Summary

Alzheimer's disease (AD), the most common type of dementia, is a progressive neurodegenerative disorder prevalent amongst elderly people. Almost 50 million people worldwide suffer from AD, and if no treatment or prevention steps are discovered soon, the number will grow up significantly to 150 million by 2050. The etiology of AD is very complex, several factors like deficit of acetylcholine (ACh), abnormal amyloid-β (Aβ) deposition and accumulation, tau hyperphosphorylation, oxidative stress and dyshomeostasis of biometals are hypothesized to play significant roles in the etiology of AD.

The multifaceted nature of AD needs treatment with multitarget-directed ligands (MTDLs) to confront the pathological aberrations. Although ChEIs offer only symptomatic relief to the patients, they still remain the drugs of choice. Neurotoxic Aβ plaques and oxidative stress perform important roles in the pathogenesis of AD. However, Aβ aggregation inhibitors or antioxidants all alone might not be sufficient to resist a multifaceted pathological condition like AD. Therefore cholinesterase inhibitors with additional Aβ aggregation inhibitory and antioxidant activities might be a desirable pick to offset the progress of this multifaceted disease.

Keeping this fact in mind, we focused our attention on designing new MTDLs as the core objective of anti-AD drug discovery. So it was planned to use two different biologically active moieties, indole and carbazole for designing of some novel MTDLs. Both of these biologically active moieties were separately fused with other biologically significant pharmacophoric moieties (i.e. 1,2,4-triazine with indole, stilbene and carbohelicene with carbazole) to design the novel scaffolds. Structural modifications were carried out on these designed scaffolds by incorporating various substituents to obtain some potential leads as anti-AD agents and to establish a logical SAR.

Chemical studies:

The work carried out for the fulfillment of the proposed plan has been discussed in two parts:

A. Triazinoindole based multifunctional anti-AD agents and,

B. Carbazole based multifunctional anti-AD agents.

A. Triazinoindole based multifunctional anti-AD agents

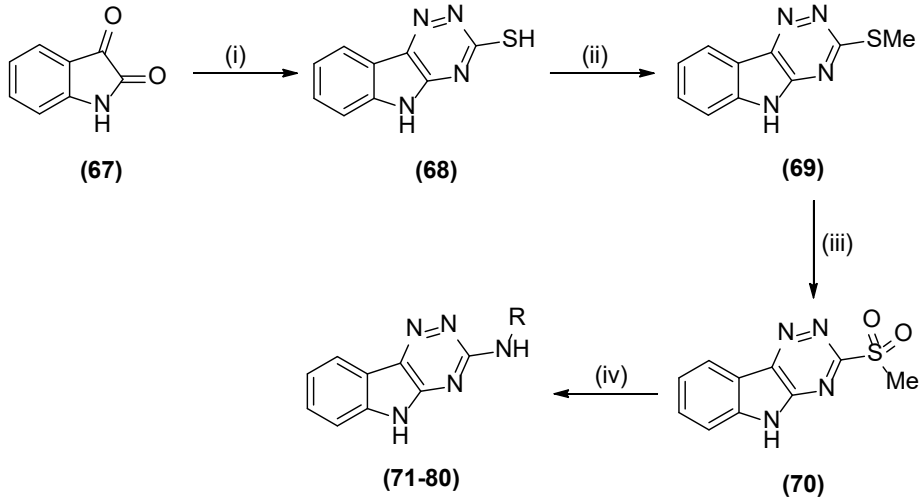
Two types of compounds have been synthesized under this category:

- 3-/5-substituted [1,2,4]triazino[5,6-*b*]indole derivatives and,
- 3- And 6-/7-/8-/9-disubstituted [1,2,4]triazino[5,6-*b*]indole derivatives.

3-/5-Substituted [1,2,4]triazino[5,6-*b*]indole derivatives

Designing of 3-/5-substituted [1,2,4]triazino[5,6-*b*]indole derivatives has been discussed in thesis. These hybrid derivatives (**71-80**, **117-126**, **179-202**) were synthesized by adopting **Schemes I-III**. Compounds (**71-80**) were synthesized from commercially available isatin (**67**) as depicted in **Scheme I**.

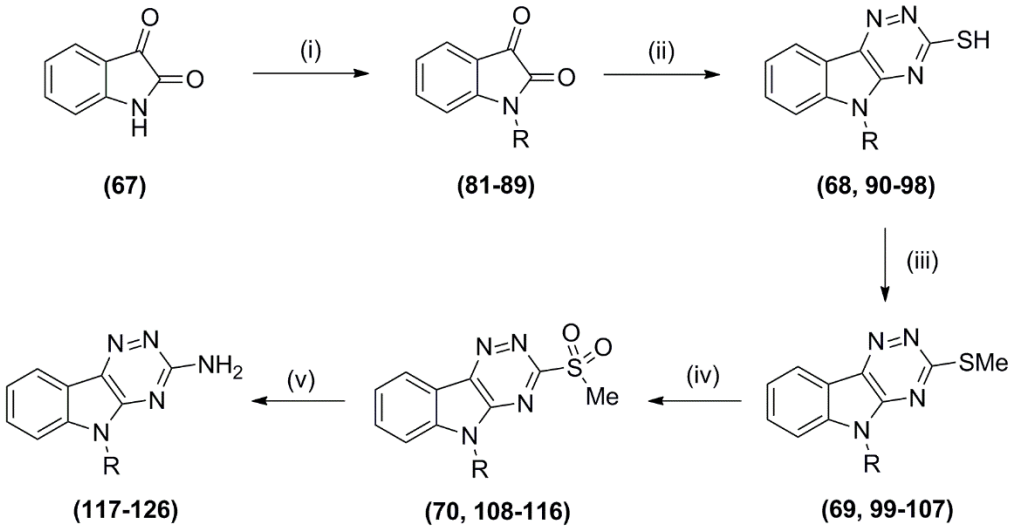
Scheme I:



Scheme I: General synthetic route for the synthesis of compounds (**71-80**); Reagents and conditions: (i) Thiosemicarbazide, K_2CO_3, H_2O, reflux, overnight; (ii) MeI, K_2CO_3, DMF. (iii) *m*CPBA, DCM, 0 °C to RT; (iv) amine, THF, reflux.

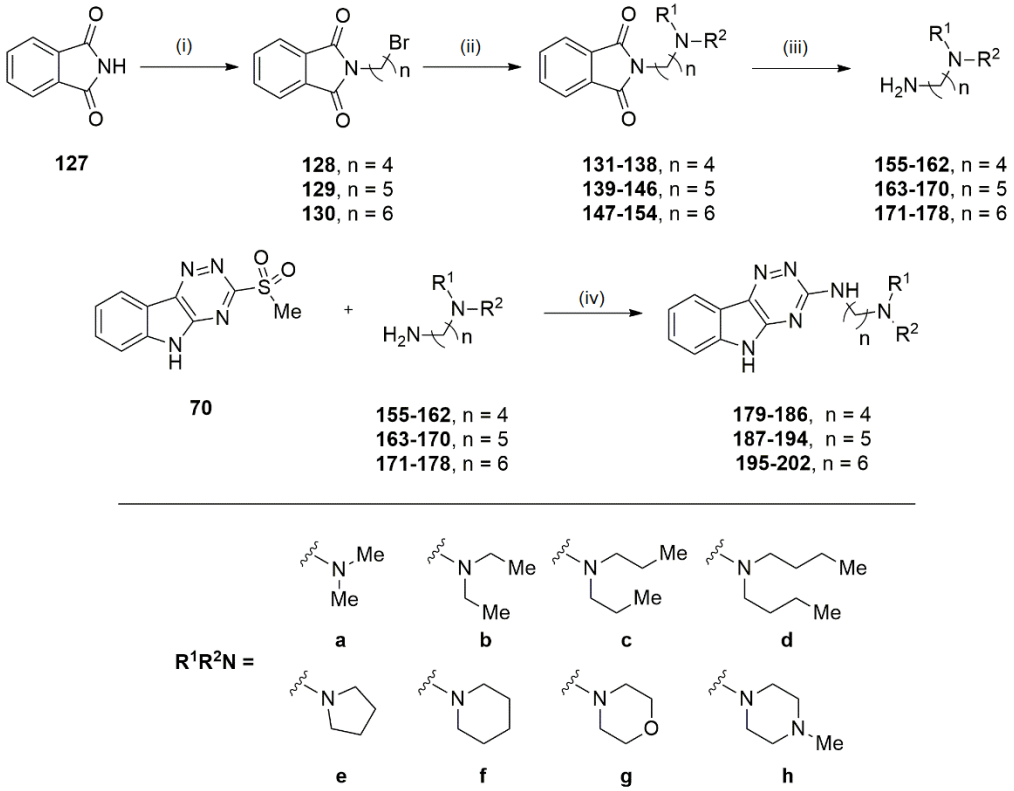
The designed 5-substituted 5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine derivatives (**117-126**) were prepared from *N*-substituted isatins (**81-89**) as depicted in **Scheme II**. Compounds (**179-202**) were synthesized by reacting compound (**70**) with aminoalkyl amines (**155-178**) as shown in **Scheme III**.

Scheme II:



Scheme II: Synthesis of 5-substituted 5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine derivatives (**117-126**). 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.

Scheme III:

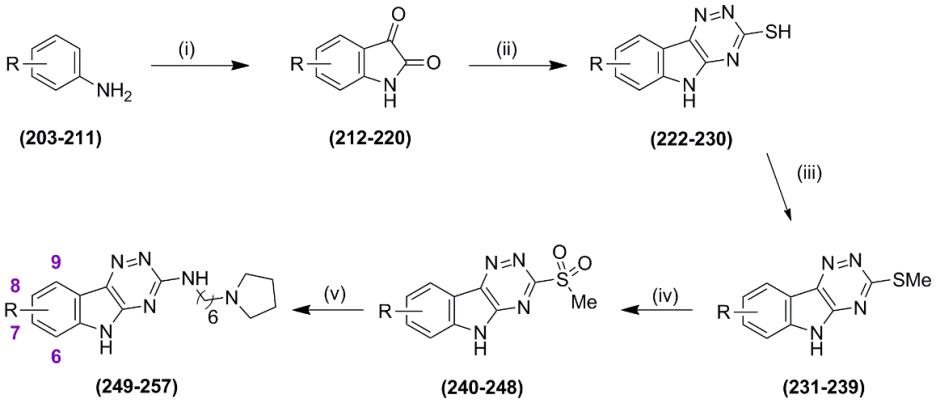


Scheme III: Synthetic route for the synthesis of *N*-(aminoalkyl)-5*H*-[1,2,4]triazino [5,6-*b*]indol-3-amine derivatives (**179-202**). Reagents and conditions: (i) $Br(CH_2)_nBr$, K_2CO_3, TEBAC, acetone, RT; (ii) HNR^1R^2, TEA, MeOH, reflux; (iii) $NH_2NH_2.H_2O$, MeOH, reflux; (iv) THF, reflux.

3- And 6-/7-/8-/9-disubstituted [1,2,4]triazino[5,6-*b*]indole derivatives

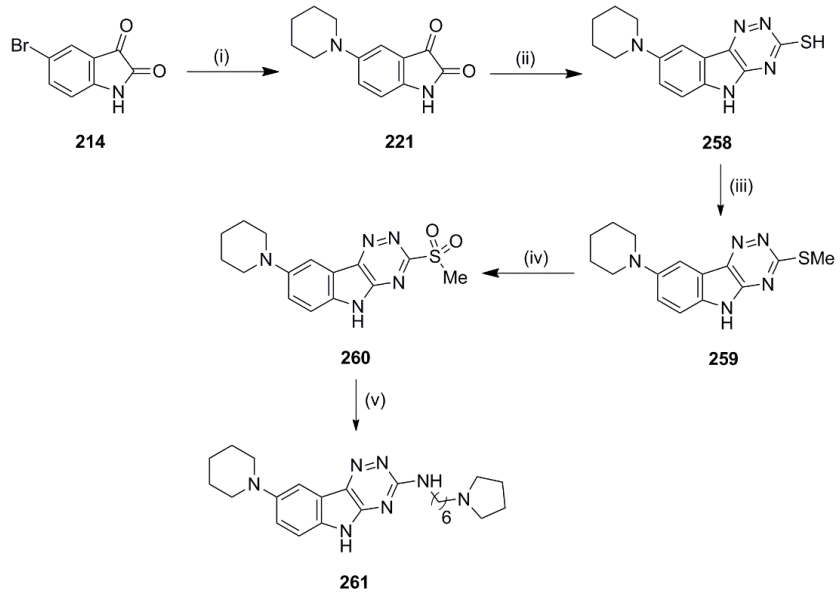
Incorporation of various substituents i.e. chloro, bromo, fluoro, methyl, ethyl, and piperidinyl at different positions (C_6-C_9) of the phenyl ring of the triazinoindole scaffold resulted in a novel series of anti-AD agents. These derivatives (**249-257, 261**) were synthesized by adopting schemes **IVa** and **IVb**.

Scheme IVa:



Scheme IVa: General synthetic route for the **s**ynthesis of C_6-C_9-substituted-*N*-(aminoalkyl)-5*H*-[1,2,4]triazino[5,6-*b*]indol-3-amine derivatives (**249-257**). Reagents and conditions: (i) (a) Chloral hydrate, sodium sulfate, hydroxylamine HCl, (b) sulphuric acid, 80 ºC; (ii) thiosemicarbazide, K_2CO_3, H_2O, reflux, overnight; (iii) MeI, K_2CO_3, DMF. (iv) *m*CPBA, DCM, 0 ºC to RT; (v) 6-(pyrrolidin-1-yl)hexan-1-amine, THF, reflux.

Scheme IVb:



Scheme IVb: Synthetic route for the **s**ynthesis of the compound (**261**). Reagents and conditions: (i) Piperidine, K_2CO_3, DMF, 80 ºC; (ii) thiosemicarbazide, K_2CO_3, H_2O, reflux, overnight; (iii) MeI, K_2CO_3, DMF. (iv) *m*CPBA, DCM, 0 ºC to RT; (v) 6-(pyrrolidin-1-yl)hexan-1-amine, THF, reflux.

B. Carbazole based multifunctional anti-AD agents.

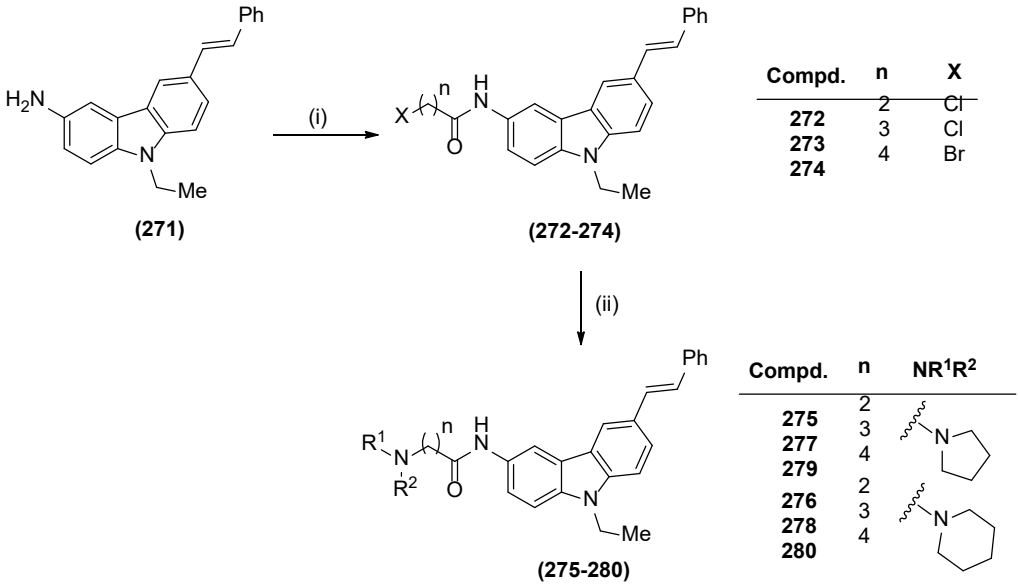
Two types of hybrid molecules have been synthesized under this category:

- Carbazole based stilbene derivatives and
- Carbazole based azahelicene derivatives.

Carbazole based stilbene derivatives

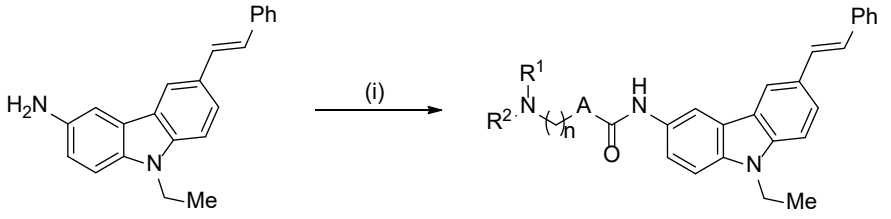
Designing of Carbazole based stilbene derivatives has been discussed in thesis. These derivatives (**275-286**, **293-307**) were synthesized by adopting schemes **V-IX**. Compounds (**275-280**) were synthesized from the amine intermediate (**271**) as illustrated in **Scheme V**. The amine intermediate (**271**) was reacted with *p*-nitrophenyl chloroformate to get compounds (**272-274**) which were further reacted with the respective aminoalkylamines to get desired compounds (**275-280**). Compounds (**282-286**) were synthesized from the amine intermediate (**271**) as depicted in **Scheme VI**.

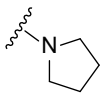
Scheme V:

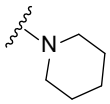


Scheme V: Synthetic route for the synthesis of compounds (**275-280**): Reagents and conditions: (i) acid chloride, K_2CO_3, acetone; (ii) NHR^1R^2, THF, reflux.

Scheme VI: (*E*)-1-(9-ethyl-6-styryl-9*H*-carbazol-3-yl)aminoalkylurea derivatives (**281-286**)



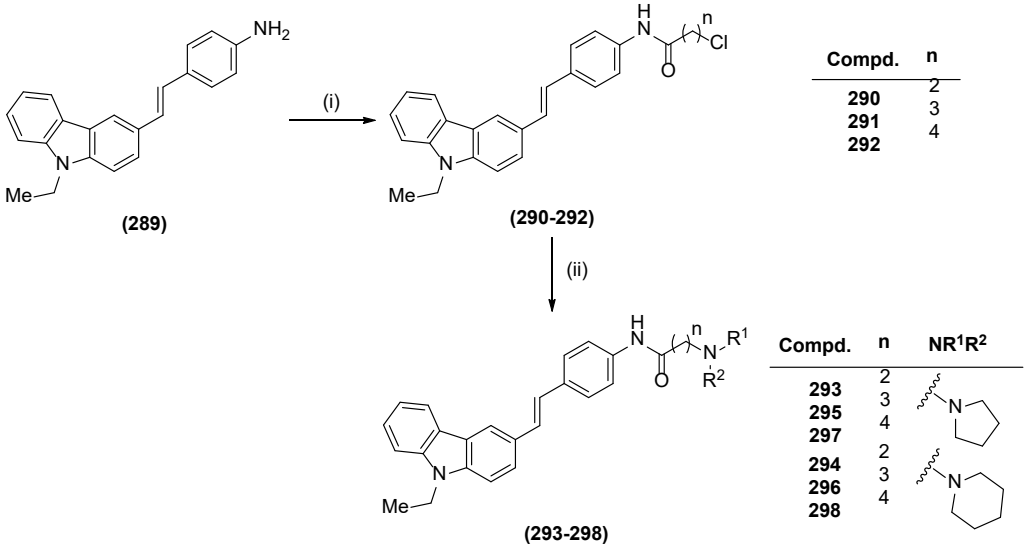
Compd.	A	n	NR^1R^2
281	--	0	
283	NH	2	
285	NH	3	

Compd.	A	n	NR^1R^2
282	--	0	
284	NH	2	
286	NH	3	

Scheme VI: Synthetic route for the synthesis of the compounds (**281-286**). Reagents and conditions: (i) (a) *p*-Nitrophenyl chloroformate, TEA, DCM:THF (1:1), 0 °C to RT; (b) pyrrolidine/piperidine/aminoalkylamines, RT.

The hybrid derivatives (**293-298**) were prepared from the amine intermediate (**289**) in two steps as depicted in Scheme **VII**. The urea derivatives (**299-304**) were prepared by the synthetic route represented in **Scheme VIII.**

Scheme VII:

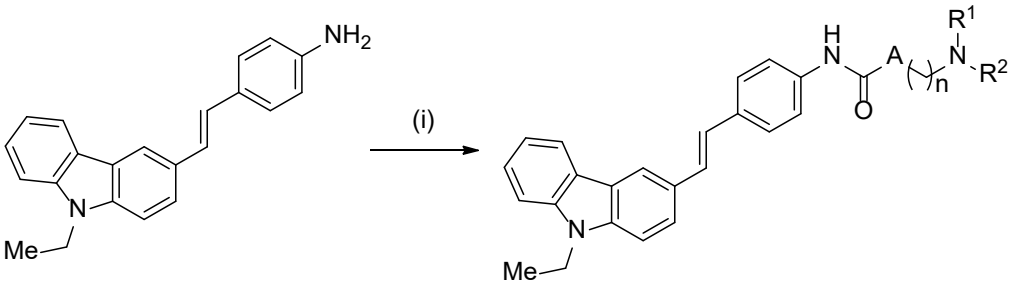


Compd.	n
290	2
291	3
292	4

Compd.	n	NR^1R^2
293	2	
295	3	
297	4	
294	2	
296	3	
298	4	

Scheme VII: Synthetic route for the synthesis of (*E*)-*N*-(4-(2-(9-ethyl-9*H*-carbazol-3-yl)vinyl)phenyl)aminoalkylamide (**293-298**). Reagents and conditions: (i) acid chloride, K_2CO_3, acetone; (ii) NHR^1R^2, THF, reflux.

Scheme VIII:



(289) (299-304)

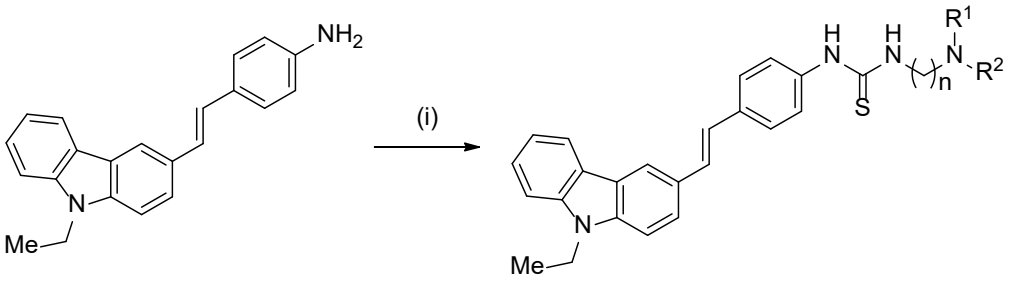
Compd.	A	n	NR^1R^2
299	--	0	
301	NH	2	N
303	NH	3	N

Compd.	A	n	NR^1R^2
300	--	0	
302	NH	2	N
304	NH	3	N

Scheme VIII: Synthetic route for the synthesis of (*E*)-*N*-(4-(2-(9-ethyl-9*H*-carbazol-3-yl) vinyl)phenyl)aminoalkylureas (**299-304**)**.** Reagents and conditions: (i) (a) *p*-nitro phenyl chloroformate, TEA, DCM:THF (1:1), 0 °C to RT; (b) pyrrolidine/ piperidine/1-pyrrolidinylalkylamines/1-piperidinylalkylamines, RT.

Synthesis of thiourea derivatives (**305-307)** was carried out as illustrated **Scheme IX.**

Scheme IX:



(289) (305-307)

Compd.	n	NR^1R^2
305	2	N
307	3	N

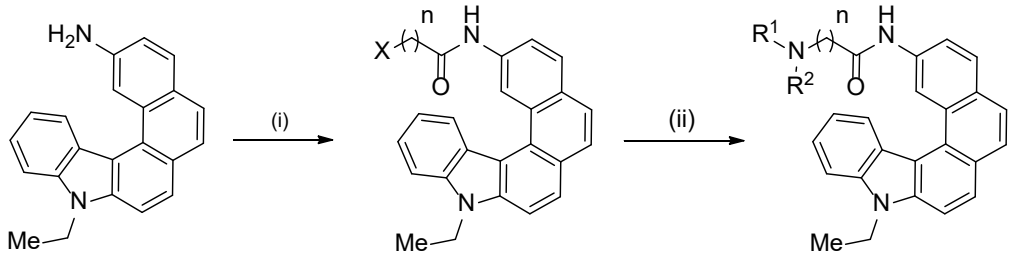
Compd.	n	NR^1R^2
306	2	N

Scheme IX: Synthetic route for the synthesis of compounds (**305-307**). Reagents and conditions: (i) (a) thiocarbonyldiimidazole, DCM:THF (1:1), 0 °C to RT; (b) 1-pyrrolidinylalkylamines/1-piperidinylethylamine, RT.

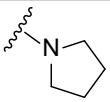
Carbazole based azahelicene derivatives

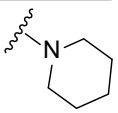
Designing of carbazole based azahelicene derivatives has been discussed in thesis. These carbazole based azahelicene derivatives (**316-329**) were synthesized by adopting schemes **Xa-Xc**.

Scheme Xa:



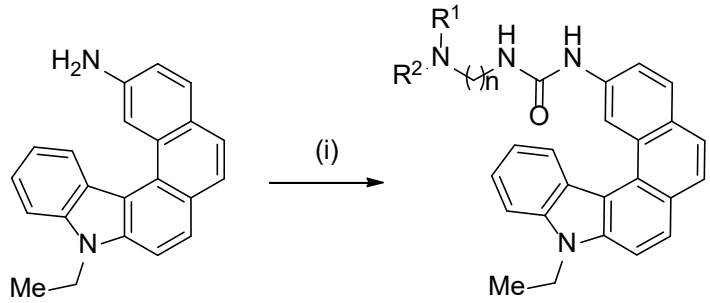
Compd.	n	X
313	3	Cl
314	4	Br
315	5	Br

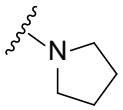
Compd.	n	NR^1R^2
316	3	
318	4	
320	5	

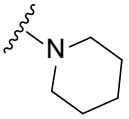
Compd.	n	NR^1R^2
317	3	
319	4	
321	5	

Scheme Xa: Synthetic route for the synthesis of compound **(316-321).** Reagents and conditions: (i) Acid chloride, K_2CO_3, acetone; (ii) NHR^1R^2, THF, reflux.

Scheme Xb:

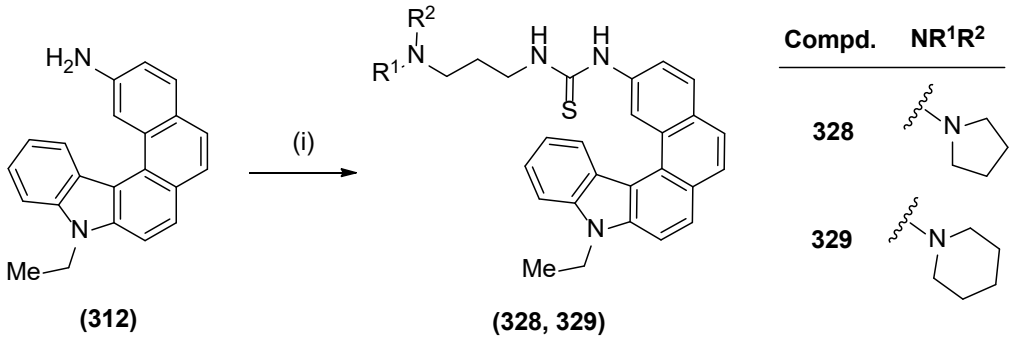


Compd.	n	NR^1R^2
322	2	
324	3	
326	4	

Compd.	n	NR^1R^2
323	2	
325	3	
327	4	

Scheme Xb: Synthetic route for the synthesis of compounds (**322-327**)**.** Reagents and conditions: (i) (a) *p*-nitrophenyl chloroformate, TEA, DCM:THF (1:1), 0 °C to RT; (b) pyrrolidine/ piperidine/aminoalkylamines, RT.

Scheme Xc:



Scheme Xc: Synthetic route for the synthesis of the compounds (**328, 329**)**.** Reagents and conditions: (i) (a) thiocarbonyldiimidazole, DCM:THF (1:1), 0 °C to RT; (b) 3-aminopropylamines, RT.

Biological studies:

The biological studies were carried out in the pharmacological division of our department. All the synthesized compounds were subjected to evaluate their anti-AD properties.

Among the triazinoindole derivatives ((**71-80**, **117-126**, **179-202**), compound (**199**) was identified as the most active compound with IC_{50} values of 0.56 μM for AChE and 1.17 μM for BuChE (SI value of 2.09). Molecular modeling studies revealed significant interactions between the most potent compound (**199**) with PAS as well as CAS sites of both the enzymes. Results from the docking studies were further validated by time-dependent molecular dynamics study. Compound (**199**) displayed excellent neuroprotective activity against H_2O_2 as well as Aβ-induced toxicity in SH-SY5Y cells in a concentration-dependent manner. Further, it did not show any significant toxicity in neuronal SH-SY5Y cells in the cytotoxicity assay. Compound (**199**) displayed high permeability in the PAMPA-BBB assay. Compound (**199**) was able to significantly reverse the cognitive impairment in both MWM and Y-Maze tests. Restoration of CAT and MDA levels to their normal values supports the antioxidant potential of compound (**199**). Additionally, compound (**199**) is endowed with the capability to restore the memory deficit through increasing glycine levels and decreasing glutamate levels in scopolamine-treated animals. It did not show any acute toxicity in rats at a dose of 2000 mg/kg. In addition,

compound (**199**) was predicted *in silico* to possess notable ADMET properties. Taken together, all these findings suggest compound (**199**) to be a potential candidate for further development as a novel anti-AD drug.

Based on the *in silico* metabolic analysis, compound (**199**) was further modified chemically to improve the potency by restricting the metabolism and elimination. Incorporation of various substituents i.e. chloro, bromo, fluoro, methyl, ethyl, and piperidinyl at different positions (C_6-C_9) of the phenyl ring of the triazinoindole scaffold resulted in a novel series of anti-AD agents. Among them, compound (**261**) was identified as the most potent compound exhibiting 1.75-fold and 3.2-fold increase in AChE and BuChE inhibitory activities, respectively (IC_{50} = 0.56 μM for AChE and 1.17 μM for BuChE) in comparison to compound (**199**). Compound (**261**) demonstrated good antioxidant activity (55.8 % inhibition at 20 μM). Compound (**261**) significantly reversed the cognitive impairment in MWM. Restoration of oxidative stress parameters (MDA and CAT) in the hippocampal region of the rat brain reflects the antioxidant potential of the compound (**261**). Moreover, compound (**261**) did not show any acute toxicity in rats at a dose of 2000 mg/kg, p.o. dose. Based on all these findings, compound (**261**) can be considered as a prime candidate for further discovery as a novel anti-AD drug.

All the synthesized carbazole based stilbene derivatives (**275-286**, **293-307**) were evaluated for their anti-AD properties, including cholinesterase inhibitory activity, $A\beta_{1-42}$ aggregation inhibitory activity, anti-oxidant activity, metal chelation activity. Amongst them, compound (**305**) having thiourea linker showed the most promising inhibitory activities against AChE (IC_{50} = 2.64 ± 0.41 uM) and BuChE (IC_{50} = 1.29 ± 0.10 uM). Compound (**305**) showed significant inhibition of self-mediated $A\beta_{1-42}$ aggregation (51.29 % at 25 μM concentration). These hybrid derivatives also possessed moderate antioxidant activity. In metal chelation study, compound (**305**) displayed specific metal (Cu^{2+}) chelation ability.

All the synthesized azahelicene derivatives (**316-329**) were evaluated for their cholinesterase inhibitory and $A\beta_{1-42}$ aggregation inhibitory activities. Among the synthesized compounds, compound (**329**) showed very good

inhibitory activities towards AChE (IC_{50} = 1.89 ± 0.25 uM) and BuChE (IC_{50} = 0.54 ± 0.12 uM). The selected hybrid derivatives (**316-329**) showed good $A\beta_{1-42}$ aggregation inhibition ranging from 53.07 to 67.93 %. Compound (**329**) also displayed specific metal (Cu^{2+}) chelation ability.

Computational studies:

The promising compounds (**199**, **261**, **305** and **329**) were subjected to predict their binding interactions with the active site of enzymes (AChE, BuChE and $A\beta_{1-42}$). Additionally, these compounds exhibited favorable in silico ADMET properties. These compounds exhibited good molecular interactions with the respective enzymes. Structure-activity relationship and molecular interaction analysis by the docking and dynamics studies within the all the series of compounds are well discussed in thesis.