6. CONCLUSION

Alzheimer's disease (AD) is the most common type of dementia. It accounts for 60% to 80% cases of dementia. The most common symptoms of AD begin with slowly worsening difficulty in memorizing new things. This is mostly because of disruption of brain cell functions mainly in regions involved in forming new memories. As neuronal damage spreads, individuals experience other difficulties which include confusion with time or place, trouble completing familiar tasks, difficulty in understanding visual images, poor judgment etc.

AD is completely related to neuronal death and the main responsible hypotheses proposed include cholinergic hypothesis which explains the role of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) enzymes in the AD. Protein misfolding and aggregation explains the role of β-amyloid and Tau protein in the AD. Apart from these two factors role of different ions and oxidative stress is also known in this condition.

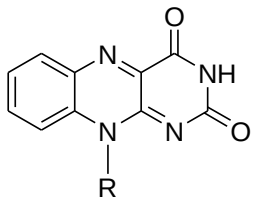
Currently, mainstream therapeutic treatments for AD are aimed to inhibit acetylcholinesterase (AChE) to enhance acetylcholine (ACh) levels in brain. AChE inhibitors like tacrine, donepezil, rivastigmine and NMDA receptor antagonists like memantine are currently available for AD treatment. There are only few drugs accepted for the treatment of AD and these are not capable of curing the cruel condition of AD. Additionally, it has been observed that increased level of BuChE activity occurs in the brain of the Alzheimer's patients. The receptor active sites analysis suggested that both AChE and BuChE are quite homologous in nature, such as a triad of Ser-His-Glu, a *p*-cation-binding site, an oxyanion hole, and an acyl-binding pocket are common features in both the active sites.

As AChE is the most successful target for symptomatic treatment of AD and BuChE also plays an important role in AD, design, syntheses and evaluation of different novel heterocyclic derivatives against AD as dual acting cholinesterase inhibitors were envisaged here.

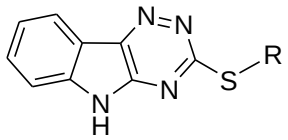
In this piece of work five different scaffolds were designed and synthesized which includes five different series as follows:

- Series I: 10-substituted benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione.
- Series II: 3-(Substituted thio)-5*H*-[1,2,4]triazino[5,6-*b*]indole.

- Series III: 5- Substituted 5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol.
- Series IV: 6-Substituted 6*H*-indolo[2,3-*b*]quinoxaline.
- Series V: 5-Substituted 5*H*-indolo[2,3-*b*]quinoxaline.



I



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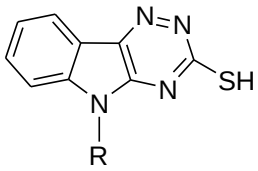

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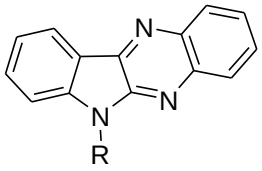
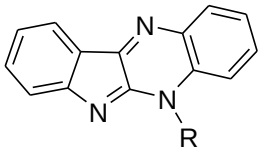
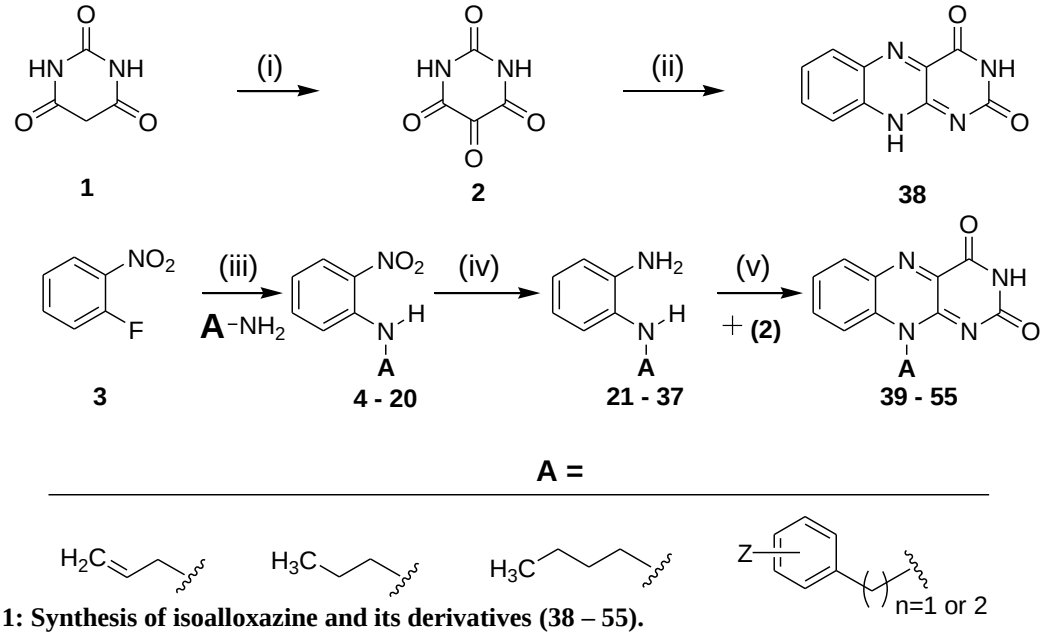


IV



V

All the synthesized compounds were characterized by different analytical techniques like ^{1}H-NMR spectroscopy and mass spectrometry whereas representative compounds were also analyzed by ^{13}C-NMR spectroscopy. Further these compounds were evaluated pharmacologically for their AChE and BuChE inhibition potencies.

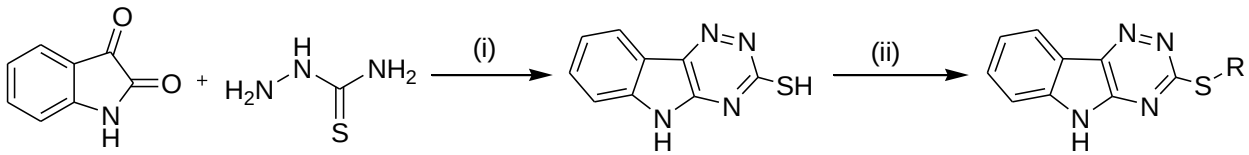


Scheme 1: Synthesis of isoalloxazine and its derivatives (38 – 55).

(i) CrO_3, AcOH; (ii) *o*-phenylenediamine, H_3BO_3, AcOH, RT, 8 hrs; (iii) K_2CO_3, DMF, 60°C, 4-6 hrs; (iv) Zn, AcOH, MeOH, RT, 6-8 hrs; (v) H_3BO_3, AcOH, RT, 8-10 hrs.

In series I, alloxan monohydrate (**2**) was prepared by oxidation of barbituric acid (**1**). The lead molecule (**38**) was prepared by cyclizing compound (**2**) with *o*-phenylenediamine in presence of boric acid and acetic acid at room temperature. In order to obtain different *N*-alkylated products (**39 – 55**) at 10 position, 1-fluoro-2-nitrobenzene (**3**) was reacted with the different alkyl/arylalkyl amines in presence of K_2CO_3 as the base in DMF to obtain the desired *N*-alkyl/arylalkyl 2-nitroanilines (**4 – 20**). Subsequently, the 2-nitro group of compounds (**4 – 20**) was reduced in presence of Zn and AcOH in methanol as solvent at room temperature to obtain *N*1-substituted 1,2-diamine intermediates (**21 – 37**). The obtained intermediates were used as such for the next step. To obtain the desired isoalloxazine derivatives (**39 – 55**), the *N*1-substituted 1,2-diamine intermediates (**21 – 37**) were reacted with **2** in presence of H_3BO_3 and AcOH at room temperature as per the procedure adopted for compound (**38**) to get the cyclized compounds (**39 – 55**). Structures of all the compounds were confirmed on the basis of spectral.

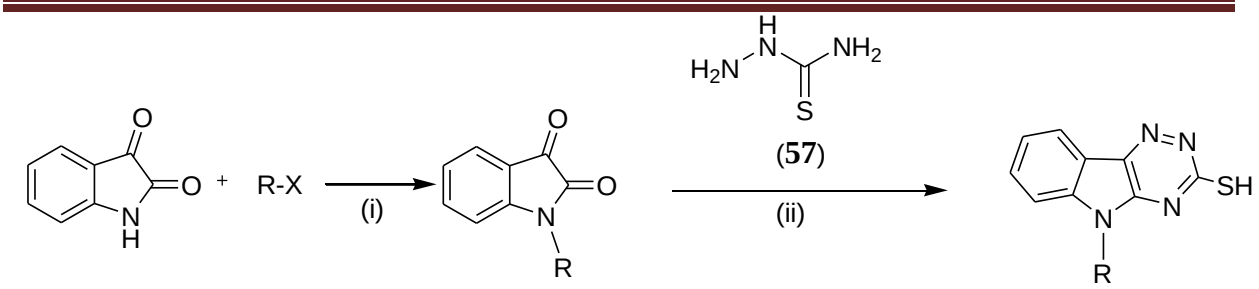
In series II, isatin (**56**) was condensed with thiosemicarbazide (**57**) to obtain 5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol (**58**). This reaction was carried out in presence of K_2CO_3 in water as protic solvent and glacial acetic acid was used to acidify the reaction mixture to obtain the condensed product (**58**). Further, substitutions were made on –SH group by using various alkyl bromides in presence of K_2CO_3 in DMF at 60 °C. This leads to the formation of various 3-substituted thio derivatives (**59** – **70**).



(**56**) (**57**) (**58**) (**59** – **70**)

Scheme 2: Synthesis of 3-substituted thio triazinoindole derivatives (58 – 70). (i) K_2CO_3, H_2O, Reflux 4 h, AcOH; (ii) R-X, K_2CO_3, DMF, 60 °C.

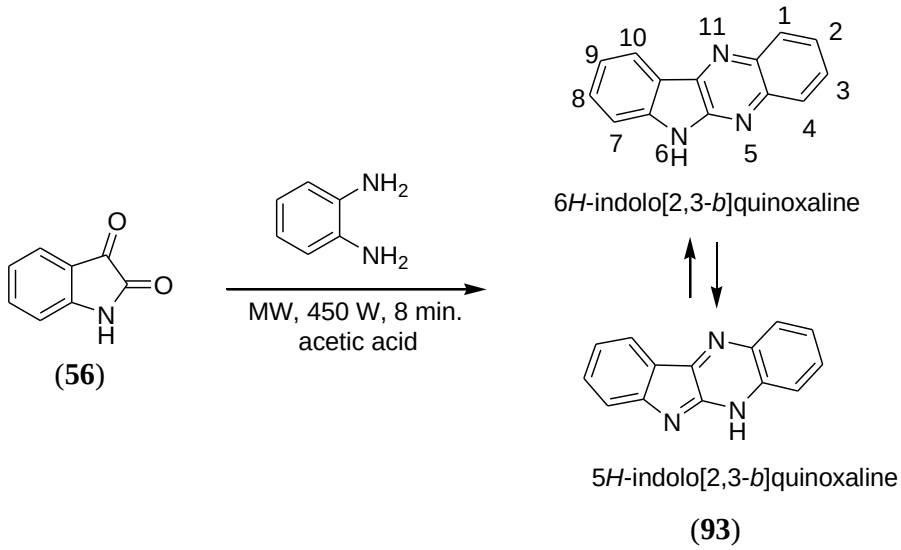
In similar way as series II, different derivatives in series III were synthesized wherein different *N*-substituted isatins were reacted with thiosemicarbazide by following the procedure as followed for preparation of compound (**58**) to get the final compounds (**82** – **92**). The *N*-substituted isatins were synthesized by treating isatin with the different alkyl halides in DMF with K_2CO_3 as base.



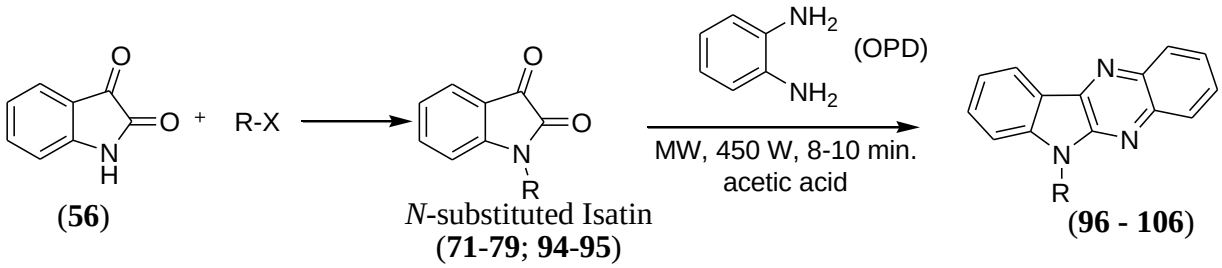
Scheme 3: Synthesis of 5- Substituted 5*H*-[1,2,4]triazino[5,6-*b*]indole-3-thiol derivatives (82 – 92). (i) K_2CO_3, DMF, alkyl halide (R-X); (ii) K_2CO_3, H_2O, Reflux 4 h, AcOH.

In series IV and V, a novel scaffold as cholinesterase inhibitor was reported. Series IV and series V are having indolo[2,3-*b*]quinoxaline (**93**) in common as a scaffold. The substitutions were made at 6th and 5th position respectively in series IV and V.

Compounds (**93**, **96** – **106**) in series IV were synthesized by using isatin and different *N*-substituted isatins which were treated with OPD under MW condition for 8-10 minutes in acetic acid to obtain the desired derivatives.

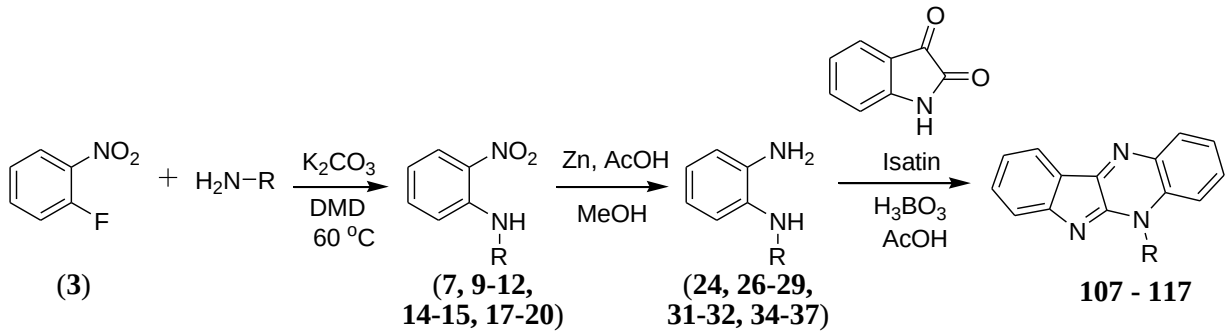


Scheme 4: Synthesis of indolo[2,3-*b*]quinoxaline (93).



Scheme 5: Synthesis of 6-substituted 6*H*-indolo[2,3-*b*]quinoxaline derivatives (96 – 106).

To initiate the synthesis of series V, 1-fluoro-2-nitrobenzene (**3**) was reacted with different amines as per the procedure followed in series I (**scheme 1**). The obtained *N*-arylalkyl 2-nitroaniline intermediates (**7**, **9-12**, **14-15**, **17-20**) were reduced using Zn and acetic acid as per the procedure followed in **scheme 1**. The intermidiates obtained from this step as *N*1-substituted 1,2-diamines (**24**, **26-29**, **31-32**, **34-37**) were used as it is for next step. The 1,2-diamine intermediates were reacted with isatin in presence of boric acid (H_3BO_3) in acetic acid medium to get desired products (**107** – **117**).



Scheme 6: Synthesis of 5-substituted 5*H*-indolo[2,3-*b*]quinoxaline derivatives (107 – 117).

All the synthesized compounds were characterized by FT-IR, ^{1}H-NMR spectroscopy and mass spectrometry while some representative compounds from each series were analyzed by ^{13}C-NMR spectroscopy.

The synthesized compounds were evaluated by means of Ellman's assay to determine their AChE and BuChE inhibition potency. All the compounds showed moderate to good activity against AChE and BuChE and were found to be effective against AD. From series I compounds (**51**) and (**55**) were found to be the most active with IC_{50} values of 4.72 μM and 5.22 μM respectively against AChE; and, 6.98 μM and 5.29 μM respectively against BuChE. These two compounds were then further evaluated for their anti-aggregatory activity for *β*-amyloid (A*β*) in presence and absence of AChE by performing Thioflavin-T (ThT) assay and Congo red (CR) binding assay. Among triazinoindole derivatives (**58** – **70** and **82** – **92**), compound (**85**) showed good activity with IC_{50} of 5.36 μM against AChE and 14.26 μM against BuChE. Compounds from indolo[2,3-*b*]quinoxaline derivatives (**93**, **96** – **106** and **107** – **117**) showed moderate activity against both the enzymes while compound (**114**) showed activity with IC_{50} of 9.42 μM and 13.50 μM against AChE and BuChE respectively. Here, all the compounds showed moderate to good activity on both the targeted enzymes i.e. AChE and

BuChE. The basic substituents as per the requirement of the receptor active sites were covered in all the series synthesized and presented in this thesis and they showed moderate to good activity. This dual nature of these compounds can be further optimized to develop more potent cholinesterase inhibitors.

To understand the ligand receptor interactions the most active compounds from three different scaffolds were docked in the active sites of AChE and BuChE enzymes and the results are presented.