease which can be increased by hydrolysis of acetylcholinesterase enzyme. Cholinesterase inhibitors are prescribed to treat symptoms related to memory, thinking, language, judgment and other thought processes. There are numerous other biologically active molecules (cholinesterase inhibitors) whose framework includes a six-membered ring with two nitrogen atoms fused to a phenyl ring. Most of these molecules are based on **quinazolinone** framework. Hence, it was planned to synthesize a novel series of compounds with a cyclic guanidine motiff incorporated into 4-quinazolinone and evaluate the synthesized compounds for their anti-Alzheimer's potential.



(50 to 91)

R_1	**R_2**
4-Bromobenzyl	Phenylethyl,
4-Methoxybenzyl	Isobutyl,
Phenylethyl	Butyl,
Methyl	4-Methylbenzyl,
Ethyl	Isopropyl,
Allyl	Propyl,
Benzyl	Cyclopropyl,
	Benzyl,
	4-Methoxybenzyl,
	2-Chlorobenzyl,
	2-Furylmethyl

Fig. 2: (1*H*)-2-imino-2,3-dihydroquinazolin-4-ones (**50-91**)

All the synthesized compounds were screened for their anti-Alzheimer's potential by determining their anticholinesterase activity. For preliminary screening of iminoquinazolin-4-one derivatives, *in vitro* anticholinesterase (anti-ChE) activity was assessed using Ellman's assay. The compound (**75**) showed the highest activity by inhibiting AChE and BuChE.

	Test	**Observation (Compound 75)**
1	AChE and BuChE inhibitory activities	5.40±2.96 μM and 7.28±0.27 μM (IC_{50} values)
2	$A\beta_{1-42}$ aggregation: ThT assay	23.67±2.98 % inhibition
3	$A\beta_{1-42}$ aggregation: CR binding assay	26.18±2.16 % inhibition
4	Neuroprotective effect in SH-SY5Y cells	27.2±2.5 at 10 μM and 43.8±3.9 at 20 μM
5	Antioxidant activity in DPPH assay	44.4±3.5 at 10 μM and 60.2±3.4 at 20 μM
6	Morris water maze test and Y maze test	Significantly reduced ELT and increased the platform area crossings
7	ROS scavenging activity	Significantly reduced $A\beta_{1-42}$-induced ROS generation.

Summary and Conclusion

Introduction

The brain is an assembly of inter-connected neural system that regulates its own activity in a dynamic and complex fashion largely through intercellular chemical neurotransmission. A neurological disorder is a disorder of the nervous system of the body that causes structural, biochemical or electrical abnormalities in the brain, spinal cord or other nerves and results in a range of symptoms. Neurological diseases like Parkinson's disease, Alzheimer's disease, Huntington's disease and Amyotrophic lateral sclerosis occur as a result of neurodegenerative processes.

Alzheimer's is a type of dementia that causes problems with memory, thinking and behavior. The most common early symptom of Alzheimer's is difficulty in remembering newly learnt information because Alzheimer's changes typically begin in the part of the brain that affects learning. As Alzheimer's advances through the brain it leads to increasingly severe symptoms, including disorientation, mood and behavior changes; deepening confusion about events, time and place; unfounded suspicions about family, friends and professional caregivers; more serious memory loss and behavior changes and difficulty in speaking, swallowing and walking. Symptoms usually develop slowly and get worse over a period of time, becoming severe enough to interfere with daily tasks. Although current Alzheimer's treatments cannot stop Alzheimer's from progressing, they can temporarily slow the worsening of dementia symptoms and improve the quality of life for the patients and their caregivers. Two abnormal structures called plaques and tangles are prime suspects in damaging and killing nerve cells. Also mechanism-based explorations of therapeutic approaches have been intensively investigated, but no effective drugs and therapies have been developed. Currently, Food and Drug Administration (FDA) approved AD drugs are still limited within two categories: cholinesterase inhibitors and memantine (an NMDA receptor antagonist)

Neurotransmitters' depletion (basically referring to acetylcholine, ACh) and synaptic dysfunction are two classical features of AD. Thus, two hypotheses have been established−cholinergic hypothesis and glutamatergic hypothesis, based on which FDA approved therapies- AchE inhibitors and NMDA receptor antagonists were developed to mitigate AD symptoms.

The proposed work has been divided in to three main sections depending upon the synthetic and pharmacological applications.

- **Part I** of the research work contains the synthesis and biological evaluation of some benzazepine derivatives as potential NMDA receptor antagonists.
- **Part II** of the research work contains the synthesis and biological evaluation of different quinazolinone derivatives as novel Cholinesterase inhibitors.

Part I : Potential NMDA Receptor Antagonist

Glutamate is a single powerful excitatory neurotransmitter that is responsible for rapidly conveying sensory information and complex motor commands from one part of the body to another, and to form thoughts and memories. There are other excitatory neurotransmitters in the brain, but glutamate is the most common and widely distributed one. Excessive release of glutamate is responsible for excitotoxic cell death within the nervous system. Excessive stimulation of the NMDA subtype of glutamate receptors is responsible for apoptotic-like excitotoxicity which afterward leads to cell death. When activated, the NMDA receptor opens a channel that allows Ca^{2+} (and other cations) to move into the cell as this activity is important for long-term potentiation (LTP), learning and memory formation. Under normal conditions of synaptic transmission, the NMDA receptor channel is blocked by Mg^{2+} ion resting in the channel and it gets activated only for brief periods of time. Under pathological conditions, overactivation of the receptor causes an excessive amount of Ca^{2+} influx into the nerve cell, which then triggers a variety of processes that can lead to apoptosis.

Competitive antagonists for glutamate or glycine site will block healthy areas of the brain before they can affect pathological areas and therefore less useful in the disease condition. Hence, an uncompetitive antagonist is beneficial approach as it is distinct from a competitive/noncompetitive antagonist as it is an inhibitor whose action depends upon prior activation of the receptor by the agonist. Hence, the same amount of antagonist blocks the higher concentrations of the agonist better than the lower concentrations of the agonist. This uncompetitive mechanism of action could yield a drug that blocks NMDA receptor-operated channels only when they are excessively open while relatively sparing the normal neurotransmission.

The tetracyclic MK-801 is a non-competitive NMDA receptor antagonist, that binds with high affinity (Ki=1.26 nM) to the PCP-binding site of the NMDA receptor. The cleavage of the C9a/C10-bond of MK-801 results in tetrahydroisoquinolines, which interact with the PCP-binding site. Extension of the amino bridge of the tetrahydroisoquinolines leads to the constitutional isomer 1-phenyltetrahydro-3-benzazepine which also binds with high affinity to the PCP-binding site of the NMDA receptor and exhibits considerable interaction with the NMDA receptor. Also, benzazepine moiety is attracting a lot of attention due to its wide spectrum of biological activity mostly on central nervous system (CNS). Synthesis of benzazepine scaffold is reported by a number of researchers and its presence is noticed in nature as a basic structure in some alkaloids. Different pharmacological actions associated with benzazepine ring-containing compounds are reviewed and majority of these actions are on CNS disorders.

Based on the literature survey, it was envisaged to prepare a conceptually new library of 3-alkylbenzazepine-2-one derivatives for the fulfilment of the need for the development of new N-containing seven membered cyclic compounds.

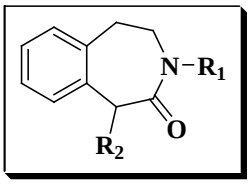


Fig. 1: Designed benzazepine-2-ones with substituents at 1 and 3 position

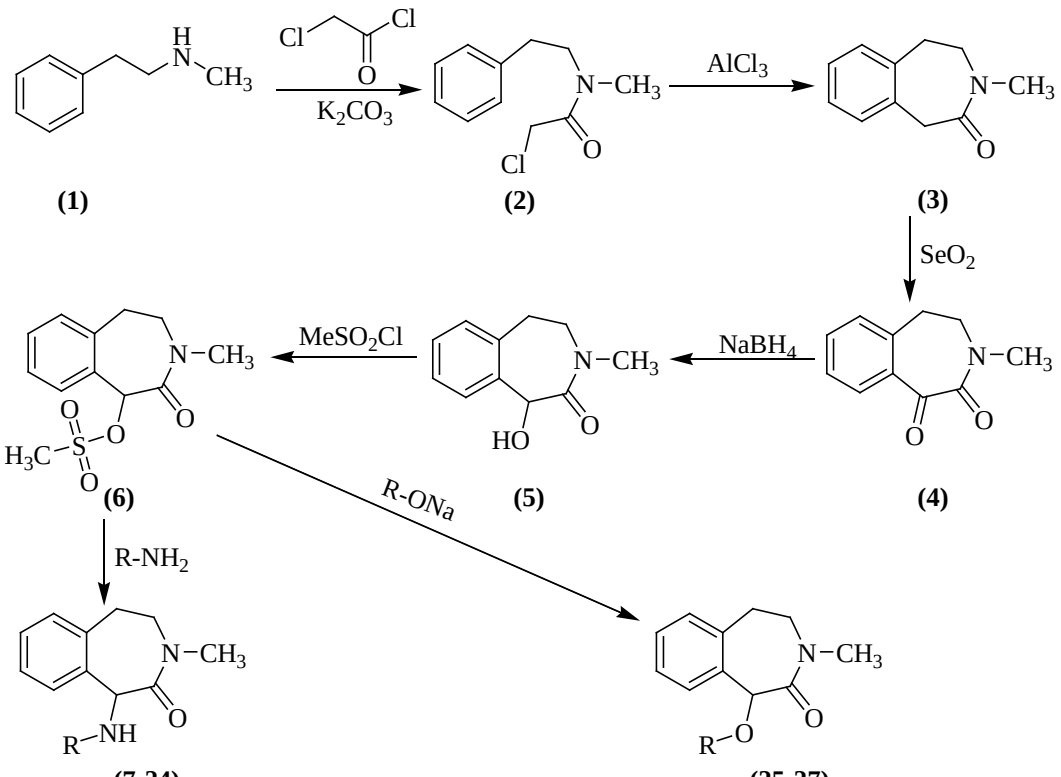
With a thorough study of various benzazepine analogs, we decided to go for the synthesis of newer benzazepine analogs with improved activity profile. From the literature study it became clear that substitution at various positions of the benzazepine ring can produce a variety of receptor interactions in the CNS. Modification at positions 1 and 3 may lead to changes in the selectivity for different receptors thus offering compounds with different activities. Hence, our main target was to make suitable substitutions on positions 1 and 3 of tetrahydro-3-benzazepin-2-one ring system.

So, our aim was to synthesize a series of 3-benzazepine derivatives and screening them for NMDA receptor antagonistic activity. Objectives of the research are:

- To develop and explore substitution methods at the desired position 1 of the azepine ring of the benzazepine scaffold.

- To screen the synthesized compounds for potential NMDA receptor antagonism by *in vitro* and *in vivo* methods.

Synthesis of 3-methyl-4,5-dihydro-3*H*-benzo[*d*]azepine-1,2-dione was carried out by the method reported by Nair *et al*. for the synthesis of 3-methylbenzazepine. Commercially available 40% aqueous solution of methylamine was taken for the neucleophilic substitution with 2-phenylethyl bromide for the synthesis of secondary amine *N*-methyl-2-phenylethylamine (**1**). The secondary amine (**1**) on reaction with chloroacetyl chloride in the biphasic chloroform:water solvent system gave 2-chloro-*N*-methyl-*N*-phenethylacetamide (**2**). A solution of 2-chloro-*N*-methyl-*N*-phenethylacetamide in 1,2-DCB was used in Friedel-Crafts alkylation using anhydrous $AlCl_3$ for the synthesis of 3-methyltetrahydro-3*H*-benzazepin-2-one (**3**) which on oxidation with SeO_2 gave 3-methyl-4,5-dihydro-3*H*-benzo[*d*]azepine-1,2-dione (**4**).



7□ R = n-Propyl
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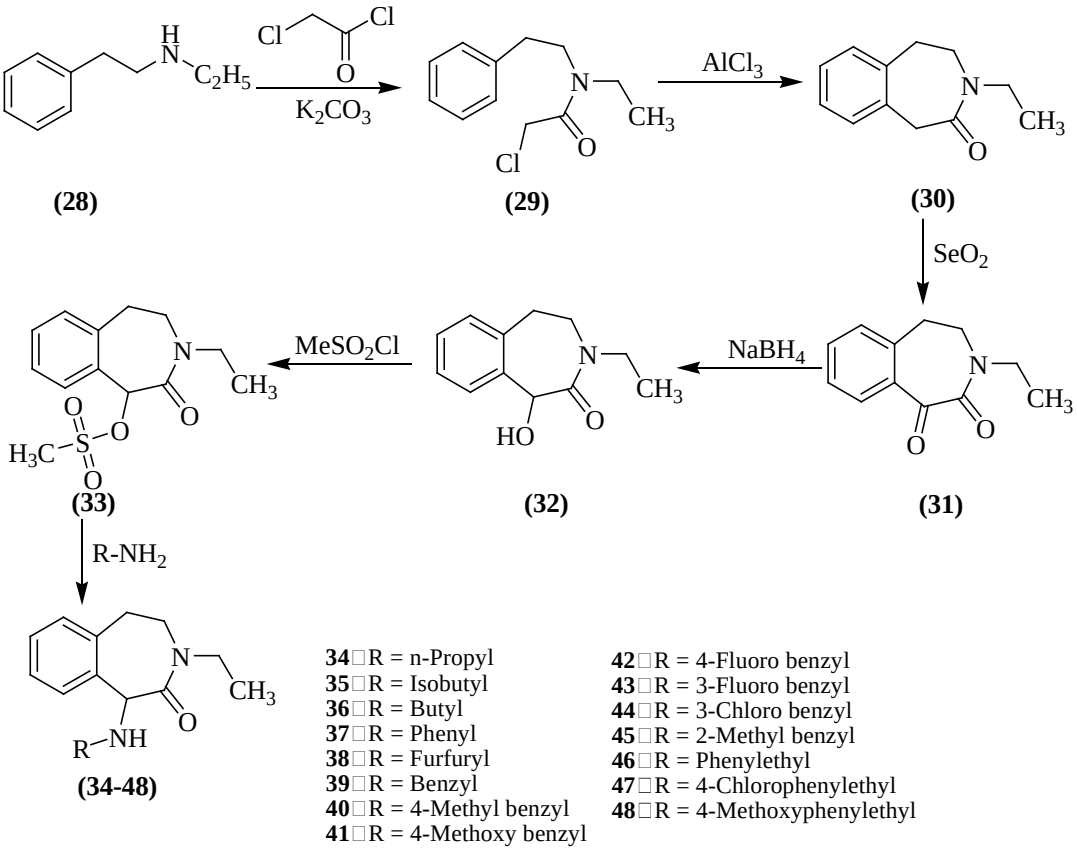
17□R = 4-Fluoro benzyl
18□R = 4-Chloro benzyl
19□R = 3-Chloro benzyl
20□R = 2-Chloro benzyl
21□R = 3,4-Dimethoxy benzyl
22□R = 2-Pydinylmethyl
23□R = Phenylethyl
24□R = 4-Chlorophenylethyl
25□R = Methyl
26□R = Ethyl
27□R = Phenyl

Scheme 1: Synthesis of 3-Methyl-4,5-dihydro-1*H*-benzo[*d*]azepin-2(3*H*)-one derivatives

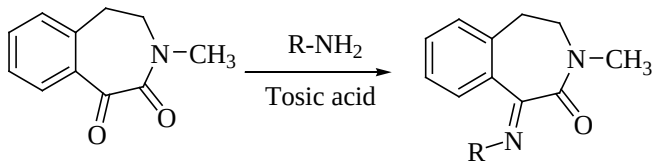
The resulted dione was reduced by $NaBH_4$ to 1-Hydroxy-3-methyl-4,5-dihydro-1*H*-benzo[*d*]azepin-2(3*H*)-one and then mesylated to 3-methyl-2-oxo-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-1-yl methanesulfonate (**6**). Neucleophilic substitution with various amines and alcohols on **6** gave 1-amino substituted 3-methyl-4,5-dihydro-1*H*-benzo[*d*]azepin-2(3*H*)-one derivatives (**7-27**) (**Scheme 1**).

The same procedures were followed for 1-amino substituted 3-ethyl-4,5-dihydro-1*H*-benzo[*d*]azepin-2(3*H*)-one derivatives (**34-48**) by replacing methyl amine with ethyl amine in initial step as shown in **scheme 2**.



Scheme 2: Synthesis of 3-ethyl-4,5-dihydro-1*H*-benzo[*d*]azepin-2(3*H*)-one derivatives

Synthesis of 1-imino derivatives of 3-methyl-4,5-dihydro-1*H*-benzo[*d*]azepin-2(3*H*)-one (**49-63**) was carried out by reaction of compound (**4**) with different amines to afford imines as a reaction of carbonyl group with amine (**Scheme 3**).



(4) **(49-63)**

49 R = n-Propyl
50 R = Isobutyl
51 R = Butyl
52 R = Cyclopropyl
53 R = Cyclohexyl
54 R = Phenyl
55 R = Benzyl
56 R = 4-Methyl benzyl
57 R = 4-Methoxy benzyl
58 R = 4-Fluoro benzyl
59 R = 4-Chloro benzyl
60 R = 4-Bromo benzyl
61 R = 3-Chloro benzyl
62 R = Phenylethyl
63 R = 4-Methoxyphenylethyl

Scheme 3: Synthesis of 1-imino derivatives of 3-methyl-4,5-dihydro-1*H*-benzo[*d*]azepin-2(3*H*)-one

The newly synthesized benzazepine derivatives were assessed as potential NMDAR antagonists using cell-based assay. From the primery *in-vitro* screening some of the test compounds (**9, 14, 19, 25, 26, 27, 37, 39** and **44**) were identified as potent NMDAR antagonists. These compounds were worthy of further evaluation for $A\beta_{1-42}$ aggregation inhibitory, neuroprotective, free radical scavenging, anti-oxidant and anti-apoptotic activities using different *in vitro* and *in vivo* experiments. Hence, from the initially synthesized 15 compounds, the most effective compounds (**9** and **14**, with % neuroprotection of 83.55 ± 3.74 and 90.42 ± 3.51 respectively) were selected for further activities although rest of the compounds might also be potential NMDA receptor antagonist. In the further tests compounds (**9** and **14**) protected primary rat hippocampal neurons against $A\beta_{1-42}$-induced excitotoxicity, exhibited significantly reduced ELT and increased the platform area crossings in Morris water maze test and Y maze test for assessing learning ability and working memory and significantly reduced $A\beta_{1-42}$-induced ROS generation in DCFH-DA assay.

In conclusion, the present study revealed the multi-target-directed potential of benzazepine derivatives against excitotoxicity as they have demonstrated efficient NMDAR antagonist, $A\beta_{1-42}$ aggregation inhibitory, neuroprotective, free radical scavenging, antioxidant and antiapoptotic activities in different *in vitro* and *in vivo* experiments. Thus, the present study reveals that these benzazepine derivatives could be promising candidates for the treatment of a variety of neurodegenerative disorders.

Part II : Novel Cholinesterase Inhibitors

As per the cholinergic hypothesis, level of acetylcholine decreases below normal level in Alzheimer's disease. Level of acetylcholine is increased to normal level in certain areas of brain by reducing its hydrolysis by acetylcholinesterase (AChE) enzyme. AChE is an important enzyme that breaks down acetylcholine in the synaptic cleft in neuronal junctions. Inhibition of this enzyme is connected with treatment of several diseases such as Alzheimer's disease, myasthenia gravis, glaucoma and anthelmintic drugs and for the control of insects. Currently available AChE inhibitors in clinical use for the treatment of AD don't have the ability to stop the progress of the disease. Drug that potentiates central cholinergic activity will control cognition and behavioural problems experienced in AD.

As per cholinergic hypothesis, a number of approaches were tried for the treatment of the cholinergic deficit in AD, one of them was replacement of ACh precursors (choline or lecithin) but these agents failed to increase central cholinergic activity. Another widely accepted approach in this hypothesis was the use of cholinesterase (ChE) inhibitors like physostigmine that reduced the hydrolysis of ACh. The newer compounds to target AD include specific M1 muscarinic or nicotinic agonists, M2 muscarinic antagonists, or improved "second generation" ChE inhibitors. Transplantation of ACh rich foetal tissue grafts is another approach, which has been shown to improve the cognitive performance of primates after excitotoxic lesions of cholinergic nuclei. Thus, such approaches may offer additional future possibilities for the treatment for AD; the use of ChE inhibitors is the most well developed and accepted approach to the disease.

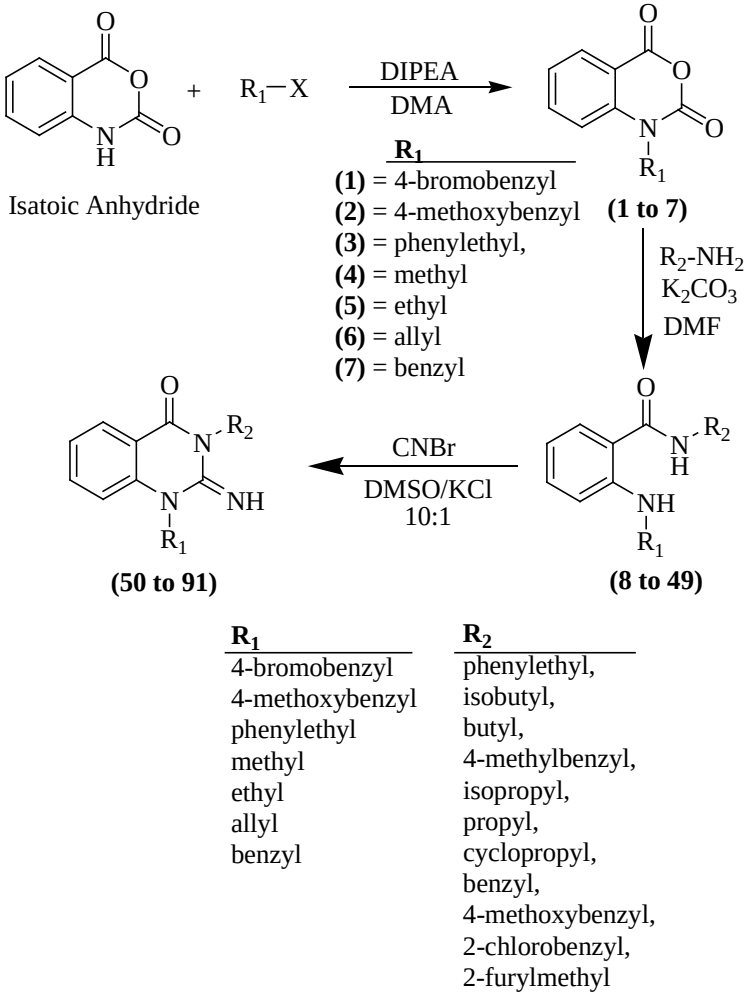
Cholinesterase inhibitors are prescribed to treat symptoms related to memory, thinking, language, judgment and other thought processes. Four drugs approved by FDA show their effects in different stages of AD and are commonly prescribed depending on the condition of the patient. Donepezil is approved to treat all stages of AD while rivastigmine and galantamine are approved for mild to moderate stages of AD. Tacrine was the first cholinesterase inhibitor that was approved in 1993 but is rarely prescribed today because of its common side effect i.e. liver damage. All approved drugs, temporarily improve symptoms of Alzheimer's disease by increasing the amount of neurotransmitters in the brain. The effectiveness of these drugs varies from person to person. However, none of the treatments available today for Alzheimer's disease slows or stops the damage to neurons that causes Alzheimer's symptoms and eventually makes the disease fatal.

Heterocycles are among the earliest organic compounds, recognised as discrete substances which gained much interest among the chemists worldwide for their diversified biological activities. 4(3*H*)-Quinazolinones and their derivatives constitute an important class of heterocyclic compounds. They occupy an important position in medicinal and pesticide chemistry, presenting a wide range of bioactivities. There are numerous biologically active molecules (choline esterase inhibitors) whose framework includes a six-membered ring containing two nitrogen atoms fused to a phenyl ring. Most of these molecules are based on the **quinazoline** or **quinazolinone** framework.

There are only five drugs approved for the treatment of Alzheimer's disease and all of these have shown side effects like seizures, hepatic discomfort, cardiac failures etc., hence, there is a great need for discovery of newer agents for the management of the disease. It has been observed that butyrylcholine esterase activity increases in the brain during the latter phase in Alzheimer's patients. Therefore, there is a need of newer agents having activity against AChE (to treat the early stage of AD) as well as BuChE (to treat late stageof AD). Hence, it was planned to synthesize compounds that would bind to both the sites for good anti-Alzheimer activity and the objective was:

- The primary aim of the study was to search for easier and faster synthetic approaches to produce multiple compounds for the development of new and more promising CNS active quinazolinones.
- To synthesize a novel series of compounds with a cyclic guanidine motiff incorporated into 4-quinazolinone as benzoannelated heterocycles on the basis of fundamental principles of synthetic chemistry using isatoic anhydride as one of the starting materials and different halides, amines, cynogen bromide etc. Also, to develop and optimize scheme under different reaction conditions for obtaining the best practical yield.
- To prepare quinazolinone scaffold with different substituents at various positions to obtain potent AChE and BuChE inhibitors.
- Further, to evaluate the synthesized compounds for their anti-Alzheimer's potential.

The compounds were synthesized as per the general **Scheme 4** in which diverse substitutions were used for the synthesis of quinazolinones having guanidine pharmacophore in the ring system.



Scheme 4: Synthetic scheme for (1*H*)-2-imino-2,3-dihydroquinazolin-4-ones (**50-91**)

The desired compounds (1*H*)-2-imino-2,3-dihydroquinazolin-4-ones (**50-91**), were synthesized from isatoic anhydride in three steps. In the first step, isatoic anhydride was reacted with alkyl halides in presence of organic base like DIPEA (*N*,*N*-diisopropylethylamine) to generate *N*-alkyl substituted isatoic anhydrides (**1-7**). The substituted isatoic anhydrides were subjected to ring opening by reaction with primary amines to afford 2-amino benzamide derivatives (**8-49**). The synthesized 2-amino benzamide derivatives were cyclised using cyanogens bromide for the synthesis of (1*H*)-2-imino-2,3-dihydroquinazolin-4-ones (**50-91**).

All the synthesized compounds were screened for their anti-Alzheimer's potential by determining their anticholinesterase activity. For preliminary screening of iminoquinazolin-4-one derivatives, *in vitro* anticholinesterase (anti-ChE) activity was assessed using Ellman's assay. The compound (**75**) showed the highest activity by inhibiting AChE and BuChE with IC_{50} values of 5.40±2.96 μM and 7.28±0.27 μM

respectively. Result showed that the compound (**75**) at 10 μM exhibited significant anti-$A\beta_{1-42}$ aggregatory effects in thioflavin-T (ThT) assay and in CR binding assay (23.67±2.98% and 26.18±2.16% inhibition respectively). The most promising compound (**75**) was further assessed for its anti-Alzheimer's potential by performing other more sophisticated tests. Test compound (**75**) showed neuroprotective effect in SH-SY5Y cells, antioxidant activity in DPPH assay, enhanced spatial learning ability in MWM test, improved "spontaneous alteration" behaviour in Y maze test and antioxidant potential in ROS scavenging activity.

In conclusion, the present study has revealed anticholinesterase potential of the novel iminoquinazoline-4-one derivatives through acetylcholinesterase and butyrylcholinesterase inhibitory activity. The most active compound (**75**) in the series was further tested for other aspects to control neuronal loss in the AD. Compound **(75)** has shown the ability to interact with different AD targets as it has shown significant multiple effects including anti-ChE, anti-Aβ aggregatory, neuroprotective, ROS scavenging and antioxidant activities in different *in vitro* and *in vivo* experimental models.