

Alzheimer's disease (AD) is a catastrophic age-related neurodegenerative illness for which there is no recognized cure at this time. It is distinguished by a sharply increasing loss of memory and cognitive function. The number of AD patients is increasing rapidly due to longer life expectancies. Memory loss is one of the most prevalent symptoms, which is followed by language impairment, confusion, mood fluctuations, motivation loss, and, in later stages of the disease, behavioural issues. The body gradually loses its ability to operate, neurons become fewer in number, and finally death occurs. Although the rate of disease progression varies, life expectancy is usually approximately 3 to 9 years.

There isn't a known treatment for AD which can actually halt disease progression. The current therapies are mostly analgesic, with only marginal benefits for symptoms. The three types of therapies that are now available are psychological, pharmacological, and caring. Currently, the USFDA has authorized four main pharmaceutical compounds to treat the cognitive issues associated with AD. These include three acetyl cholinesterase inhibitors, namely galantamine (4), rivastigmine (3), and donepezil (2), and one N-methyl-D-aspartate (NMDA) receptor blocker, memantine (5). The cholinergic inhibitor tacrine (1) was taken off the market in 2013 because it was too harmful to the liver. The chronology of discovery for the marketed anti-AD agents is given in **Fig 1**.

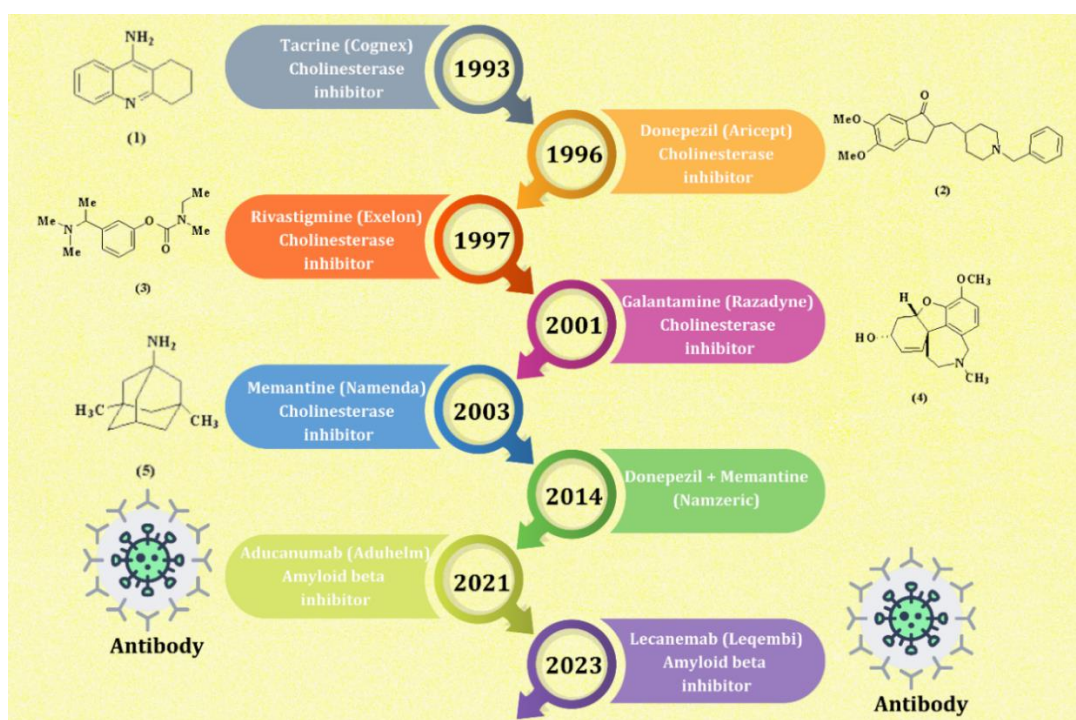


Fig 1: Chronology of approved drugs for the treatment of AD.

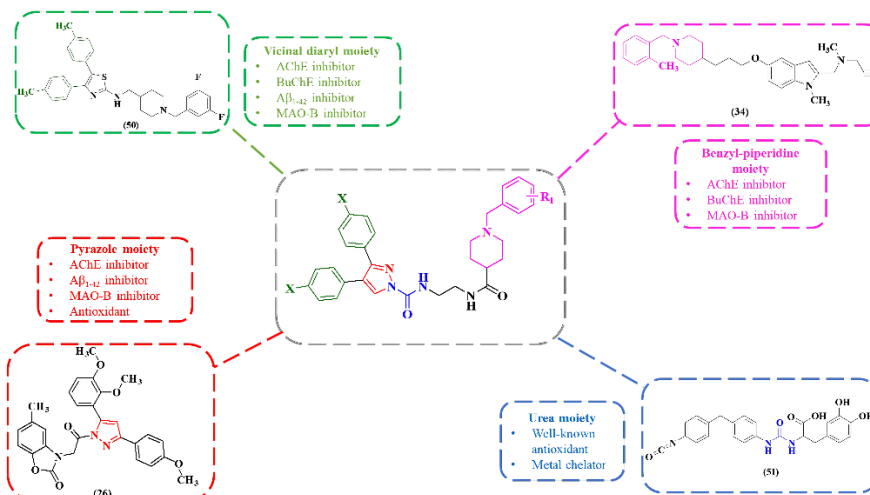
Owing to the complex nature of the illness, no drug has been proven to slow or stop the disease's progression¹⁰. These therapies are mostly successful in treating modest cognitive deficits and offer momentary symptom alleviation; nevertheless, they are unable to stop the course of AD or cure it. Consequently, rather than just providing symptomatic relief for patients, it is crucial to concentrate on creating molecules that truly address the condition, either by treating the disease itself or by delaying the onset of AD.

Pathophysiology:

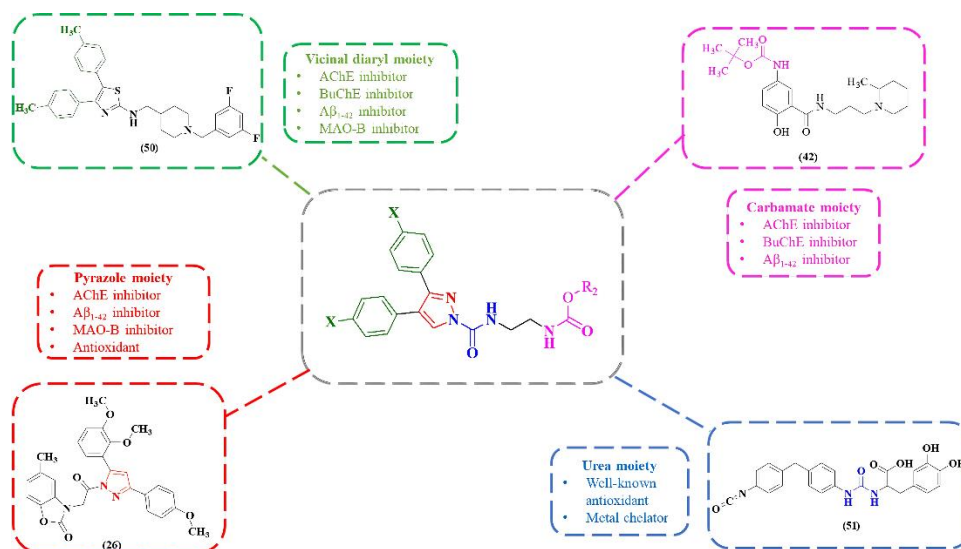
- Cholinergic hypothesis
- Amyloid β -cascade and Tau (τ) hypothesis
- Oxidative stress and Metal ion dyshomeostasis
- Novel targets like MAO-B.

Following a review on recent literature on AD, we concentrated on developing novel MTDLs as the main goal of anti-AD drug development *via* incorporating pharmacophores in order to target multiple etiologies in one single molecule. Cholinesterase inhibitors continue to be the preferred medication for treating AD, despite extensive research on novel targets, even if these treatments are just temporary and symptomatic for patients. Numerous clinical and preclinical findings suggest that the ROS mechanism and A β aggregation are important players in the pathophysiology of AD. Antioxidants or A β aggregation inhibitors by themselves, however, could not be enough to withstand a complex disease situation like as AD. As a result, cholinesterase inhibitors with added antioxidant and MAO-B inhibitory properties may be a good choice to slow the progression of this complex illness thereby acting as neuroprotective and prophylactic agent to lower the progression of disease. The designed molecules are follows:

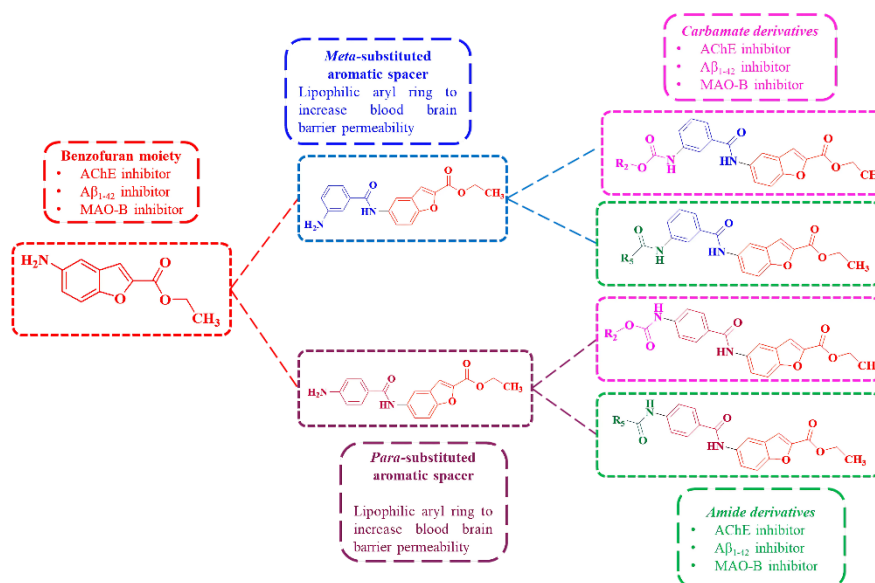
1. Vicinal-diaryl pyrazole fused benzyl piperidine molecules:



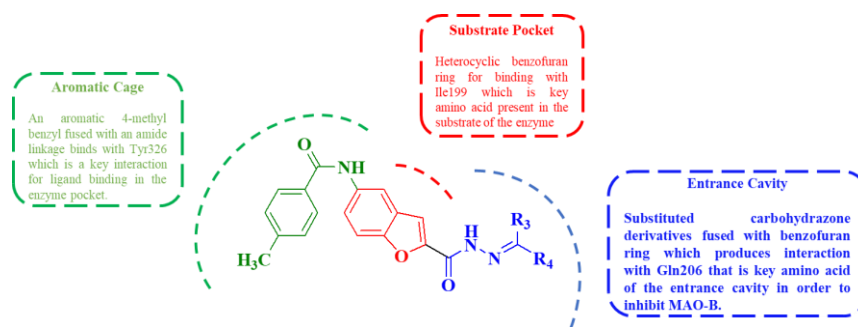
2. Vicinal-diaryl pyrazole fused benzyl piperidine molecules:



3. Benzofuran fused carbamate and amide molecules:



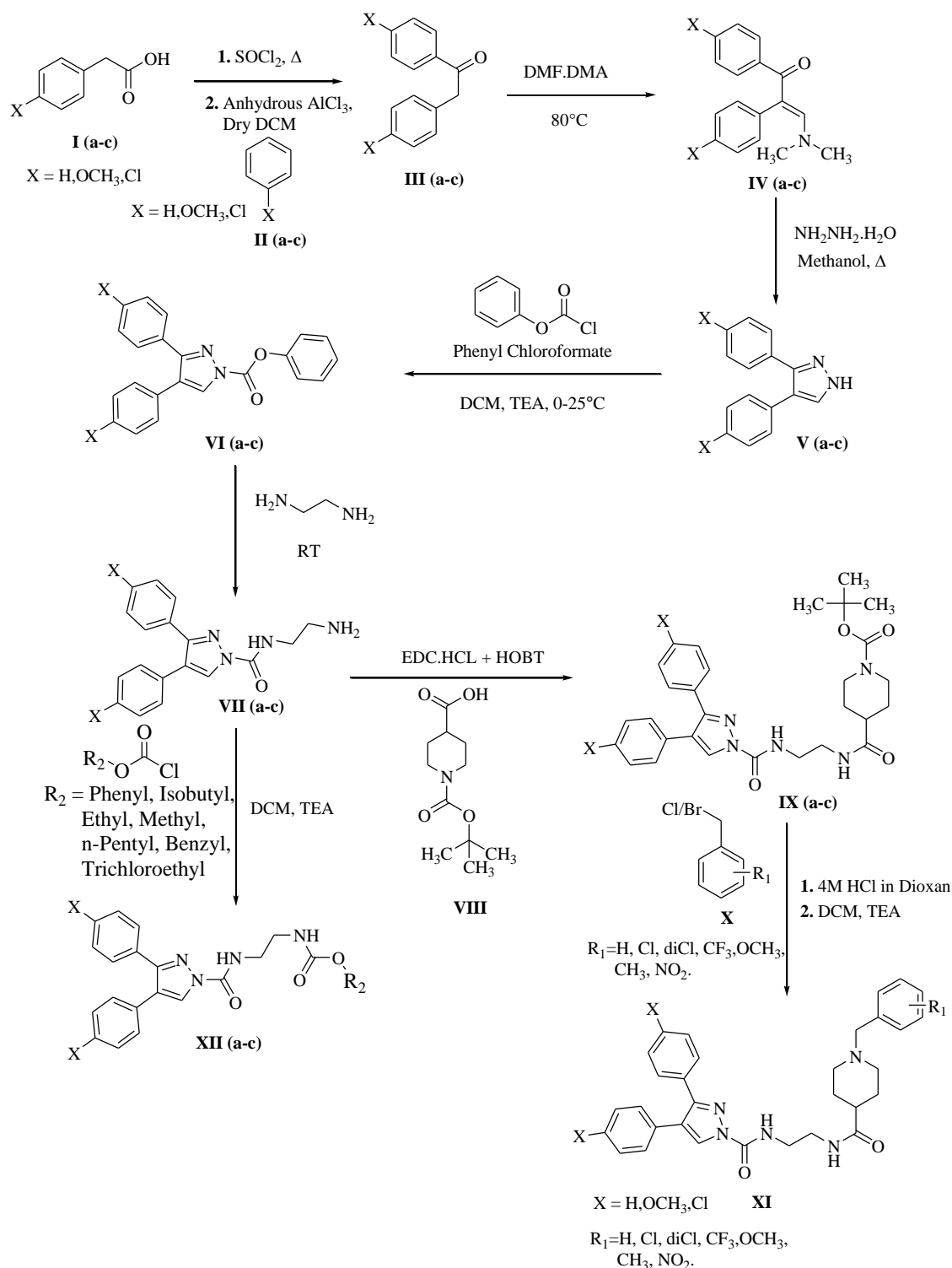
4. Benzofuran fused carbohydrazone molecules:



The proposed compounds were synthesized using synthetic schemes as depicted in **general schemes 1-3**.

Using **general scheme-1**, two different series of vicinal diaryl substituted pyrazole *viz.* *N*-(2-(3,4-diphenyl-1*H*-pyrazole-1-carboxamido)ethyl)-1-benzylpiperidine-4-carboxamide derivatives [**XI (a-c)**] and 2-(3,4-diphenyl-1*H*-pyrazole-1-carboxamido)ethylcarbamate derivatives [**XII (a-c)**] were prepared.

Using thionyl chloride, the acid chlorides were initially made from substituted phenylacetic acids [**I (a-c)**]. 1,2-Diarylethanones [**III (a-c)**] were subsequently generated by Friedel-Crafts acylation employing substituted benzenes [**II (a-c)**] and the acid chlorides. Further, *N,N*-dimethylformamide dimethylacetal (DMF.DMA) was added and heated in solvent free condition to produce substituted (*E*)-3-(dimethylamino)-1,2-diarylprop-2-en-1-one (**IV (a-c)**) which was cyclized to substituted 3,4-diphenyl-1*H*-pyrazole [**V (a-c)**] using hydrazine hydrate. In the next step this compound was treated with phenyl chloroformate to produce substituted phenyl 3,4-diaryl-1*H*-pyrazole-1-carboxylate [**VI (a-c)**] which was then substituted with ethylene diamine to synthesize intermediate *N*-(2-aminoethyl)-3,4-diaryl-1*H*-pyrazole-1-carboxamide substituents [**VII (a-c)**]. This derivative was coupled with 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid (**VIII**) via acid amine coupling in presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC.HCl) and hydroxybenzotriazole (HOBt) under basic condition to prepare *tert*-butyl 4-(2-(3,4-diaryl-1*H*-pyrazole-1-carboxamido)ethylcarbamoyl)piperidine-1-carboxylate derivatives [**IX (a-c)**]. The protected amine was deprotected using 4M HCl in dioxan and was further reacted with different benzyl halides (**X**) to obtain final compound *N*-(2-(3,4-diaryl-1*H*-pyrazole-1-carboxamido)ethyl)-1-benzylpiperidine-4-carboxamide derivatives [**XI (a-c)**].

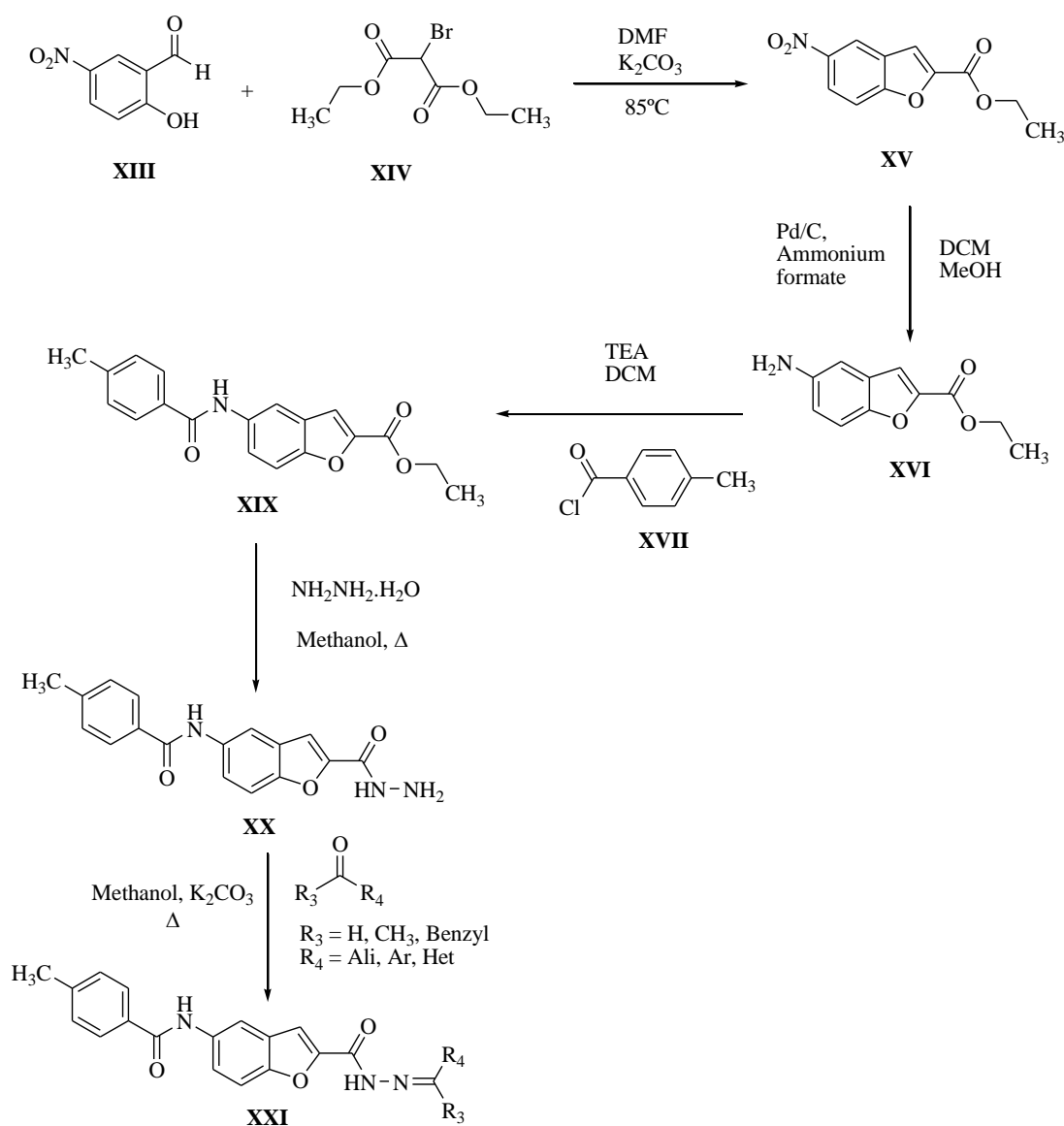


General Scheme-1

On the other hand, [**VII (a-c)**] reacted with various chloroformate derivatives to produce final compounds [**XII (a-c)**] containing carbamate derivatives.

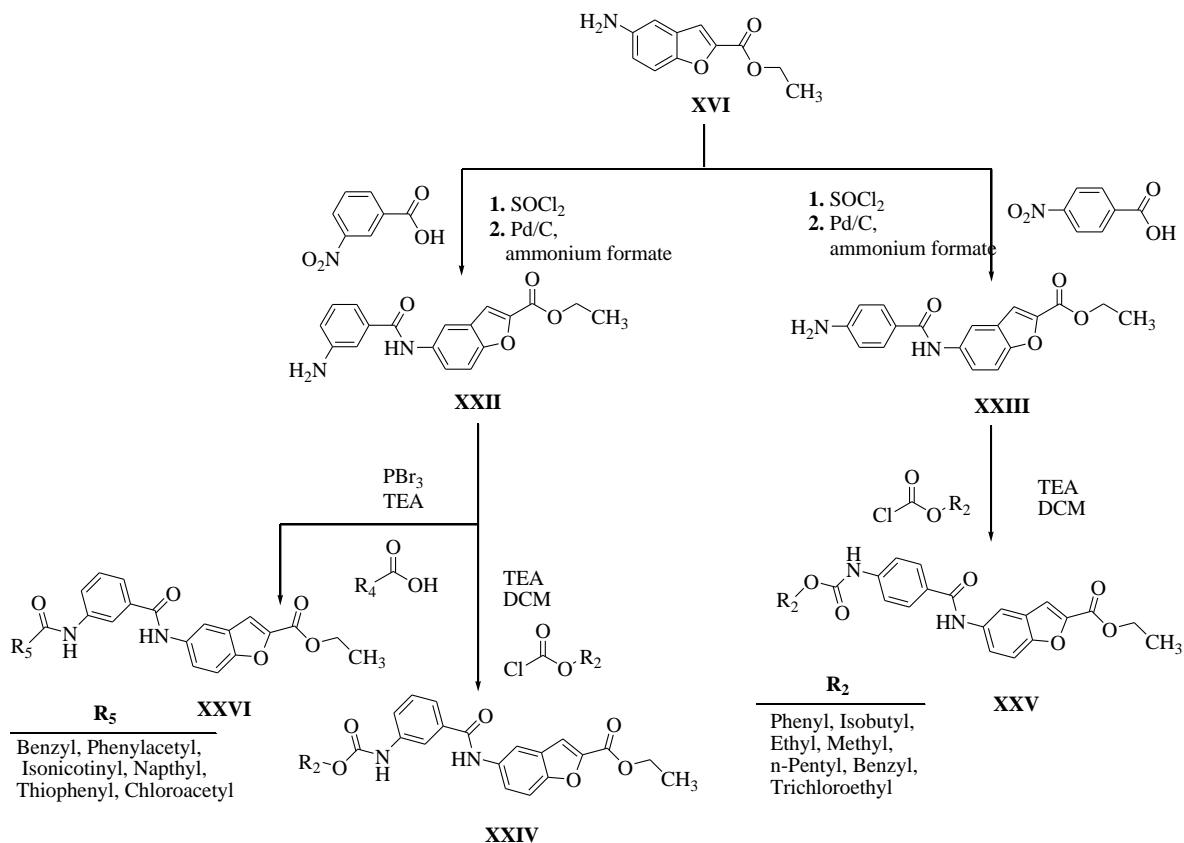
To synthesize the envisaged compounds, **general scheme-2** was adopted, wherein 2-hydroxy-5-nitrobenzaldehyde (**XIII**) reacted with potassium carbonate. Subsequently, cyclization using diethyl 2-bromomalonate (**XIV**) produced ethyl 5-nitrobenzofuran-2-carboxylate (**XV**). Further reduction was carried out using ammonium formate and Pd/C to obtain ethyl 5-aminobenzofuran-2-carboxylate (**XVI**).

Ethyl 5-aminobenzofuran-2-carboxylate (**XVI**) and 4-methylbenzoyl chloride (**XVII**) produced ethyl 5-(4-methylbenzamido)benzofuran-2-carboxylate (**XVIII**) which when further reacted with hydrazine hydrate afforded 5-(4-methylbenzamido)benzofuran-2-carbohydrazide (**XIX**). The final step included generation of final Schiff base compounds (**XX**).



General Scheme 2

The acid chloride was prepared from 3-aminobenzoic acid using thionyl chloride followed by subsequent, nucleophilic substitution (S_N2) using ethyl 5-aminobenzofuran-2-carboxylate (**XVI**) and acid chloride to produce ethyl 5-(3-nitrobenzamido)benzofuran-2-carboxylate which was then reduced with Pd/C to produce intermediate 5-((3-amino)benzamido)benzofuran-2-carboxylate (**XXII**). In the next step, substituted chloroformates were treated with Ethyl 5-((3-amino)benzamido)benzofuran-2-carboxylate (**XXII**) in the presence of triethyl amine in dichloromethane at room temperature by the nucleophilic substitution (S_N2) to obtain substituted Ethyl 5-((3-carbamate)benzamido)benzofuran-2-carboxylate (**XXIV**) on the other hand, various carboxylic acid derivatives were coupled with ethyl 5-((3-amino)benzamido)benzofuran-2-carboxylate (**XXII**) to produce ethyl 5-(3-substitutedbenzamido)benzofuran-2-carboxylate (**XXVI**).



Another acid chloride was prepared from 4-aminobenzoic acid using thionyl chloride and ethyl 5-aminobenzofuran-2-carboxylate **XVI** to produce ethyl 5-(4-nitrobenzamido)benzofuran-2-carboxylate subsequently getting reduced in the presence of Pd/C to produce ethyl 5-((4-amino)benzamido)benzofuran-2-carboxylate **XXIII**. In the next step, substituted chloroformates were treated with ethyl 5-((4-amino)benzamido)benzofuran-

2-carboxylate **XXIII** in the presence of triethyl amine and dichloromethane at room temperature by the nucleophilic substitution (SN_2) to obtained substituted ethyl 5-((4-carbamate)benzamido)benzofuran-2-carboxylate **XXV**.

Biological evaluation:

The proposed derivatives from general scheme 1, containing a vicinal diaryl-pyrazole scaffold combined with substituted benzylpiperidine (**XI**) and carbamates (**XII**) through urea linker were evaluated for inhibition of cholinesterase (ChEs) and monoamine oxidase B enzymes (MAO-B). The biological activity data proved that the proposed compounds possess potent MAO-B inhibitory activity having >90% inhibition at 10 μ M concentration. Compound (**152**) showed the most potent MAO-B having IC_{50} value of 92.86 ± 7.66 nM inhibitory activity. Compounds (**121**, **96**, **111** and **140**) showed IC_{50} values of 101.4 ± 10.62 nM, 109.3 ± 20.78 nM, 146.8 ± 22.15 nM and 1448.5 ± 18.25 nM respectively. The compounds from this series (**144**, **152**) showed good AChE inhibitory activity having inhibition of 89.5 % and 94.7% respectively. Various other activities including neurotoxicity studies, metal chelation and anti-oxidant studies are reported for the synthesized compounds.

Proposed derivatives from general scheme-2 and 3, containing benzofuran derivatives wherein the benzofuran scaffold is clubbed with carbohydrazide (**XXI**), carbamate (**XXIV**), (**XXV**) and amide (**XXVI**) functional groups. The compounds were also evaluated for their ChEs and MAO-B inhibitory activities. Surprisingly, this benzofuran-containing series was found to be a negative inhibitor of MAO-B. Furthermore, these compounds were found to possess moderate to low activity against ChE enzymes. Metal chelation and anti-oxidant studies were performed and are reported for the synthesized compounds.

Conclusion:

With an aim to design and develop novel therapeutics for the treatment of Alzheimer's disease, three general schemes were adopted to synthesize proposed compounds. According to the literature survey conducted, combination of various scaffolds was designed and docked to check the interaction with AChE, BuChE and MAO-B enzyme. Four derivatives were finalized and proposed for further development as anti-AD agents. All the proposed compounds have been confirmed for their structures by IR, 1H & ^{13}C -NMR, HRMS and mass analysis. Using general scheme 1, a vicinal diaryl-pyrazole scaffold was combined with substituted

benzylpiperidine (91-121) and carbamates (130-153) through urea linker. It was envisaged that the newly synthesized compounds would be potential anti-Alzheimer's agents via inhibition of cholinesterase (ChEs) and monoamine oxidase B enzymes (MAO-B). Presence of nitrogen containing pyrazole heterocycle could be helpful in controlling the basicity of the resulting compounds which would be crucial for the BBB permeation. The biological activity data proved that the proposed compounds possess potent MAO-B inhibitory activity having >90% inhibition at 10 μ M concentration. Compound (152) showed the most potent MAO-B having IC₅₀ value of 92.86 \pm 7.66 nM inhibitory activity. Compounds (121, 96, 111 and 140) showed IC₅₀ values of 101.4 \pm 10.62 nM, 109.3 \pm 20.78 nM, 146.8 \pm 22.15 nM and 1448.5 \pm 18.25 nM respectively. The compounds from this series (144, 152) showed good AChE inhibitory activity having inhibition of 89.5 % and 94.7% respectively. Various other activities including neurotoxicity studies, metal chelation and anti-oxidant studies are reported for the synthesized compounds.

Using general scheme-2 and 3, an attempt was also been made to prepare novel benzofuran derivatives wherein the benzofuran scaffold is clubbed with carbohydrazide (181-199), carbamate (203-208), (212-215) and amide (222-227) functional groups. The compounds so synthesized were also evaluated for their ChEs and MAO-B inhibitory activities. Surprisingly, this benzofuran-containing series was found to be a negative inhibitor of MAO-B. These compounds were found to possess moderate to low activity against ChE enzymes. Metal chelation and anti-oxidant studies were performed and are reported for the synthesized compounds.

Finally, with all the work carried out it can be concluded that the vicinal diaryl pyrazole-based molecules are proved as a lead molecule and can be further investigated to develop as small molecules to treat Alzheimer's disease.