A Synopsis on

DESIGN AND DEVELOPMENT OF NOVEL POTENTIAL THERAPEUTICS FOR ALZHEIMER'S DISEASE

Submitted

To

THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA

For the submission of Ph. D. Thesis

By

Dushyantkumar V. Patel

Research Guide

Prof. M. R. Yadav

Research Co-guide

Prof. A.V. Bedekar

Faculty of Pharmacy,
The Maharaja Sayajirao University of Baroda,
Vadodara-390 001.

1. Introduction

Alzheimer's disease (AD) is an irrevocable age-related neurodegenerative disorder clinically identified by progressive deterioration in memory, cognitive deficit, abnormal behaviour and incoherent language.[1] It is the most prominent form of dementia. More than 50 million people are suffering from it worldwide, and the number will significantly rise up to 152 million by 2050 if no cure or preventive measures are found.[2] This burgeoning number of people suffering from AD, in both developed and developing countries, has drawn the attention of medicinal chemists to accelerate research on drug discovery in this area.

1.1. Pathophysiology of AD

The etiology of AD is still enigmatic. Different factors, like low levels of neurotransmitter acetylcholine (ACh),[3] aggregation of the β-amyloid peptide,[4,5] accumulation of hyperphosphorylated tau protein,[6,7] dyshomeostasis of biometals,[8] oxidative stress,[9] mitochondrial dysfunction[10] and neuroinflammation[11-13] are proposed to play pivotal roles in the pathogenesis of AD.

1.1.1. Cholinergic hypothesis

The oldest, on which most currently available drug therapies are based, is the cholinergic hypothesis, which proposes that AD is caused by reduced level of the neurotransmitter acetylcholine in brain. Consequently it was suggested that degeneration of cholinergic neurons inside basal forebrain and consequent cholinergic neurotransmission loss in the cerebral cortex and different zones contributed to the decay in psychological capacity seen in patients with AD.[3]

Two types of cholinesterase enzymes (ChEs) namely acetylcholinesterase (AChE) (EC 3.1.1.7) and butyrylcholinesterase (BuChE) (EC 3.1.1.8), are found in the central nervous system. Both these enzymes belong to the carboxylesterase family of enzymes and play an important role in cholinergic transmission through hydrolysis of the neurotransmitter ACh. Although AChE and BuChE are produced by different genes they are highly homologous with more than 65 % similarity in their active sites.[14,15] AChE has two major binding sub-sites, a peripheral anionic site (PAS) and the other a catalytic active site (CAS).[16] The CAS of the enzyme is actively involved in the maintenance of cholinergic neurotransmission. PAS is involved in the formation of β-

amyloid fibrils that are associated with plaque deposition.[17,18] AChE inhibitors blocking both CAS and PAS simultaneously could alleviate the cognitive defect in AD patients by elevating ACh levels and have also been endowed with disease modifying ability by inhibiting the amyloid plaque formation.[19] In healthy brains, AChE is more active than BuChE and can hydrolyze about 80 % of ACh. Current studies have demonstrated that as the disease progresses, the ability of BuChE increases by 40-90 %, and that of AChE declines in the hippocampus and temporal cortex areas of the brain.[20-22] BuChE plays several roles both in neural and non-neural functioning. Clinical data suggested that the high cortical levels of BuChE were associated with some important AD hallmarks, such as extracellular deposition of the Aβ and aggregation of hyper-phosphorylated tau protein.[14,23,24]

1.1.2 Amyloid hypothesis

The amyloid hypothesis postulates that beta-amyloid (Aβ) deposits are the fundamental cause of the disease. AD has been identified as a protein misfolding disease (proteopathy), caused by accumulation of abnormally folded amyloid beta proteins in the brain.[4]

Amyloid precursor protein (APP) is a protein found widely throughout the body. The amyloid hypothesis states that a fault with the processing of APP in the brain leads to the production of a short fragment of APP known as Aβ **(Figure 1)**. The theory rests on the idea that it is the accumulation of this sticky protein fragment in the brain that triggers the disruption and destruction of nerve cells that cause AD. The accumulated clumps of beta amyloid are known as amyloid plaques. The hypothesis thus says that there is a fault with the over production of beta amyloid or with the mechanism that usually clears it from the brain, or possibly both.[5]

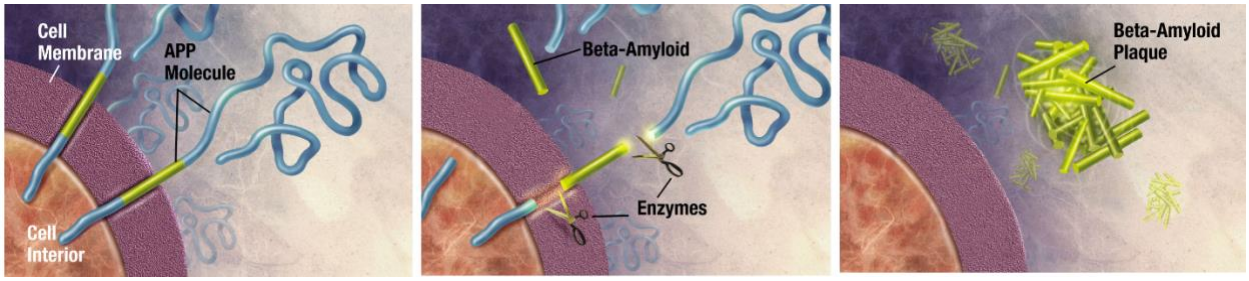


Figure 1. Enzymes act on the APP (amyloid precursor protein) and cut it into fragments. The β-amyloid fragment is crucial in the formation of plaques in AD.

1.1.3 Tau hypothesis

The tau hypothesis states that excessive or abnormal phosphorylation of tau results in the transformation of normal adult tau into PHF-tau (paired helical filament) and neurofibrillary tangles. Tau protein is a highly soluble microtubule-associated protein. Through its isoforms and phosphorylation tau protein interacts with tubulin to stabilize microtubule assembly. Tau proteins constitute a family of six isoforms with the range from 352-441 amino acids. All of the six tau isoforms are often present in a hyperphosphorylated state in paired helical filaments from AD.[6]

Mutations that alter function and isoform expression of tau lead to hyper-phosphorylation. The process of tau aggregation in the absence of mutations is not known but might result from increased phosphorylation, protease action or exposure to polyanions, such as glycosaminoglycans. Hyperphosphorylated tau disassembles microtubules and sequesters normal tau, MAP 1 (microtubule associated protein1), MAP 2, and ubiquitin into tangles of PHFs **(Figure 2)**. This insoluble structure damages cytoplasmic functions and interferes with axonal transport, which can lead to cell death.[7]

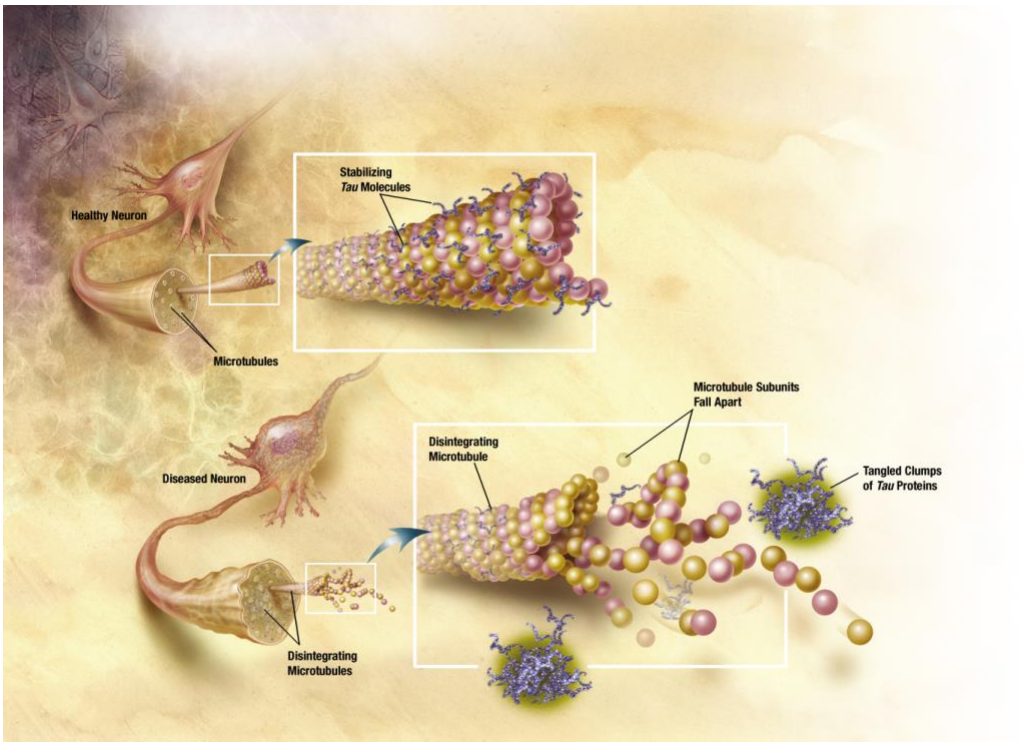


Figure 2. Changes in tau protein lead to the disintegration of microtubules in brain cells of AD patient.

1.1.4 Oxidative stress hypothesis

Recent research has emphasized the significance of oxidative stress in the fundamental molecular mechanism of AD.[8,9] Oxidative stress occurs when there is an inequity between the formation and quenching of free radicals formed from oxygen species. These reactive oxygen species (ROS) are regarded as the other major etiological factor of AD, since the role of the ROS in the formation of both amyloid plaques and neurofibrillary tangles is confirmed.[25] Through pathological reduction-oxidation steps, ROS can denature biomolecules like proteins, lipids and nucleic acids. This can cause tissue damage through necrosis and apoptosis (**Figure 3**).[26] Thus, oxidative stress plays a central role in the pathogenesis of AD leading to neuronal dysfunction and cell death.[27]

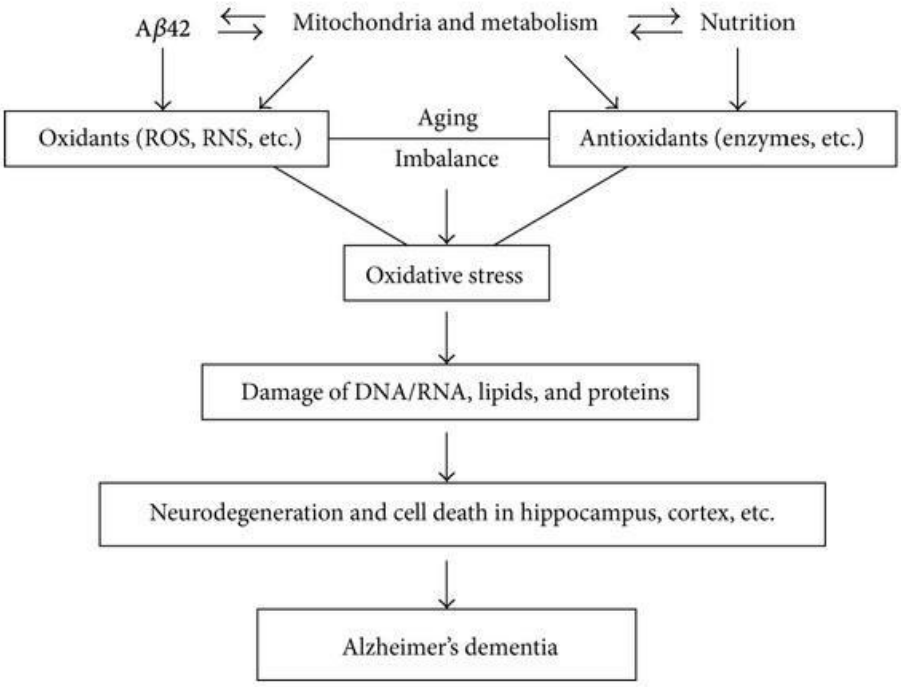


Figure 3. Role of oxidative stress in AD pathophysiology

1.1.5 Metal ion dyshomeostasis

High levels and dysregulation of biometal ions were closely implicated in the pathogenesis of AD. Metal ions such as Cu^{2+}, Zn^{2+} and Fe^{2+} have been shown to facilitate Aβ aggregation, leading to the generation of toxic Aβ oligomers. In particular, redox-active Cu (I/II) and Fe (II/III)

are implicated in the generation of reactive oxygen species (ROS) leading to an increased oxidative stress which ultimately result in cell death (**Figure 4**).[8]

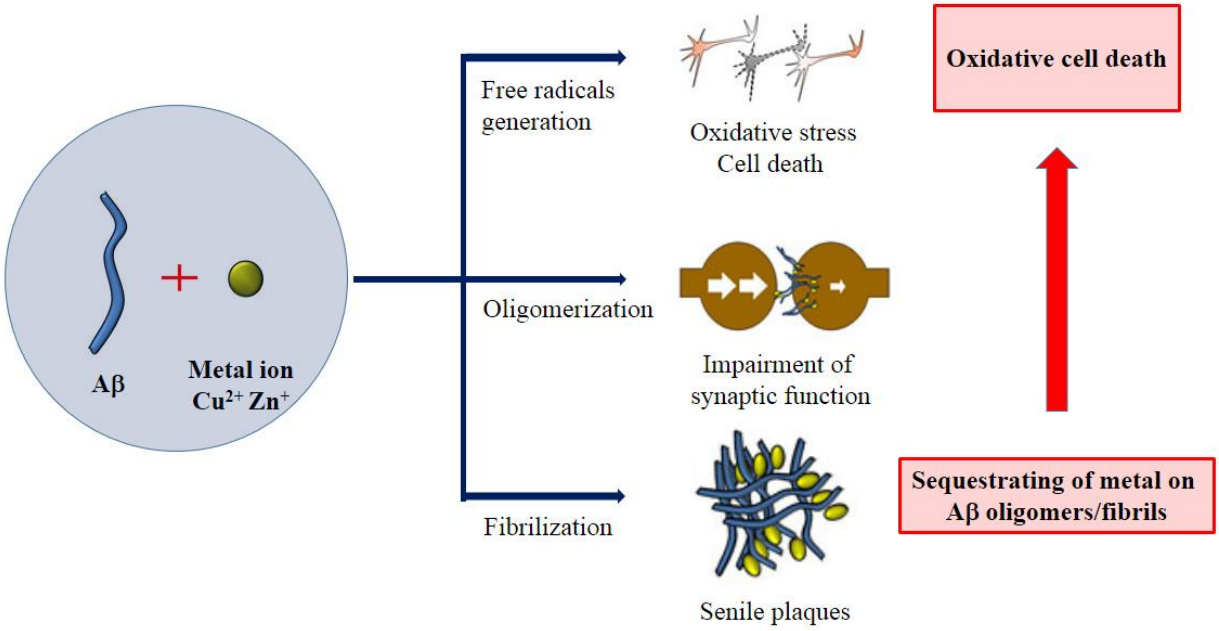


Figure 4. Metal ion hypothesis

1.2. Treatment for AD

Currently there are only four FDA approved medications available to treat AD. Three of them are AChE inhibitors (donepezil, galantamine, and rivastigmine),[28,29] and the fourth memantine[30] is *N*-methyl-D-aspartate (NMDA) antagonist. These medications ameliorate the symptoms and can improve the functioning of patients with AD, but they are not curative, nor do they significantly change the course of the illness.

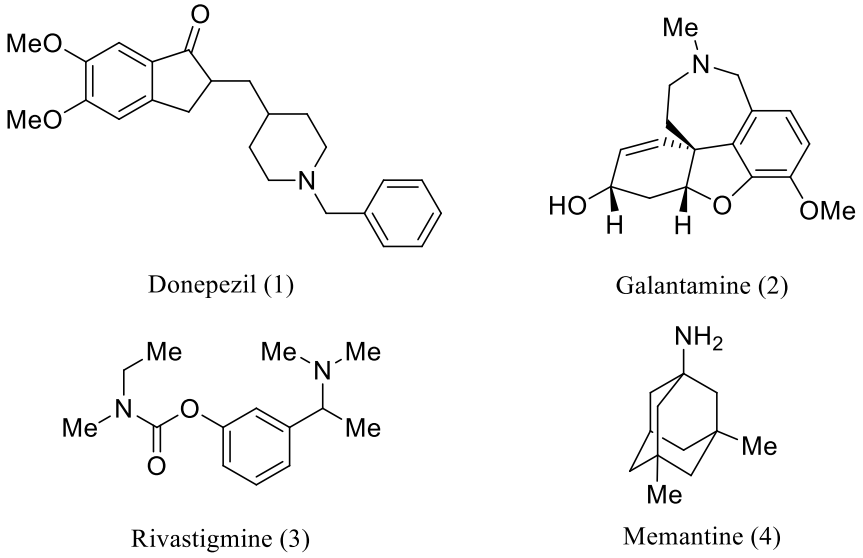
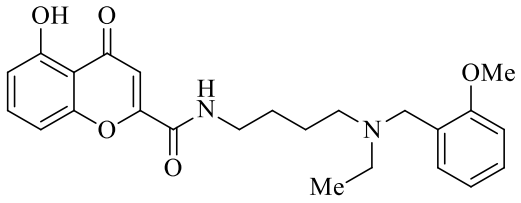


Figure 5. FDA approved drugs for the treatment of AD.

2. Literature review

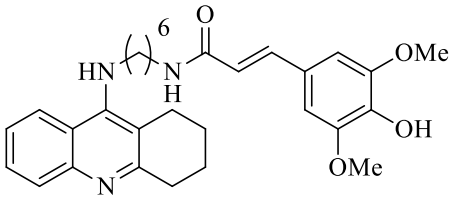
Apart from the drugs approved by FDA, recently many research groups have reported diverse scaffolds with anti-AD property. These include cholinesterase inhibitors (ChEIs) combined with Aβ aggregation inhibitors, β-secretase inhibitors or multifunctional agents. In the following section various ChEIs endowed with additional anti-AD activities are summarized.



(V)

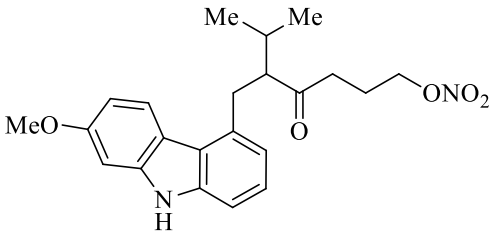
Liu Q. *et. al.* reported novel chromone-2-carboxamido-alkylbenzylamines as multifunctional agents against AD.[31] Most of the compounds exhibited potent inhibitory activity towards AChE and displayed high selectivity for AChE over BuChE. Compound (**V**) showed the most potent inhibition toward AChE with IC_{50} value of 0.07 μM. It showed excellent self-induced

Aβ aggregation inhibitory activity (59.2%) and good Cu^{2+}-induced Aβ aggregation inhibitory activity (48.3%). Moreover, compound (**V**) also endowed with bio-metal chelation property.



(VI)

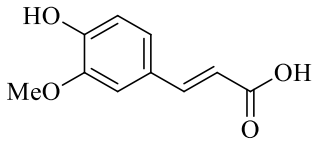
Guoliang L. *et. al.* reported a series of novel tacrine-phenolic acid hybrids as multifactorial anti-AD agents.[32] Compound (**VI**) showed good AChE inhibition (*ee*AChE, IC_{50} value of 3.9 nM; *h*AChE, IC_{50} value of 65.2 nM) in Ellman's assay. It could also effectively block Aβ self-aggregation with percentage inhibition of 47% at 20 μM. The strong anti-oxidation activity of the compound could protect PC12 cells from $CoCl_2$-induced damage in the experimental condition with no neurotoxicity.



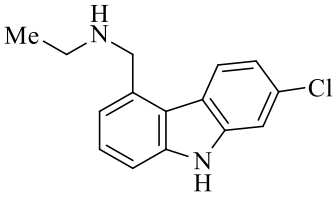
(VII)

Fang. L. *et. al*. reported dibenzofuran/carbazole derivatives which can be considered as the D-ring opened analogs of galantamine, as multifunctional anti-AD agents.[33] *In vitro* enzyme inhibition study revealed that compound (**VII**) having a nitrate moiety in the structure showed a good inhibitory activity for AChE (IC_{50} value of 2.21 μM) and BuChE (IC_{50} value of 2.50 μM). Compound (**VII**) also release a relative low concentration of NO *in vitro* and it did not show toxicity to neuronal cells, while exerted a neuroprotective effect against the Aβ-induced toxicity.

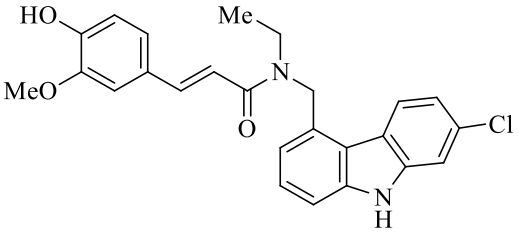
Fang L. *et. al.* designed and synthesized a series of ferulic acid-carbazole hybrid molecules with the aim to gain a synergic action of a ferulic acid moiety as anti-oxidant and carbazole moiety as ChE inhibitor.[34] Most of the synthesized compounds showed moderate to potent ACHE and BuChE inhibitory activities with IC_{50} values ranging from 1.9-88.2 μM in Ellman's assay.



Ferulic acid, VIII

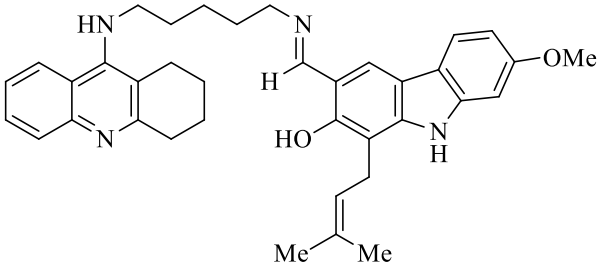


(IX)



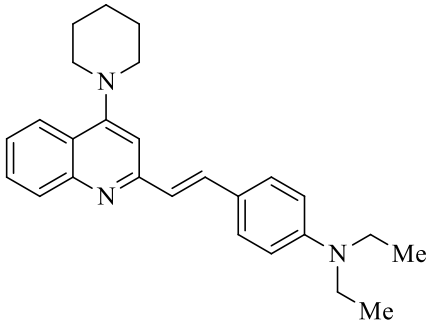
(X)

Among them, compound (**IX**) and (**X**) showed good AChE inhibitory activity (IC_{50} values of 2.1 µM and 1.9 µM, respectively) and BuChE inhibitory activity (IC_{50} values of 1.9 µM and 3.1 µM, respectively) which was even higher than that of galantamine (AChE, IC_{50} = 8.5 µM; BuChE, IC_{50} = 28.1 µM). The hybrid molecule (**X**) showed good anti-oxidant activity with FRSA value of 91.1 ± 8.0% at 100 µM concentration which is similar to that of ferulic acid (**8**) with FRSA value of 92.2 ± 7.0% at 100 µM concentration.



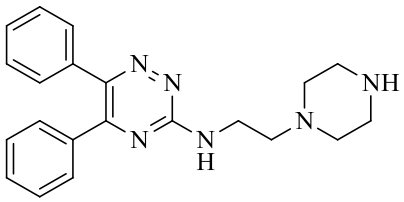
(XI)

Thiratmatrakul S. *et. al.* reported novel tacrine-carbazole hybrids as potential multifunctional anti-AD agents for their cholinesterase inhibitory and radical scavenging activities.[35] Compound (**XI**) exhibited good inhibition of AChE and moderate inhibition of BuChE with IC_{50} values of 0.48 µM and 52.12 µM, respectively. Moreover it also showed potent ABTS radical scavenging activity (IC_{50} value of 8.34 µM). Furthermore, it also reduced oxidative stress and Aβ-induced neuronal cell death.

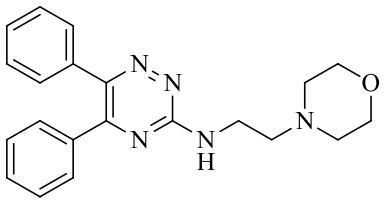


(XII)

Wang X. *et. al.* reported a series of novel 2-arylethenylquinoline derivatives as potential multifunctional agents for the treatment of AD.[36] Amongst them, compound (**XII**) showed potent BuChE and AChE inhibitory activities (IC_{50} values of 0.2 μM and 64.1 μM, respectively). It was also capable of disassembling the self-induced $A\beta_{1\text{-}42}$ aggregation fibrils and had a good metal chelating activity. The anti-oxidative activity of the compound (**XII**) was 3.9-fold higher than that of trolox.



(XIII)



(XIV)

Sinha. A. *et. al.* reported piperazinoethyl and morpholinoethyl substituted triazine derivatives as novel ChEIs. Compound (**XIII**), showed IC_{50} values of 4.23 μM and 13.3 μM for AChE and BuChE respectively, and compound (**XIV**) showed IC_{50} values of 5.79 μM and 163.4 μM for AChE and BuChE respectively.[38]

3. Aims and objectives

To combat the diseases like AD having complex etiology, development of MTDLs is recognized as one of the most assuring drug discovery approaches. In spite of considerable research on new targets available for AD treatment, the cholinesterase inhibitors still remain the drugs of choice, although they provide symptomatic and transient benefits to the patients. Oxidative stress plays an important role in pathogenesis of AD. However antioxidant molecules

all alone might not be enough to treat such highly complex pathologies like AD. So dual cholinesterase inhibitors endowed with additional anti-oxidant and neuroprotective properties could increase the probability of success to combat the AD.

Hence, objective of the study was to design, synthesis and evaluation of some novel hybrid molecules which act on multiple targets of AD.

4. Results and discussion

The work has been divided into two parts:

A. Substituted triazinoindole derivatives as anti-AD agent.

B. Carbazole based stilbene and azahelicene derivatives as anti-AD agents.

A. Substituted triazinoindole derivatives as anti-AD agent.

4.1.1. Designing of triazinoindole derivatives:

Indole ring is prodigiously present in many natural compounds possessing invaluable medicinal and biological properties.[39,40] Melatonin is an indole ring containing pineal neurohormone whose levels decrease during aging, especially in AD patients.[41-43] It is endowed with strong free radical scavenging properties.[44] The high reactivity of melatonin with ROS is apparently due to the electron-rich indole ring, allowing it to act readily as an electron donor. Recent studies have also shown that melatonin offers protective effects against Aβ-induced apoptosis,[45] glutamate-induced excitotoxicity,[46] nitric oxide toxicity,[47] and decreases neurofilament hyperphosphorylation and augments learning and memory in rats. In recent years, many indole based hybrids e.g. tacrine-melatonin hybrids,[48] donepezil-chromone-melatonin hybrids,[49] melatonin-*N*,*N*-dibenzyl(*N*-methyl)amine hybrids,[50] and carbamate derivatives of indolines,[51] have been designed to act as multifunctional agents for the treatment of AD. Furthermore, the indole moiety is present in several CNS-active drugs like rizatriptan, oxypertine etc. Hence, the indole moiety manifests a privileged profile for CNS-active drugs which could prove to be useful in the search for new therapeutics for AD.

1,2,4-Triazine nucleus is another important structural system present in several biologically active compounds.[52] The significance of triazines in neuropharmacology is explored progressively for their potential anti-AD,[53-56] antianxiety,[57] antiepileptic[58] and anti-depressant[59]

activities. Some triazine derivatives have been reported to be potent neuroprotective agents against H_2O_2-induced cell death in PC12 cell line.[60] Recently our research group has also reported substituted diaryltriazines as potential entities for the treatment of AD.[38]

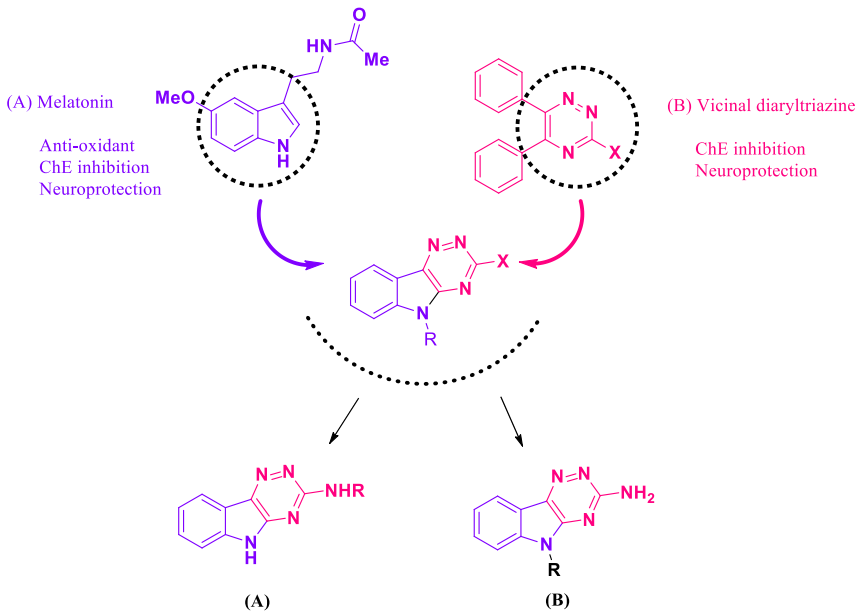


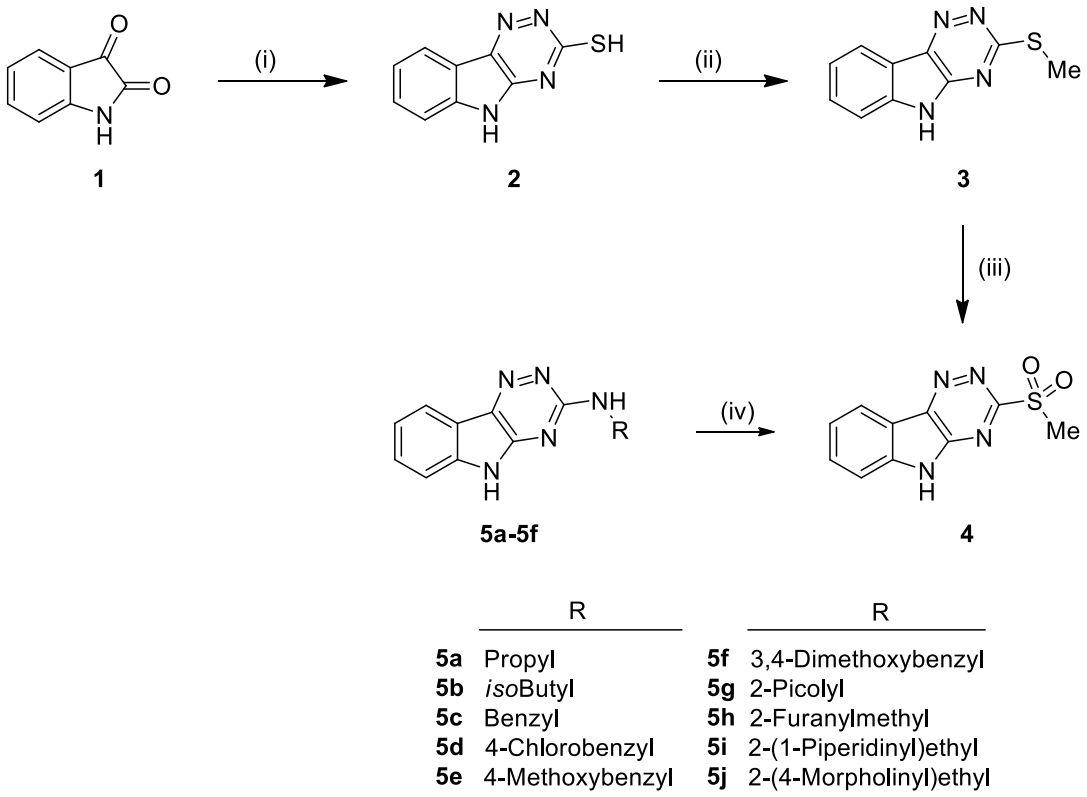
Figure 5: Molecular hybridization approach to design triazinoindole derivatives (**A, B**).

The overabundance of remarkable biological activities associated with the indole ring and the 1,2,4-triazine nucleus prompted us to fuse these two privileged scaffolds into a single scaffold which could offer multiple favorable activities for the treatment of AD. Hence, the objective of the study was to synthesize novel substituted triazinoindole derivatives and to evaluate the synthesized compounds for their potential as anti-AD agents.

4.1.2. Chemical Work

Compounds **5a-5j** were synthesized as depicted in **Scheme 1**. Compound **2** was obtained by condensation of commercially available isatin (**1**) with thiosemicarbazide in aqueous potassium carbonate solution at reflux conditions. The clear liquid obtained was acidified by glacial acetic acid to get the condensed product **2**.

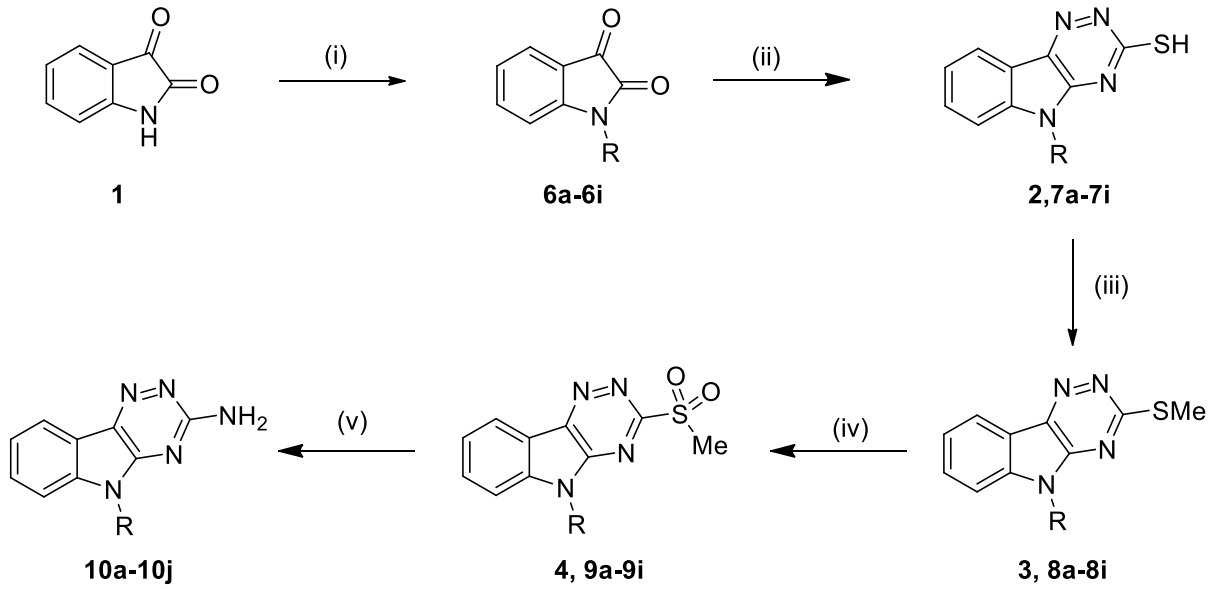
Scheme 1. Synthesis of compounds 5a-5f.



Reagents and conditions. (i) Thiosemicarbazide, K_2CO_3, H_2O, reflux, overnight; (ii) Alkyl/substituted benzyl halides, K_2CO_3, DMF; (iii) MeI, K_2CO_3, DMF; (iv) *m*CPBA, DCM, 0 °C to RT; (v) Amines, THF, reflux.

Methylation of compound **2** with methyl iodide gave thiomethyl derivative **3**. The thiomethyl group was converted to sulfone, as it has more electron withdrawing ability compared to the parent thiomethyl group. This was achieved by oxidizing the thiomethyl group by *m*CPBA to sulfone. This sulfone derivative **4** was reacted with various amines in THF at refluxing conditions, the sulfone group was substituted by the amines offering the desired substituted [1,2,4]triazino[5,6-*b*]indol-3-amine derivatives **5a**-**5j**.

Scheme 2. Synthesis of compounds 2, 3a-3l and 5a-5k.

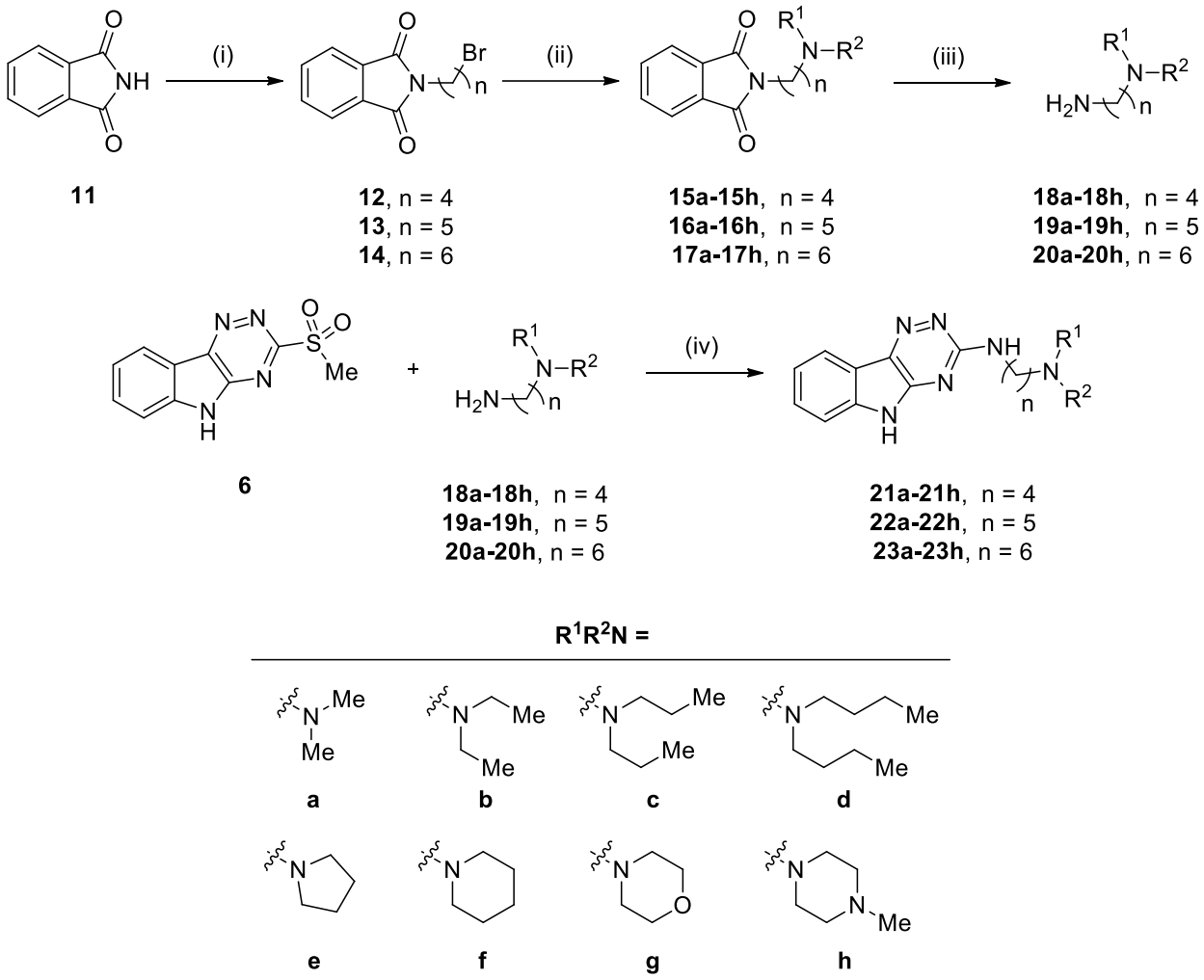


			R				R
8a	**9a**	**10a**	Methyl	**8f**	**9f**	**10f**	2-Methylbenzyl
8b	**9b**	**10b**	Ethyl	**8g**	**9g**	**10g**	4-Methylbenzyl
8c	**9c**	**10c**	Propyl	**8h**	**9h**	**10h**	4-Chlorobenzyl
8d	**9d**	**10d**	Butyl	**8i**	**9i**	**10i**	3-Fluorobenzyl
8e	**9e**	**10e**	Benzyl	**3**	**4**	**10j**	H

Reagents and conditions. (i) Alkyl/substituted benzyl halides, K_2CO_3, DMF; (ii) Thiosemicarbazide, K_2CO_3, H_2O, reflux, overnight; (iii) MeI, K_2CO_3, DMF; (iv) *m*CPBA, DCM, 0 °C to RT; (v) Ammonia, THF, reflux.

Compounds **5a-5j** were synthesized as depicted in **Scheme 1**. *N*-Alkylation or *N*-benzylation of the isatin was achieved with the corresponding alkyl/benzyl halides in the presence of potassium carbonate in DMF to obtain compound **6a-6i**. These substituted isatins were reacted with thiosemicarbazide as previously discussed to obtain compound **7a-7i.** Similarly, compounds **7a-7i** were methylated and further oxidized to the corresponding sulfone compounds **9a-9j**. These sulfone compounds were reacted with ammonia to get the desired 5-substituted[1,2,4]triazino[5,6-*b*]indol-3-amine derivatives **10a-10j** (**Scheme 2**).

Scheme 3. Synthesis of compounds 21a-23h.



Reagents and conditions. (i) $Br(CH_2)_nBr$, K_2CO_3, TEBAC, acetone, RT; (ii) HNR^1R^2, TEA, MeOH, reflux; (iii) $NH_2NH_2.H_2O$, MeOH, reflux; (iv) THF, reflux.

The aminoalkylamines **18a-20h** required for the synthesis of compounds **21a-23h** were prepared through Gabriel synthesis using phthalimide as the starting material. Phthalimide (**11**) was reacted with dibromoalkanes to form *N*-(bromoalkyl)phthalimides **12-14**. The desired basic amines (**a-h**; R^1R^2NH) were reacted with *N*-(bromoalkyl)phthalimides **12-14** to give compounds **15a-17h**. Hydrazinolysis of compounds **15a-17h** in ethanol gave the desired aminoalkylamines

18a-20h in 67–87 % yields. These aminoalkylamines **18a-20h** were reacted with compound 6 as discussed previously to obtain the desired final products **21a-23h** (**Scheme 3).**

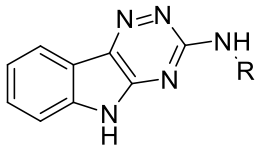
All the synthesised compounds were characterised by IR, ^{1}H-NMR, ^{13}C-NMR and MS. The detailed data will be provided in the thesis.

4.1.3. Biological Evaluation

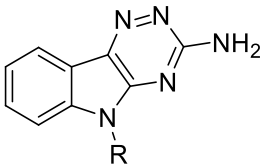
4.1.3.1. Inhibition studies on AChE and BuChE

The potential of the synthesized compounds to inhibit cholinesterases (ChEs) was evaluated in vitro using a spectrophotometric method of Ellman et al. using donepezil and tacrine as reference drugs as previously reported by our group.[59,61,62] The obtained IC_{50} values of the compounds and their selectivity for AChE over BuChE were summarized in **Table 1, 2**.

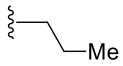
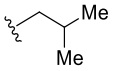
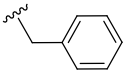
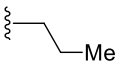
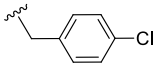
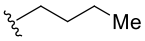
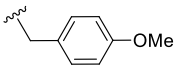
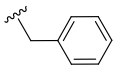
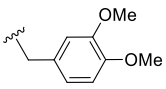
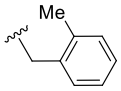
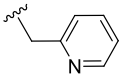
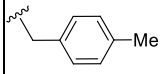
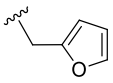
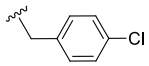
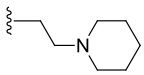
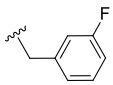
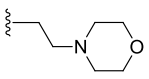
Table 1. *In vitro* Inhibition of AChE, BuChE and Selectivity Index of compounds 5a-5j and 10a-10j.



(5a-5j)



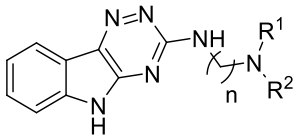
(10a-10j)

Compd	R	IC_{50}± SEM (μM)		SI	Compd	R	IC_{50}± SEM (μM)		SI
		AChE[a]	BuChE[b]				AChE[a]	BuChE[b]	
5a		11.07 ± 0.86	52.19 ± 2.01	4.71	**10a**		7.81 ± 1.01	52.23 ± 1.76	6.69
5b		9.21 ± 0.54	61.23 ± 1.12	6.64	**10b**		8.13 ± 1.32	29.42 ± 1.21	3.62
5c		8.06 ± 0.75	56.41 ± 1.65	6.99	**10c**		10.12 ± 1.16	32.78 ± 1.47	3.24
5d		41.22 ± 1.83	9.09 ± 0.65	0.22	**10d**		9.26 ± 0.82	16.02 ± 1.10	1.73
5e		36.63 ± 1.22	67.32 ± 1.32	1.84	**10e**		20.57 ± 1.34	24.87 ± 1.38	1.21
5f		10.75 ± 0.62	13.12 ± 1.02	1.22	**10f**		17.23 ± 1.22	32.72 ± 1.32	1.90
5g		14.71 ± 1.37	26.08 ± 1.21	1.77	**10g**		22.09 ± 1.78	22.64 ± 1.49	1.02
5h		20.22 ± 1.21	33.02 ± 1.65	1.63	**10h**		24.63 ± 2.01	29.45 ± 1.62	1.20
5i		6.16 ± 0.53	20.53 ± 2.01	3.33	**10i**		20.22 ± 1.25	25.32 ± 1.23	1.25
5j		6.61 ± 0.69	9.14 ± 0.54	1.38	**10j**		9.76 ± 1.25	51.25 ± 1.32	5.25

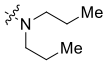
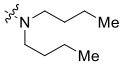
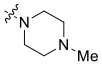
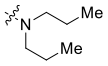
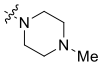
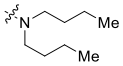
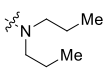
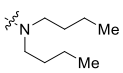
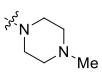
[a] AChE from human erythrocytes; IC_{50}, 50% inhibitory concentration (means ± SEM of three experiments).

[b] BuChE from equine serum.
[c] Selectivity Index = IC_{50} (BuChE)/IC_{50} (AChE).

Table 2. *In vitro* Inhibition of AChE, BuChE and Selectivity Index of compounds 21a-23h.



(21a-23h)

Compd	n	R^1R^2N	IC_{50}± SEM (μM)		SI	Compd	n	R^1R^2N	IC_{50}± SEM (μM)		SI
			AChE[a]	BuChE[b]					AChE[a]	BuChE[b]	
21a	4		33.70 ± 1.12	6.39 ± 0.22	0.19	**22e**	5		0.85 ± 0.05	17.01 ± 0.40	20.0
21b	4		6.31 ± 0.43	11.09 ± 0.27	1.75	**22f**	5		0.96 ± 0.04	2.77 ± 0.05	2.89
21c	4		2.47 ± 0.11	8.05 ± 0.51	3.26	**22g**	5		18.58 ± 0.41	6.74 ± 0.21	0.36
21d	4		20.70 ± 0.76	0.47 ± 0.03	0.02	**22h**	5		1.65 ± 0.11	29.19 ± 1.10	17.7
21e	4		2.69 ± 0.08	5.89 ± 0.70	2.19	**23a**	6		1.25 ± 0.09	4.43 ± 0.27	3.54
21f	4		3.17 ± 0.10	18.01± 1.31	5.68	**23b**	6		2.40 ± 0.11	12.70 ± 0.42	5.29
21g	4		15.01 ± 0.54	1.92 ± 0.15	0.13	**23c**	6		1.48 ± 0.08	3.43 ± 0.24	2.32
21h	4		2.61 ± 0.07	32.19 ± 1.02	12.3	**23d**	6		1.43 ± 0.07	4.01 ± 0.32	2.80
22a	4		3.58 ± 0.28	23.24 ± 0.67	6.49	**23e**	**6**		**0.56 ± 0.02**	**1.17 ± 0.09**	**2.09**
22b	4		6.79 ± 0.22	0.34 ± 0.03	0.05	**23f**	6		0.67 ± 0.02	0.84 ± 0.03	1.25
22c	4		2.77 ± 0.07	0.48 ± 0.03	0.17	**23g**	6		4.16 ± 0.15	23.65 ± 1.38	5.69
22d	4		2.76 ± 0.18	0.38 ± 0.05	0.14	**23h**	6		0.79 ± 0.04	3.92 ± 0.21	4.96

[a] AChE from human erythrocytes; IC_{50}, 50% inhibitory concentration (means ± SEM of three experiments).
[b] BuChE from equine serum.
[c] Selectivity Index = IC_{50} (BuChE)/IC_{50} (AChE).

All the tested compounds showed IC_{50} values for both the enzymes in micromolar to submicromolar ranges. Compounds **23e** and **23f** showed the highest inhibition of AChE (IC_{50} values of 0.56 µM and 0.67 µM, respectively) and BuChE (IC_{50} values of 1.17µM and 0.84 µM, respectively).

4.1.3.2. Antioxidant activity

The DPPH radical scavenging assay is commonly used as a rapid and reliable method to assess the antioxidant/free radical scavenging potential of compounds.[64] DPPH is a stable free radical that can accept a hydrogen radical or an electron to become a stable molecule. The antioxidant activity of the selected compounds was estimated by their ability to reduce DPPH· radical (purple color) to DPPHH (yellow) and the corresponding radical-scavenging potential was evaluated by the decrease in the absorbance at 517 nm.[65]

Table 3. DPPH Radical Scavenging Activity of the Selected Compounds.[a]

Compd	RP of DPPH (%)[b]		Compd	RP of DPPH (%)[b]	
	10 µM	20 µM		10 µM	20 µM
21c	45.5 ± 3.1	57.3 ± 2.9	**23b**	43.7 ± 2.5	60.3 ± 3.4
21e	52.1 ± 2.4	63.1 ± 2.3	**23c**	42.7 ± 3.7	58.4 ± 3.1
21f	51.5 ± 1.6	62.7 ± 1.7	**23d**	47.3 ± 2.0	59.6 ± 2.2
21h	53.7 ± 2.7	65.4 ± 1.3	**23e**	**54.9 ± 1.8**	**64.3 ± 2.8**
22a	43.2 ± 3.3	60.2 ± 2.7	**23f**	54.3 ± 2.1	66.4 ± 2.4
22c	44.3 ± 2.4	59.4 ± 3.1	**23g**	56.7 ± 1.6	67.3 ± 1.7
22d	46.7 ± 1.9	60.7 ± 3.3	**23h**	55.1 ± 3.4	64.2 ± 1.9
22e	53.2 ± 2.4	62.4 ± 2.5	**Tacrine**	44.6 ± 2.1	68.9 ± 3.7
22f	52.8 ± 2.9	60.7 ± 2.9	**Done.**	50.0 ± 2.8	70.2 ± 2.4
22h	54.1 ± 1.7	65.1 ± 2.1	**Ascorbic acid**	36.5 ± 2.9	61.8 ± 3.2

23a	44.1 ± 3.4	61.3 ± 2.3		

[a]Data are expressed as Mean ± SE (three independent experiments)
[b]RP of DPPH (%) = reduction percentage of DPPH.

Ascorbic acid was used as the positive control in this assay. All the test compounds exhibited notable free radical scavenging activity ranging from 40−55 % and 56-70 % at 10 μM and 20 μM concentrations respectively (**Table 3**).

The results of the biological activity of the most active compound **23e** in animal models will be discussed in detail in the thesis.

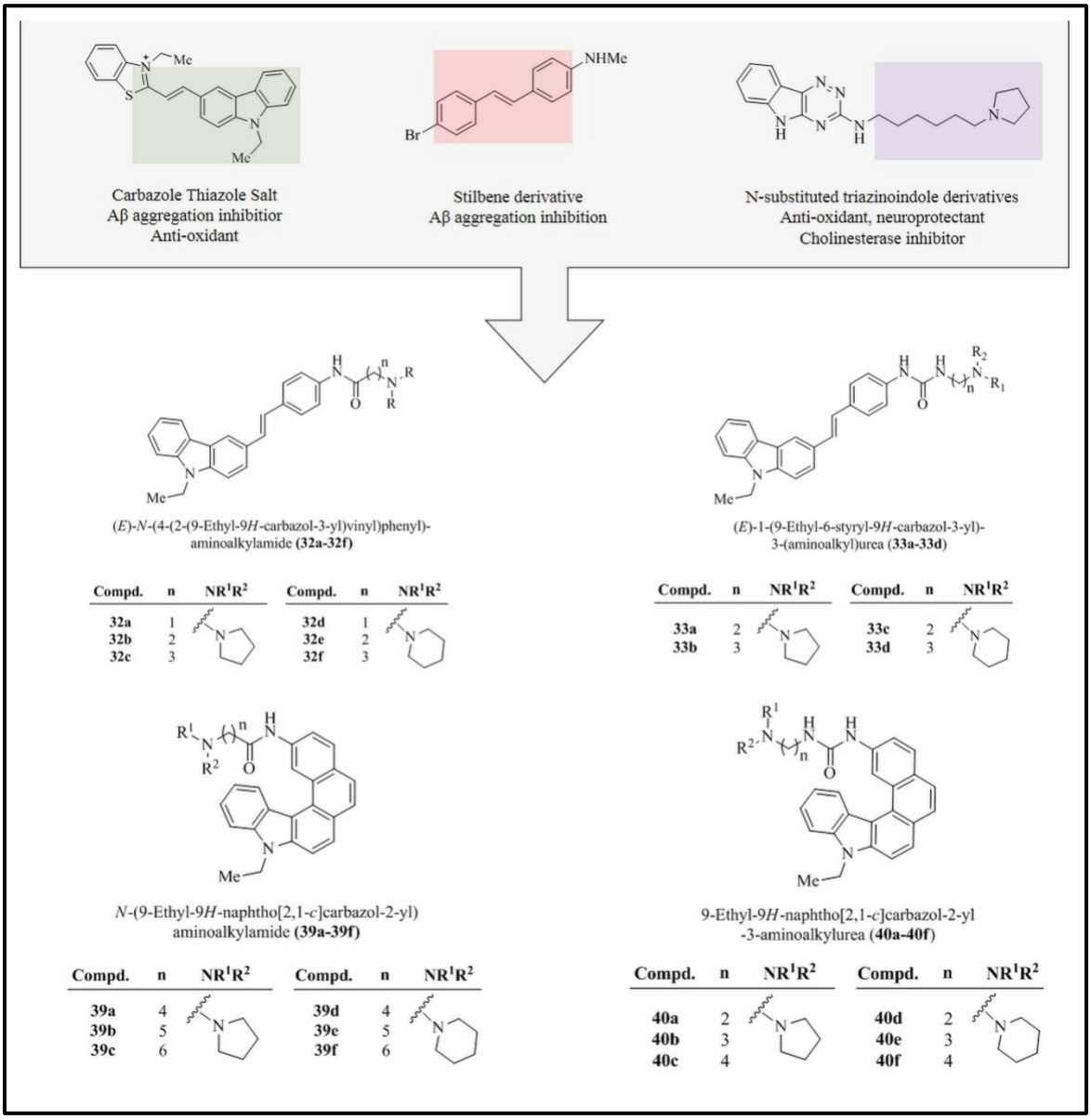
4.2. Carbazole based stilbene and azahelicene derivatives as anti-AD agents

4.2.1 Designing of carbazole based stilbene and azahelicene derivatives as anti-AD agents

Carbazole, naturally occurring phytochemical, is widely present in many plant species and possess a wide range of biological activities associated with AD. It has been reported that naturally occurring carbazole derivatives are able to directly scavenge a variety of reactive oxygen species and possess strong antioxidant actions[34]. Moreover, a recent study has also shown that carbazole derivatives have the capacity of inhibiting Aβ aggregation[35]. As the cholinesterase and oxidative stress are important targets for the treatment of AD, some research group reported various hybrids of carbazole as cholinesterase inhibitor with additional activities for treatment of AD (**Figure 4.1**).

Stilbene derivatives showed a broad range of biological responses[36,37] such as anti-leukemic, anti-bacterial, anti-fungal, antiplatelet aggregation, coronary vasodilator activities and anti-cancer activities. Among them, SB-13 is one of the promising lead compounds for Aβ plaque detection, however, its application has been limited in positron emission tomography (PET) imaging due to its strong binding affinity to amyloid aggregates and low fluorescence responses (**Figure 4.1**).

The plethora of remarkable biological activities associated with the carbazole ring and stilbene nucleus prompt us to fuse these two active scaffolds into the single entity and attaching various heterocyclic amines to get series of compounds with multiple beneficial activities for treatment of AD.



Compd.	n	NR^1R^2	Compd.	n	NR^1R^2
32a	1		32d	1	
32b	2		32e	2	
32c	3		32f	3	

Compd.	n	NR^1R^2	Compd.	n	NR^1R^2
33a	2		33c	2	
33b	3		33d	3	

Compd.	n	NR^1R^2	Compd.	n	NR^1R^2
39a	4		39d	4	
39b	5		39e	5	
39c	6		39f	6	

Compd.	n	NR^1R^2	Compd.	n	NR^1R^2
40a	2		40d	2	
40b	3		40e	3	
40c	4		40f	4	

Figure 4.2. Designing of carbazole based stilbene and azahelicene derivatives as anti-AD agents.

4.2.2 Synthesis and characterization of the designed stilbene derivatives (32a-32f, 33a-33d)

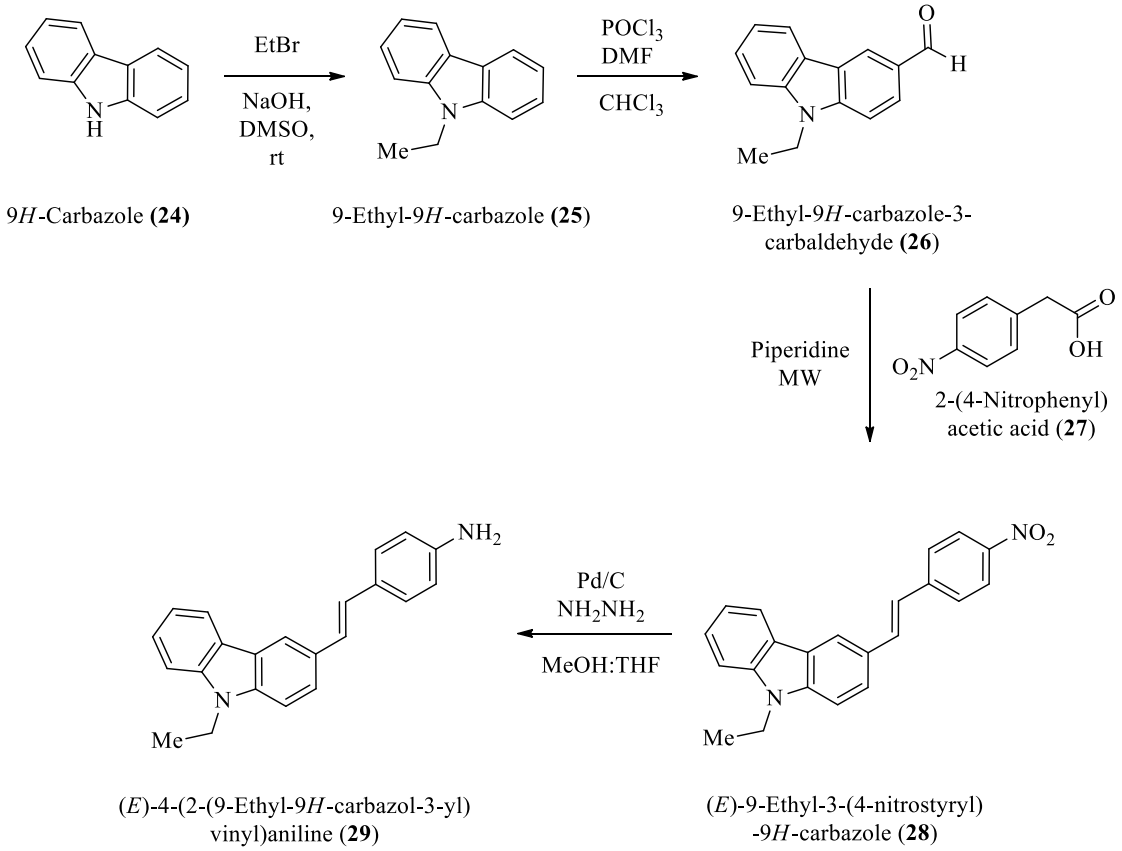
The designed compounds were synthesized according to following general **Schemes (4-6)** and characterized by IR, NMR and MS.

Commercially available carbazole **(24)** was reacted with ethyl bromide in the presence of aqueous NaOH solution in DMSO as a solvent to give 9-ethyl-9*H*-carbazole (**25**). Formylation of

the compound (**25**) by phosphorus oxychloride and *N*, *N*-dimethyl formamide gave mono-formylated product 9-ethyl-9*H*-carbazole-3-carbaldehyde (**26**).

A mixture of 2-(4-nitrophenyl)acetic acid (**27**) and 9-ethyl-9*H*-carbazole-3-carbaldehyde (**26**) was irradiated under microwave at 800 W in a microwave reactor in presence of piperidine to give (*E*)-9-ethyl-3-(4-nitrostyryl)-9*H*-carbazole (**28**). Reduction of the nitro group in **28** to amine was carried out by tin chloride in methanol and tetrahydrofuran at refluxed condition to give desired (*E*)-4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)aniline (**29**) (**Scheme 4**).

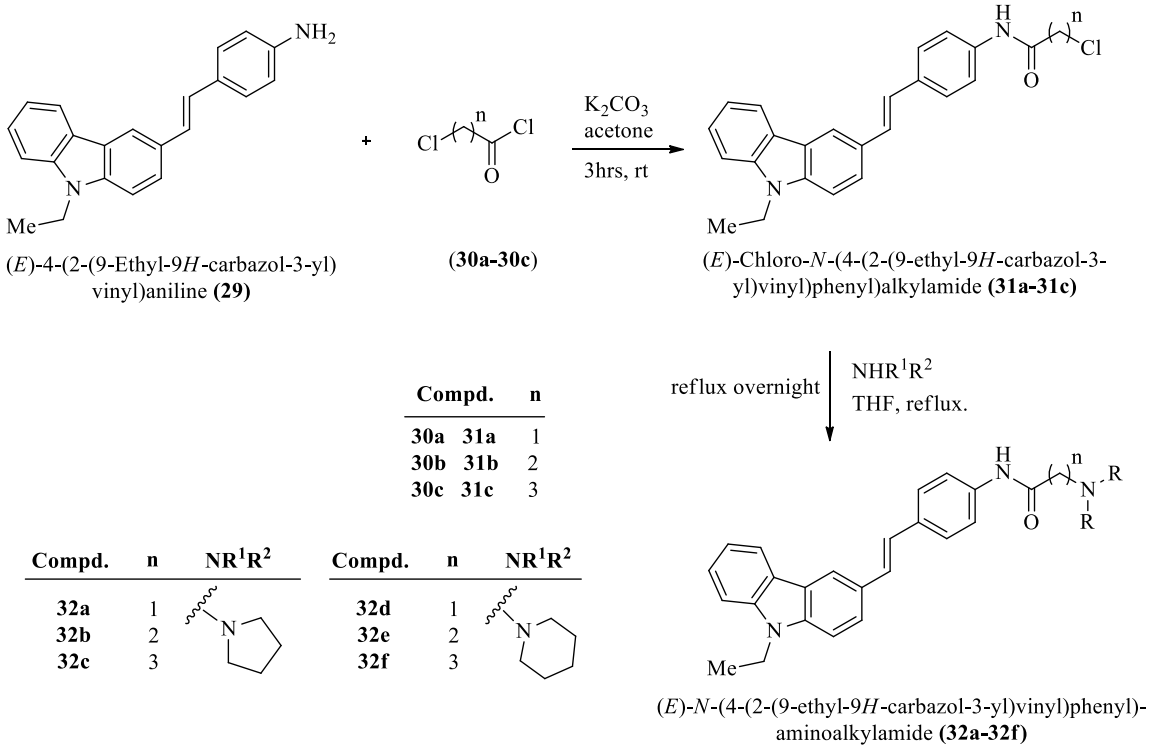
Scheme 4. Synthesis of (*E*)-4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)aniline (29)



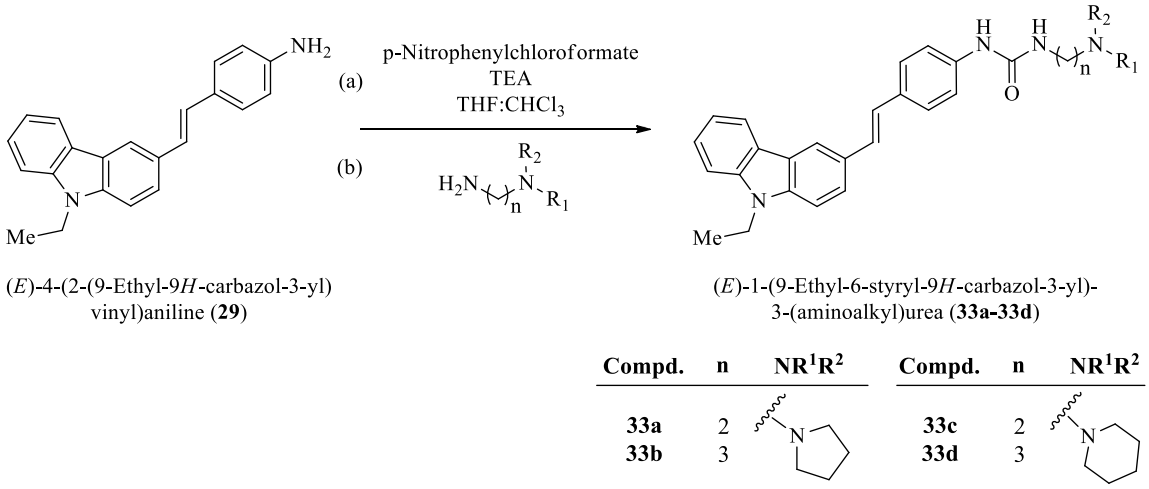
Synthesis of designed (*E*)-*N*-(4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)aminoalkyl amide **(32a-32f)** from the amine derivative **(29)** was carried out as shown in **Scheme 5.** The **(***E*)-4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)aniline (**29**) was reacted with various acid chlorides (**30a-30c**) in the presence potassium carbonate in acetone to give compound (**31a-31c**). Reaction of

these compounds (**31a-31c**) with excess of heterocyclic amines (pyrrolidine and piperidine) in the tetrahydrofuran as a solvent at refluxed condition gave final compounds (**32a-32f**).

Scheme 5. Synthesis of (*E*)-*N*-(4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)aminoalkylamide (32a-32f)



Scheme 6. Synthesis of (*E*)-1-(9-ethyl-6-styryl-9*H*-carbazol-3-yl)-3-(aminoalkyl)urea (33a-33d)

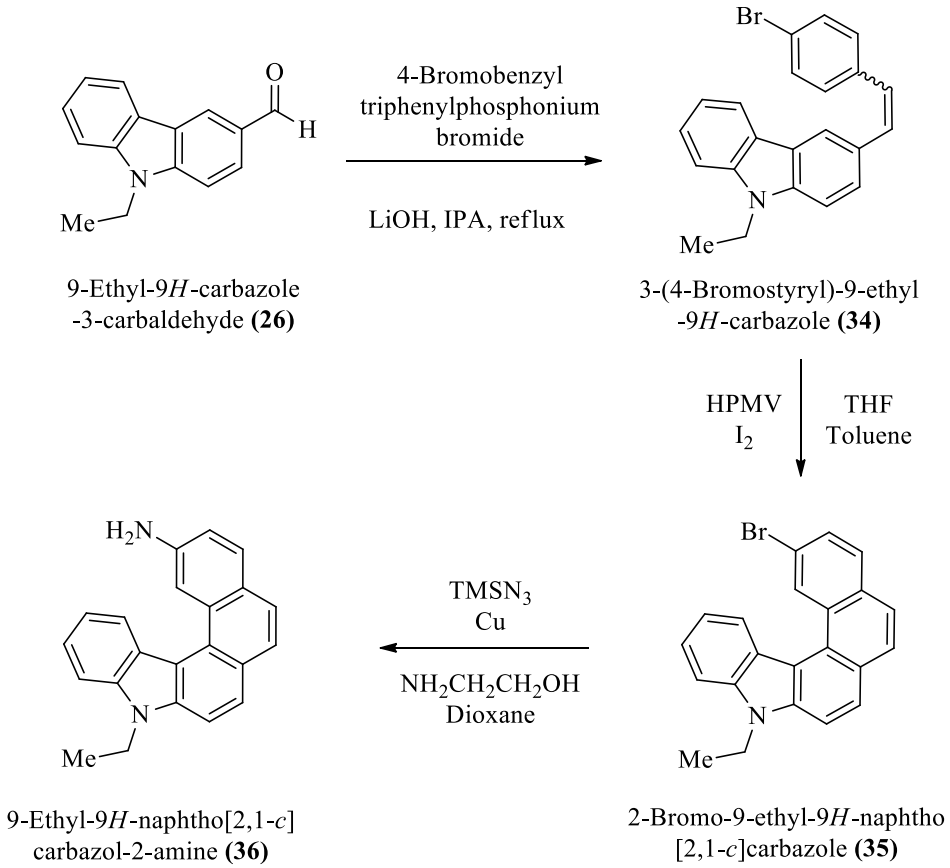


Synthesis of designed (*E*)-1-(9-ethyl-6-styryl-9*H*-carbazol-3-yl)-3-(aminoalkyl)urea **(33a-33d)** from the amine derivative **(29)** was carried out as shown in **Scheme 6.** The (*E*)-4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)aniline **(29)** was reacted with *p*-nitrophenylchloroformate in the presence of triethylamine in tetrahydrofuran. Later, excess of various aminoalkylamines were added to the above mixture. After the completion of the reaction, solvent was recovered and methanol was added to precipitate the componunds (*E*)-1-(9-ethyl-6-styryl-9*H*-carbazol-3-yl)-3-(aminoalkyl) urea **(33a-33d).**

4.2.2. Synthesis of carbazole based azahelicene derivatives

The designed carbazole based azahelicenes were synthesized as depicted in **Scheme (7-9)**. Wittig reaction of aldehyde **26** with 4-bromobenzyltriphenylphosphonium bromide in presence of lithium hydroxide in IPA gave cis and trans isomers of stilbene derivative **(34)**.

Scheme 7. Synthesis of 9-ethyl-9*H*-naphtho[2,1-*c*]carbazole-2-amine (36)



9-Ethyl-9*H*-carbazole -3-carbaldehyde **(26)**

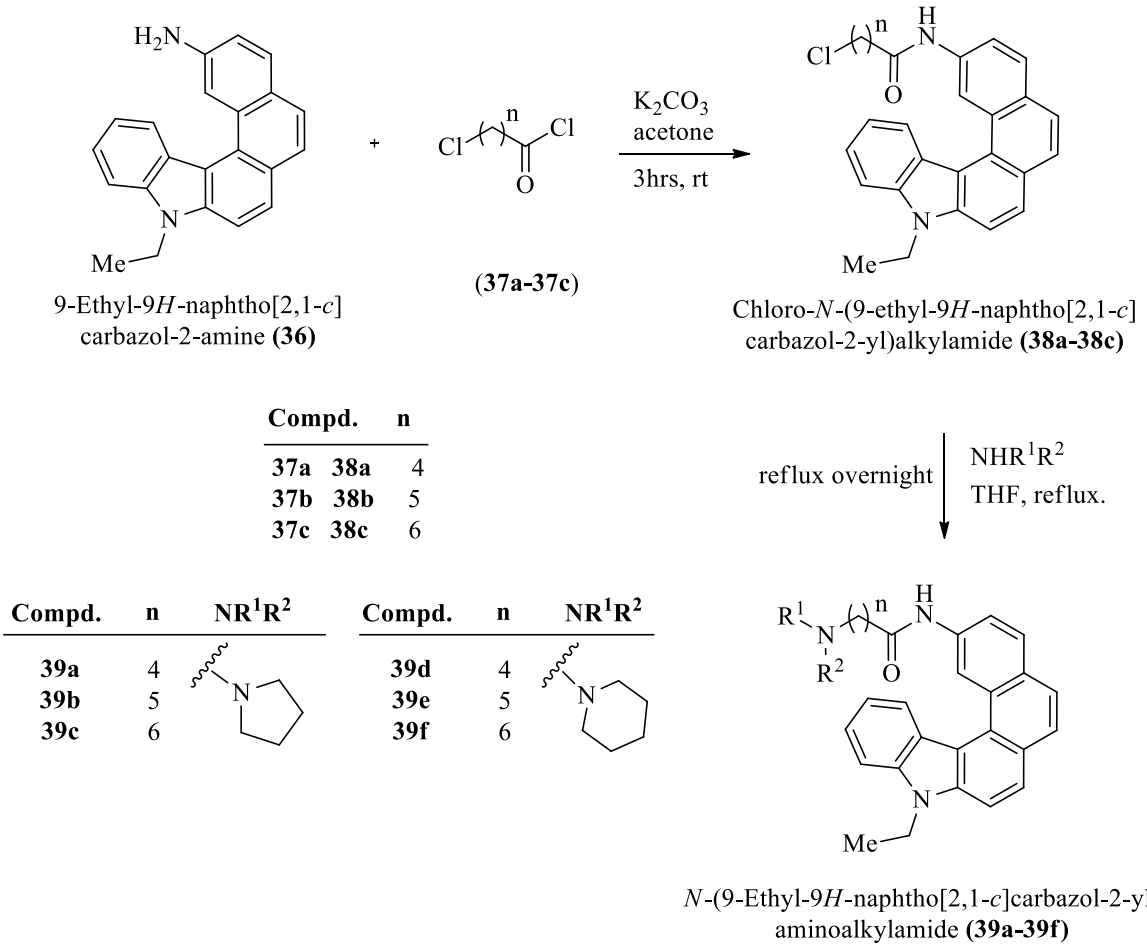
3-(4-Bromostyryl)-9-ethyl -9*H*-carbazole **(34)**

9-Ethyl-9*H*-naphtho[2,1-*c*] carbazol-2-amine **(36)**

2-Bromo-9-ethyl-9*H*-naphtho [2,1-*c*]carbazole **(35)**

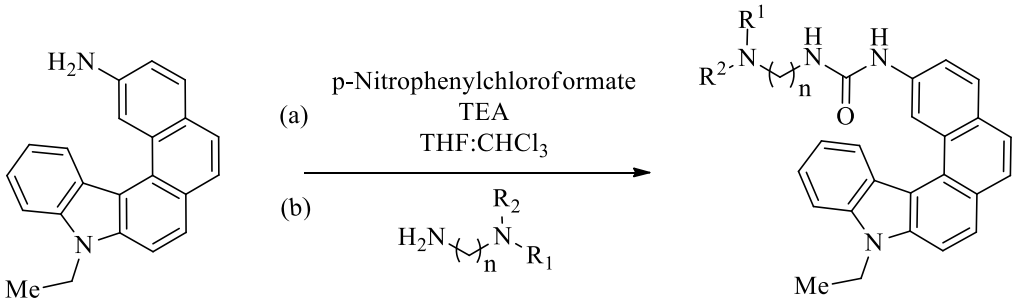
Photodehydrocyclization of these isomers by high pressure mercury lamp in presence of iodine and THF in toluene gave the cyclised compound **(35)**. Bromo group in the compound **(35)** was substituted with amino group by reaction of bromo derivative with trimethylsilyl azide in presence of copper and aminoethanol to give 9-ethyl-9*H*-naphtho[2,1-*c*]carbazole-2-amine **(36) (Scheme 7).**

Scheme 8. Synthesis of *N*-(9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)aminoalkylamide (39a-39f)



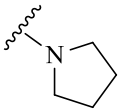
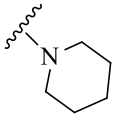
Synthesis of designed *N*-(9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl)aminoalkylamide **(39a-39f)** from the amine derivative **(36)** was carried out as shown in **Scheme 8.** The 9-ethyl-9*H*-naphtho[2,1-c]carbazol-2-amine **(36)** was reacted with various acid chlorides (**37a-37c**) in the presence potassium carbonate in acetone to give compound (**38a-38c**). Reaction of these compounds (**38a-38c**) with excess of heterocyclic amines (pyrrolidine and piperidine) in THF as a solvent at refluxed condition gave final compounds (**39a-39f**).

Scheme 9. Synthesis of 9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl-3-aminoalkylurea (40a-40f)



9-Ethyl-9*H*-naphtho[2,1-*c*] carbazol-2-amine **(36)**

9-Ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl -3-aminoalkylurea (**40a-40f**)

Compd.	n	NR^1R^2	Compd.	n	NR^1R^2
40a	2		**40d**	2	
40b	3		**40e**	3	
40c	4		**40f**	4	

Synthesis of designed 9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl-3-aminoalkylurea **(40a-40f)** from the amine derivative **(36)** was carried out as shown in **Scheme 9.** The 9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-amine **(36)** was reacted with *p*-nitrophenylchloroformate in the presence of triethylamine in tetrahydrofuran. Later excess of various aminoalkylamines were added to the above mixture. After the completion of the reaction, solvent was recovered and methanol was added to precipitate the componunds 9-ethyl-9*H*-naphtho[2,1-*c*]carbazol-2-yl-3-aminoalkylurea **(40a-40f).**

The detailed characterisation data and biological results will be provided in the thesis.

Publications:

1. **Novel Multi-Target Directed Triazinoindole Derivatives as Anti-Alzheimer Agents.**
   Dushyant V. Patel, Nirav R. Patel, Ashish M. Kanhed, Sagar P. Patel, Anshuman Sinha, Deep D. Kansara, Annie R. Mecwan, Sarvangee B. Patel, Pragnesh N. Upadhyay, Kishan B. Patel, Dharti B. Shah, Navnit K. Prajapati, Prashant R. Murumkar, Kirti V. Patel, Mange Ram Yadav*
   Submitted to *ACS Chemical Neuroscience* (Revision requested).

2. **2-Aminobenzamide-Based Factor Xa Inhibitors with Novel Mono- and Bi-Aryls as S4 Binding Elements.**
   Nirav R. Patel, Dushyant V. Patel, Ashish M. Kanhed, Sagar P. Patel, Kirti V. Patel, Daniel K. Afosah, Umesh R. Desai, Rajshekhar Karpoormath, Mange Ram Yadav*. *ChemistrySelect* 4.3 (2019): 802-809.

3. **Contemporary Developments in the Discovery of Selective Factor Xa Inhibitors: A Review**.
   Nirav Patel, Dushyant Patel, Prashant Murumkar, Mange ram Yadav*. *Eur. J. Med. Chem.* 2016 Oct 4; 121, 671-98.

References

(1) Scheltens, P.; Blennow, K.; Breteler, M. M. B.; de Strooper, B.; Frisoni, G. B.; Salloway, S.; Van der Flier, W. M. Alzheimer's disease. *The Lancet* **2016,** *388*, 505-517.

(2) Patterson, C. World Alzheimer Report 2018. *The State of the Art of Dementia Research: New Frontiers* **2018**, pp. 32-36.

(3) Talesa, V. N. Acetylcholinesterase in Alzheimer's disease. *Mech. Ageing Dev.* **2001,** *122*, 1961-1969.

(4) Selkoe, D. J. Folding proteins in fatal ways. *Nature* **2003,** *426*, 900.

(5) Hardy, J. A.; Higgins, G. A. Alzheimer's disease: the amyloid cascade hypothesis. *Science* **1992,** *256*, 184.

(6) Maccioni, R. B.; Farías, G.; Morales, I.; Navarrete, L. The revitalized tau hypothesis on Alzheimer's disease. *Arch. Med. Res.* **2010,** *41*, 226-231.

(7) Chu, D.; Liu, F. Pathological changes of tau related to Alzheimer's Disease. *ACS Chem. Neurosci.* **2018,** *10*, 931-944.

(8) Greenough, M. A.; Camakaris, J.; Bush, A. I. Metal dyshomeostasis and oxidative stress in Alzheimer's disease. *Neurochem. Int.* **2013,** *62*, 540-555.

(9) Bonda, D. J.; Wang, X.; Perry, G.; Nunomura, A.; Tabaton, M.; Zhu, X.; Smith, M. A. Oxidative stress in Alzheimer disease: a possibility for prevention. *Neuropharmacology* **2010,** *59*, 290-294.

(10) Maruszak, A.; Żekanowski, C. Mitochondrial dysfunction and Alzheimer's disease. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2011,** *35*, 320-330.

(11) Heneka, M. T.; Carson, M. J.; El Khoury, J.; Landreth, G. E.; Brosseron, F.; Feinstein, D. L.; Jacobs, A. H.; Wyss-Coray, T.; Vitorica, J.; Ransohoff, R. M. Neuroinflammation in Alzheimer's disease. *Lancet Neurol* **2015,** *14*, 388-405.

(12) Fu, W. Y.; Wang, X.; Ip, N. Y., Targeting neuroinflammation as a therapeutic strategy for alzheimer's disease: mechanisms, drug candidates, and new opportunities. *ACS Chem. Neurosci.* **2019**. *10*, 872-879.

(13) Del Pino, J.; Marco-Contelles, J.; Lopez-Munoz, F.; Romero, A.; Ramos, E. Neuroinflammation signaling modulated by Ass234, a multitarget small molecule for Alzheimer's disease therapy. *ACS Chem. Neurosci.* **2018,** *9*, 2880-2885.

(14) Greig, N. H.; Lahiri, D. K.; Sambamurti, K. Butyrylcholinesterase: an important new target in Alzheimer's disease therapy. *Int Psychogeriatr* **2002,** *14*, 77-91.

(15) Jing, L.; Wu, G.; Kang, D.; Zhou, Z.; Song, Y.; Liu, X.; Zhan, P. Contemporary medicinal-chemistry strategies for the discovery of selective butyrylcholinesterase inhibitors. *Drug Discov. Today.* **2019,** *24*, 629-635.

(16) Sussman, J. L.; Harel, M.; Frolow, F.; Oefner, C.; Goldman, A.; Toker, L.; Silman, I. Atomic structure of acetylcholinesterase from Torpedo californica: a prototypic acetylcholine-binding protein. *Science* **1991,** *253*, 872-879.

(17) De Ferrari, G. V.; Canales, M. A.; Shin, I.; Weiner, L. M.; Silman, I.; Inestrosa, N. C. A structural motif of acetylcholinesterase that promotes amyloid β-peptide fibril formation. *Biochemistry* **2001,** *40*, 10447-10457.

(18) Dinamarca, M. C.; Sagal, J. P.; Quintanilla, R. A.; Godoy, J. A.; Arrázola, M. S.; Inestrosa, N. C. Amyloid-β-Acetylcholinesterase complexes potentiate neurodegenerative changes induced by the Aβ peptide. Implications for the pathogenesis of Alzheimer's disease. *Mol. Neurodegener.* **2010,** *5*, 4.

(19) Wang, Y.; Wang, H.; Chen, H. AChE inhibition-based multi-target-directed ligands, a novel pharmacological approach for the symptomatic and disease-modifying therapy of Alzheimer's disease. *Curr. Neuropharmacol.* **2016,** *14*, 364-375.

(20) Hartmann, J.; Kiewert, C.; Duysen, E. G.; Lockridge, O.; Greig, N. H.; Klein, J. Excessive hippocampal acetylcholine levels in acetylcholinesterase-deficient mice are moderated by butyrylcholinesterase activity. *J. Neurochem.* **2007,** *100*, 1421-1429.

(21) Arendt, T.; Bigl, V.; Walther, F.; Sonntag, M. Decreased ratio of CSF acetylcholinesterase to butyrylcholinesterase activity in Alzheimer's disease. *The Lancet* **1984,** *323*, 173.

(22) Kosak, U.; Brus, B.; Knez, D.; Zakelj, S.; Trontelj, J.; Pislar, A.; Sink, R.; Jukic, M.; Zivin, M.; Podkowa, A. The magic of crystal structure-based inhibitor optimization: development of a butyrylcholinesterase inhibitor with picomolar affinity and in vivo activity. *J. Med. Chem.* **2017,** *61*, 119-139.

(23) Geula, C.; Mesulam, M. Cholinesterases and the pathology of Alzheimer disease. *Alzheimer Dis. Assoc. Disord.* **1995**.

(24) Guillozet, A.; Mesulam, M. M.; Smiley, J.; Mash, D. Butyrylcholinesterase in the life cycle of amyloid plaques. *Ann. Neurol.* **1997,** *42*, 909-918.

(25) Jakob-Roetne, R.; Jacobsen, H. Alzheimer's disease: from pathology to therapeutic approaches. *Angew. Chem. Int. Ed.* **2009,** *48*, 3030-3059.

(26) Uttara, B.; Singh, A. V.; Zamboni, P.; Mahajan, R. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Curr. Neuropharmacol.* **2009,** *7*, 65-74.

(27) Zhao, Y.; Zhao, B. Oxidative stress and the pathogenesis of Alzheimer's disease. *Oxid Med Cell Longev* **2013,** *2013*.

(28) Marksteiner, J.; Schmidt, R., Treatment strategies in Alzheimer's disease with a focus on early pharmacological interventions. *Drug & aging*. **2004**, 21 (7), 415- 426.

(29) Meunier, J.; Ieni, J.; Maurice, T., The anti-amnesic and neuroprotective effects of donepezil against amyloid β_{25-35} peptide-induced toxicity in mice involve an interaction with the σ1receptor. *Br. J. Pharmacol*. **2006**, 149 (8), 998-1012.

(30) Wilkinson, D. G.; Francis, P. T.; Schwam, E.; Payne-Parrish, J., Cholinesterase inhibitors used in the treatment of Alzheimer's disease. *Drug & aging*. **2004**, 21 (7), 453-478.

(31) Liu, Q.; Qiang, X.; Li, Y.; Sang, Z.; Li, Y.; Tan, Z.; Deng, Y.; Design, synthesis and evaluation of chromone-2-carboxamido-alkylbenzylamines as multifunctional agents for the treatment of Alzheimer's disease. *Bioorg. Med. Chem*. **2015** 1; 23(5): 911-23

(32) Li, G.; Hong, G.; Li, X.; Zhang, Y.; Xu, Z.; Mao, L.; Feng, X.; Liu, T.; Synthesis and activity towards Alzheimer's disease in vitro: Tacrine, phenolic acid and ligustrazine hybrids. *Eur. J. Med. Chem.* 2**018**, 148, 238-54.

(33) Fang, L.; Fang, X.; Gou, S.; Lupp, A.; Lenhardt, I.; Sun, Y.; Huang, Z.; Chen, Y.; Zhang, Y.; Fleck, C.; Design, synthesis and biological evaluation of D-ring opened galantamine analogs as multifunctional anti-Alzheimer agents. *Eur. J. Med. Chem.* **2014,** 9; 76:376-86.

(34) Fang, L.; Chen, M.; Liu, Z.; Fang, Gou, S.; Chen, L.; Ferulic acid–carbazole hybrid compounds: Combination of cholinesterase inhibition, antioxidant and neuroprotection as multifunctional anti-Alzheimer agents. *Bioorganic Med. chem*. **2016**; 24(4):886-93.

(35) Thiratmatrakul, S.; Yenjai, C.; Waiwut, P.; Vajragupta, O.; Reubroycharoen, P.; Tohda , M.; Boonyarat, C.; Synthesis, biological evaluation and molecular modeling study of novel tacrine–carbazole hybrids as potential multifunctional agents for the treatment of Alzheimer's disease. *Eur. J. Med. Chem*. **2014**, 21; 75: 21-30.

(36) Xia, CL.; Wang, N.; Guo, QL.; Liu, ZQ.; Wu, JQ.; Huang, SL.; Ou, TM.; Tan, JH.; Wang HG, Li D, Huang ZS. Design, synthesis and evaluation of 2-arylethenyl-N-methylquinolinium derivatives as effective multifunctional agents for Alzheimer's disease treatment. *Eur. J. Med. Chem.* **2017**, 21; 130: 139-53.

(37) Li, Y.; Qiang, X.; L, Y.; Yang, X.; Luo, L.; Xiao, G.; Cao, Z.; Tan, Z.; Deng, Y.; Pterostilbene-O-acetamidoalkylbenzylamines derivatives as novel dual inhibitors of cholinesterase with anti-β-amyloid aggregation and antioxidant properties for the treatment of Alzheimer's disease. *Bioorgnic Med. Chem. lett.* **2016**; 26(8): 2035-9.

(38) Sinha, A.; Tamboli, R. S.; Seth, B.; Kanhed, A. M.; Tiwari, S. K.; Agarwal, S.; Nair, S.; Giridhar, R.; Chaturvedi, R. K.; Yadav, M. R. Neuroprotective role of novel triazine derivatives by activating Wnt/β catenin signaling pathway in rodent models of Alzheimer's disease. *Mol. Neurobiol.* **2015,** *52*, 638-652.

(39) Sharma, V.; Kumar, P.; Pathak, D. Biological importance of the indole nucleus in recent years: a comprehensive review. *J. Heterocycl. Chem.* **2010,** *47*, 491-502.

(40) Kaushik, N.; Kaushik, N.; Attri, P.; Kumar, N.; Kim, C.; Verma, A.; Choi, E. Biomedical importance of indoles. *Molecules* **2013,** *18*, 6620-6662.

(41) Zhou, J. N.; Liu, R. Y.; Kamphorst, W.; Hofman, M. A.; Swaab, D. F. Early neuropathological Alzheimer's changes in aged individuals are accompanied by decreased cerebrospinal fluid melatonin levels. *J. Pineal Res.* **2003,** *35*, 125-130.

(42) Wu, Y. H.; Feenstra, M. G.; Zhou, J. N.; Liu, R. Y.; Toranõ, J. S.; Van Kan, H. J.; Fischer, D. F.; Ravid, R.; Swaab, D. F. Molecular changes underlying reduced pineal melatonin levels in Alzheimer disease: alterations in preclinical and clinical stages. *J. Clin. Endocrinol. Metab.* **2003,** *88*, 5898-5906.

(43) Wu, Y. H.; Swaab, D. F. The human pineal gland and melatonin in aging and Alzheimer's disease. *J. Pineal Res.* **2005,** *38*, 145-152.

(44) Pandi-Perumal, S. R.; Srinivasan, V.; Maestroni, G.; Cardinali, D.; Poeggeler, B.; Hardeland, R. Melatonin: Nature's most versatile biological signal. *FEBS J.* **2006,** *273*, 2813-2838.

(45) Feng, Z.; Zhang, J. T. Melatonin reduces amyloid β-induced apoptosis in pheochromocytoma (PC12) cells. *J. Pineal Res.* **2004,** *37*, 257-266.

(46) Espinar, A.; García-Oliva, A.; Isorna, E. M.; Quesada, A.; Prada, F. A.; Guerrero, J. M. Neuroprotection by melatonin from glutamate-induced excitotoxicity during development of the cerebellum in the chick embryo. *J. Pineal Res.* **2000,** *28*, 81-88.

(47) Aydogan, S.; Yerer, M. B.; Goktas, A. Melatonin and nitric oxide. *J. Endocrinol. Invest.* **2006,** *29*, 281-287.

(48) Rodríguez-Franco, M. I.; Fernández-Bachiller, M. I.; Pérez, C.; Hernández-Ledesma, B.; Bartolomé, B. Novel tacrine-melatonin hybrids as dual-acting drugs for Alzheimer disease, with improved acetylcholinesterase inhibitory and antioxidant properties. *J. Med. Chem.* **2006,** *49* , 459-462.

(49) Pachón-Angona, I.; Refouvelet, B.; Andrýs, R.; Martin, H.; Luzet, V.; Iriepa, I.; Moraleda, I.; Diez-Iriepa, D.; Oset-Gasque, M. J.; Marco-Contelles, J. Donepezil-chromone-melatonin hybrids as promising agents for Alzheimer's disease therapy. *J. Enzyme Inhib. Med. Chem.* **2019,** *34*, 479-489.

(50) López-Iglesias, B.; Pérez, C. N.; Morales-García, J. A.; Alonso-Gil, S.; Pérez-Castillo, A.; Romero, A.; López, M. G.; Villarroya, M.; Conde, S.; Rodríguez-Franco, M. I. New melatonin-*N*, *N*-dibenzyl (*N*-methyl) amine hybrids: Potent neurogenic agents with antioxidant, cholinergic, and neuroprotective properties as innovative drugs for Alzheimer's disease. *J. Med. Chem.* **2014,** *57*, 3773-3785.

(51) Yanovsky, I.; Finkin-Groner, E.; Zaikin, A.; Lerman, L.; Shalom, H.; Zeeli, S.; Weill, T.; Ginsburg, I.; Nudelman, A.; Weinstock, M. Carbamate derivatives of indolines as cholinesterase inhibitors and antioxidants for the treatment of Alzheimer's disease. *J. Med. Chem.* **2012,** *55*, 10700-10715.

(52) Kumar, R.; Sirohi, T.; Singh, H.; Yadav, R.; Roy, R.; Chaudhary, A.; Pandeya, S. 1,2,4-triazine analogs as novel class of therapeutic agents. *Mini Rev. Med. Chem.* **2014,** *14*, 168-207.

(53) Irannejad, H.; Amini, M.; Khodagholi, F.; Ansari, N.; Tusi, S. K.; Sharifzadeh, M.; Shafiee, A. Synthesis and in vitro evaluation of novel 1,2,4-triazine derivatives as neuroprotective agents. *Bioorg. Med. Chem.* **2010,** *18*, 4224-4230.

(54) Veloso, A. J.; Chow, A. M.; Dhar, D.; Tang, D. W.; Ganesh, H. V.; Mikhaylichenko, S.; Brown, I. R.; Kerman, K. Biological activity of sym-triazines with acetylcholine-like substitutions as multitarget modulators of Alzheimer's disease. *ACS Chem. Neurosci.* **2013,** *4*, 924-929.

(55) Veloso, A. J.; Dhar, D.; Chow, A. M.; Zhang, B.; Tang, D. W.; Ganesh, H. V.; Mikhaylichenko, S.; Brown, I. R.; Kerman, K. sym-Triazines for directed multitarget modulation of cholinesterases and amyloid-β in Alzheimer's disease. *ACS Chem. Neurosci.* **2012,** *4*, 339-349.

(56) Tripathi, P. N.; Srivastava, P.; Sharma, P.; Tripathi, M. K.; Seth, A.; Tripathi, A.; Rai, S. N.; Singh, S. P.; Shrivastava, S. K. Biphenyl-3-oxo-1,2,4-triazine linked piperazine derivatives as potential cholinesterase inhibitors with anti-oxidant property to improve the learning and memory. *Bioorg. Chem.* **2019,** *85*, 82-96.

(57) Russell, M. G.; Carling, R. W.; Street, L. J.; Hallett, D. J.; Goodacre, S.; Mezzogori, E.; Reader, M.; Cook, S. M.; Bromidge, F. A.; Newman, R. Discovery of imidazo[1,2-*b*][1,2,4]triazines as GABAA α2/3 subtype selective agonists for the treatment of anxiety. *J. Med. Chem.* **2006,** *49*, 1235-1238.

(58) Leach, M. J.; Marden, C. M.; Miller, A. A. Pharmacological studies on lamotrigine, a novel potential antiepileptic drug. *Epilepsia* **1986,** *27*, 490-497.

(59) Misra, U.; Hitkari, A.; Saxena, A.; Gurtu, S.; Shanker, K. Biologically active indolylmethyl-1,3,4-oxadiazoles, 1,3,4-thiadiazoles, 4*H*-1,3,4-triazoles and 1,2,4-triazines. *Eur. J. Med. Chem.* **1996,** *31*, 629-634.

(60) Ansari, N.; Khodagholi, F.; Ramin, M.; Amini, M.; Irannejad, H.; Dargahi, L.; Amirabad, A. D. Inhibition of LPS-induced apoptosis in differentiated-PC12 cells by new triazine derivatives through NF-κB-mediated suppression of COX-2. *Neurochem. Int.* **2010,** *57*, 958-968.