3. Aim and Objectives

Alzheimer's disease (AD), the most common form of dementia, is a progressive neurological illness that primarily affects the elderly. Around 50 million people globally suffer from AD, and if no cure or preventative measures are discovered quickly, that figure will sharply increase to 150 million by the year 2050. Etiology of AD is complex; several variables, including tau hyperphosphorylation, oxidative stress, low levels of ACh, Aβ aggregation, free radicals and dyshomeostasis of biometals play role in AD.

Donepezil **(1)**, a blockbuster drug for treatment of AD still has significant problems even with its great safety, tolerance, and pharmacological profile. For the treatment of early to middle stages of AD, donepezil may be useful. However, it fails to stop or totally eradicate the cognitive deficits; it can only be helpful in managing the symptoms or delaying the course of AD as the disease-causing root factors are not addressed with the use of donepezil hydrochloride. Donepezil's poor effectiveness makes it unsuitable for treating severe and advanced stages of AD. This is the reason that medicinal chemists working on AD research have been working so hard to create rather innovative drug molecules targeting multiple etiologies. to treat the disease, that are safe and effective over the long run.

The reaction catalysed by monoamine oxidase-B (MAO-B) leads to the production of reactive aldehydes, hydrogen peroxide (H_2O_2), and either an alkyl-substituted amine or ammonia in brain[1]. These byproducts are neurotoxic as it regulates APP gene metabolism by reacting with PS1 thereby affecting the Aβ formation in brain. On the other hand, the oxidative species formed due MAO is responsible for hyperphosphorylation of tau protein and oxidative stress leading to neuron disintegration and cell death. Research, both in preclinical and clinical settings, has suggested that the metabolites of MAO oxidation contribute to neurotoxicity, thereby playing a role in the pathophysiology of AD. Therefore, MAO-B becomes a crucial target for drug discovery of AD.[2]

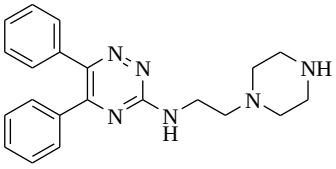
One of the most promising drug discovery strategies for treating AD-like illnesses with a complicated etiology is the creation of multitarget-directed ligands, or MTDLs.[3] Even with great affinity and selectivity, drugs that only operate on one target may not have a sufficient impact on the intricate etiology of the illness. More advantageous effects can still be obtained from an MTDL with balanced, moderate affinity for the targets than from a

mono-targeted ligand. Higher safety and a lower chance of therapeutic resistance may be achieved by a gentle and well-balanced action on several therapeutic targets.[4]

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. MAO-B is one novel target of AD which is widely considered and developed due to its role in multiple pathophysiologies in progression of AD. On the other hand, 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. 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.

3.1. Design and development of vicinal diaryl pyrazole based anti-AD inhibitors

- Our research group has been actively involved in the design and development of various vicinal diaryl **(48-50)** and have successfully reported vicinal diaryl thiazoles[5] and triazines[6] containing heterocyclic systems as anti-Alzheimer agents. The reports suggest that vicinal diaryls, because of its lipophilic nature binds at the peripheral anionic site (PAS) via interaction with Trp286 of AChE enzyme. Moreover, according to results obtained from *in silico* molecular docking studies performed, the data suggests vicinal diaryl having a prominent role in binding at the hydrophobic substrate part and interact with Try326 similar to aryl ring of marketed drug safinamide. Therefore, vicinal diaryl was considered as one of the active pharmacophores for the design and synthesis of MTDL.



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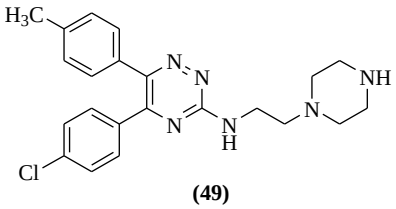

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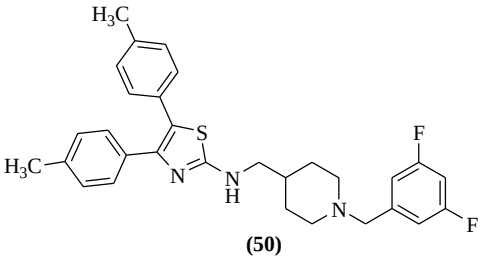
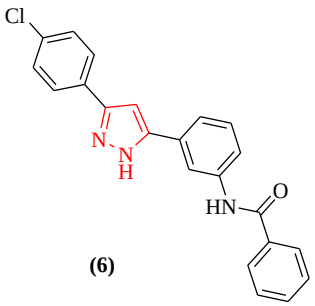


(50)

Compound	AChE IC_{50}	BuChE IC_{50}
48	4.23 µM	13.3 µM
49	5.79 µM	163.4 µM
50	0.30 ± 0.01 µM	0.03 µM

- According to literature survey conducted, compound **(6-26)** containing pyrazole moiety have been reported for various anti-AD targets including cholinesterase inhibitors, amyloid inhibitors and potent monoamine oxidase inhibitors along with a decent antioxidant property. Therefore, pyrazole was considered as a heterocycle.



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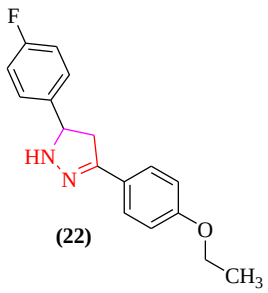

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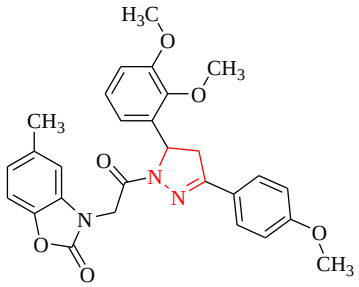


(26)

Compound	AChE IC_{50}	BuChE IC_{50}	MAO-A IC_{50}	MAO-B IC_{50}
6	1.937 ± 0.066 µM	1.166 ± 0.088 µM	-	-
22	-	-	-	0.063 ± 0.0042 µM
26	-	-	0.001± 0.022 µM	-

- Urea functional group is reported as a potent free radical scavenger **(**compound **51-52)** and therefore, urea was considered and incorporated in as a part of spacer in the designed molecules.

- Benzyl piperidine, a well-known pharmacophore of Donepezil **(1)** which is supposed to bind at CAS site of AChE was considered. Furthermore, literature **(**compound **28-29, 32-35)** suggests benzyl piperidine to posses MAO inhibitory activity thus, benzyl piperidine was added.

Considering above discussed points designing of vicinal diaryl pyrazole fused benzyl piperidine derivatives as shown in **Fig 3.1,** that has a vicinal diaryl fused with pyrazole moiety which is separated from benzyl piperidine pharmacophore with a spacer having urea functional group.

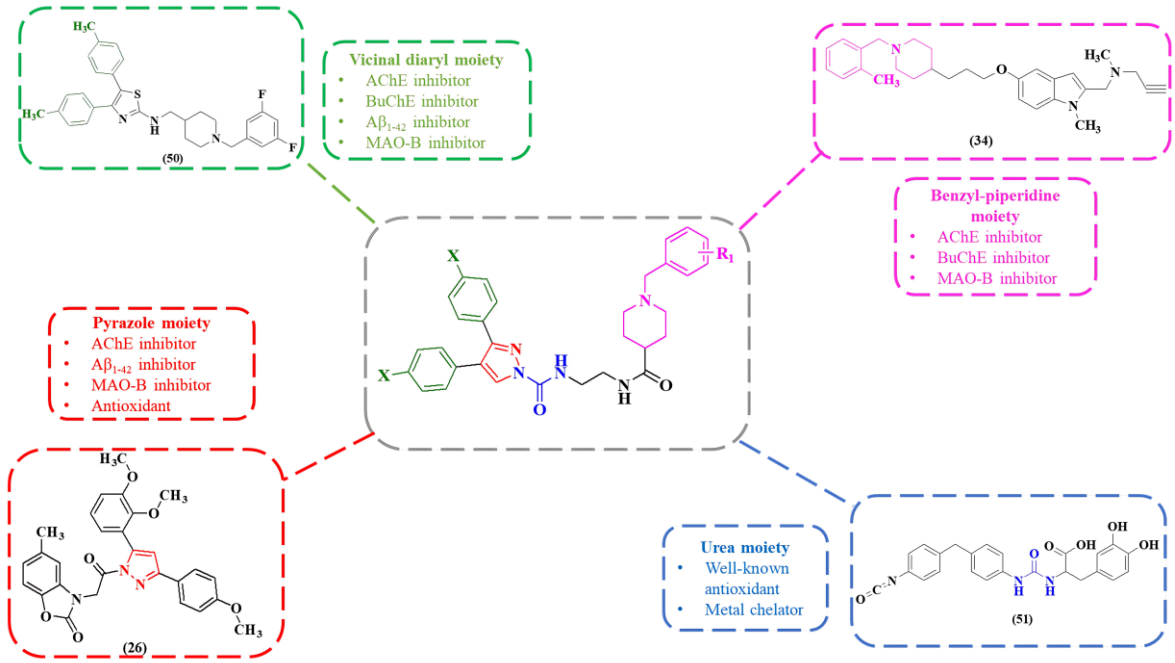


Fig 3.1: Designing of vicinal diaryl pyrazole fused benzyl piperidine derivatives.

- Carbamate moiety is also reported to be active in management of AD. Rivastigmine, a well-known marketed drug contains a carbamate functional group, which is responsible for binding at CAS site of AChE. Moreover, literature survey (compound **37-42**) also confirms carbamate group to have potent activity on AChE and Aβ inhibition. Therefore, carbamate group was considered in designing of novel molecules.

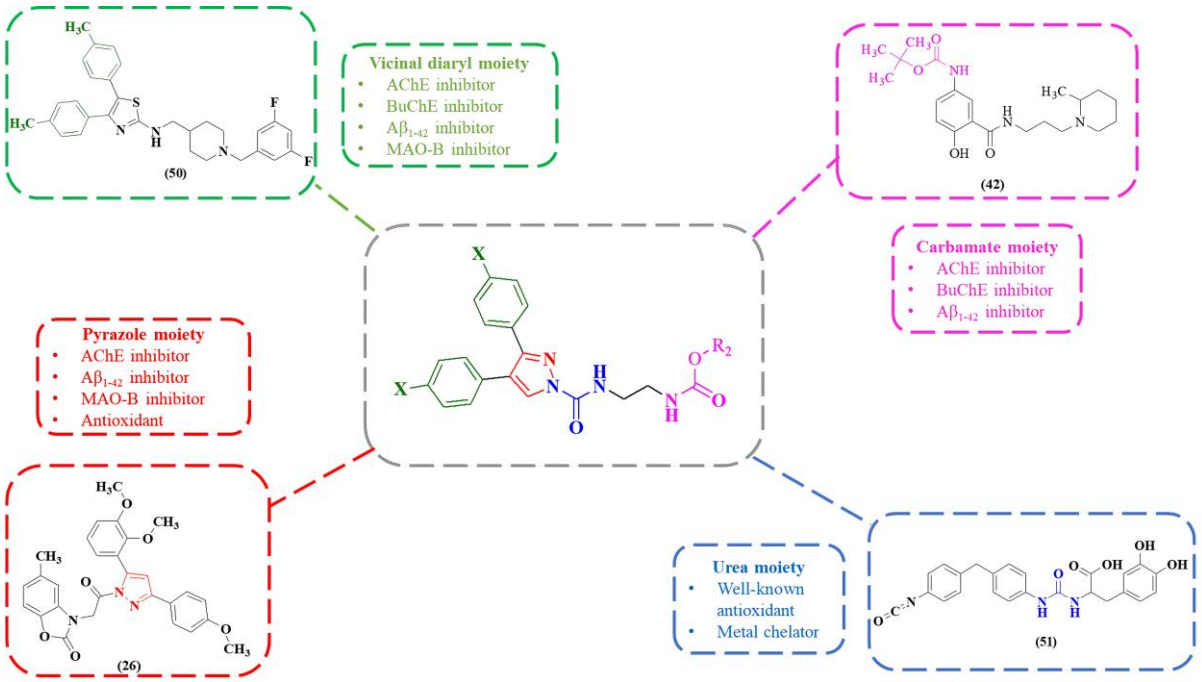


Fig 3.2: Designing of vicinal diaryl pyrazole fused carbamate derivatives.

A vicinal diaryl pyrazole fused carbamate derivatives were designed which is shown in (**Fig 3.2).** A vicinal diaryl fused with pyrazole moiety is separated from carbamate functional group with a spacer having urea functional group.

3.2. Design and development of benzofuran based amide and carbamate derivatives as anti-AD inhibitors

- Galantamine **(3)** along with other benzofuran derivatives covered in literature survey **(**compound **43-47)** have been reported for various targets of AD including cholinesterase inhibitors, β-secretase-1 inhibition, antioxidants and $A\beta_{25-35}$ aggregation inhibition. Benzofurans have been reported for MAO inhibitory activity and therefore benzofuran was considered as basic pharmacophore having MTDL property.

- Rivastigmine **(2)** possessing a carbamate moiety is responsible for controlling AD having significant biological properties such as β-secretase-1 inhibition and cholinesterase inhibitory activity along with MAO inhibition, brought researcher's attention to carbamate as a primary scaffold for building new anti-AD drugs. During *in silico* molecular docking studies conducted by us, the carbamate functional group

was replaced with amide group and better interactions were observed therefore both the functional groups were considered in order to establish correlation.

- An aromatic spacer separating two pharmacophores, benzofuran and carbamate was considered in order to increase lipophilicity of the compound and substitution on *meta* and *para* position was considered to evaluate the effect of position.

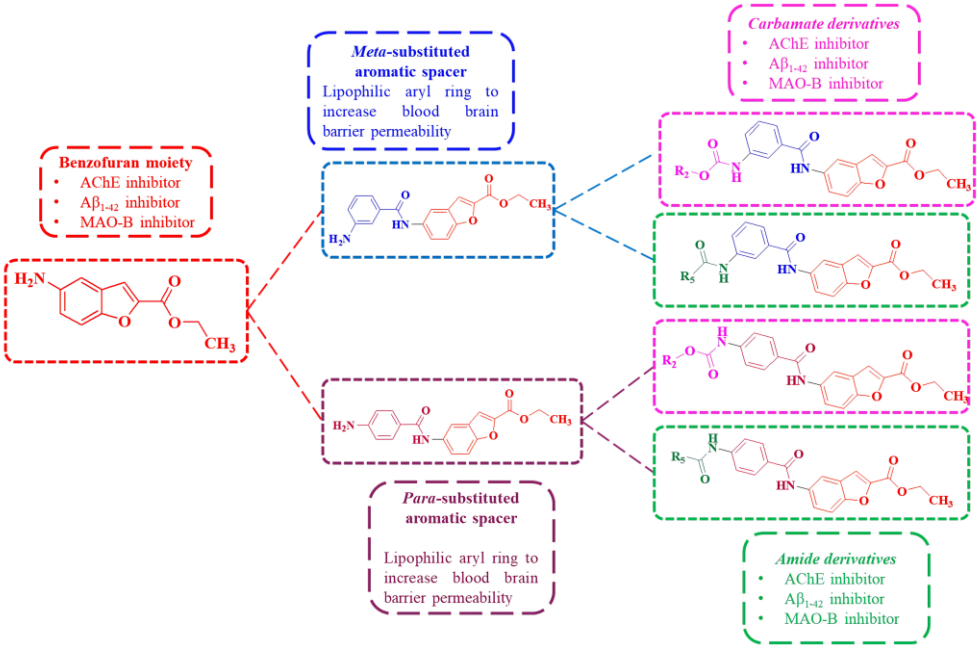


Fig 3.3: Designing of benzofuran fused carbamate and amide derivatives.

Considering above discussed points designing of benzofuran fused carbamate and amide derivatives was done which is shown in (**Fig 3.3).** A benzofuran fused with various carbamate and amide derivatives which separated by aromatic ring as a spacer to increase the overall lipophilicity of the compounds were designed to increase the BBB permeability.

3.3. Design and development of benzofuran based carbohydrazide derivatives as anti-AD inhibitors

Another derivative from benzofuran was designed specifically for MAO-B inhibitory activity which was optimized with the help of molecular docking procedure. The MAO-B receptor is essential to be discussed along with its active sites (**Fig 3.4)** before designing the molecule for the same.

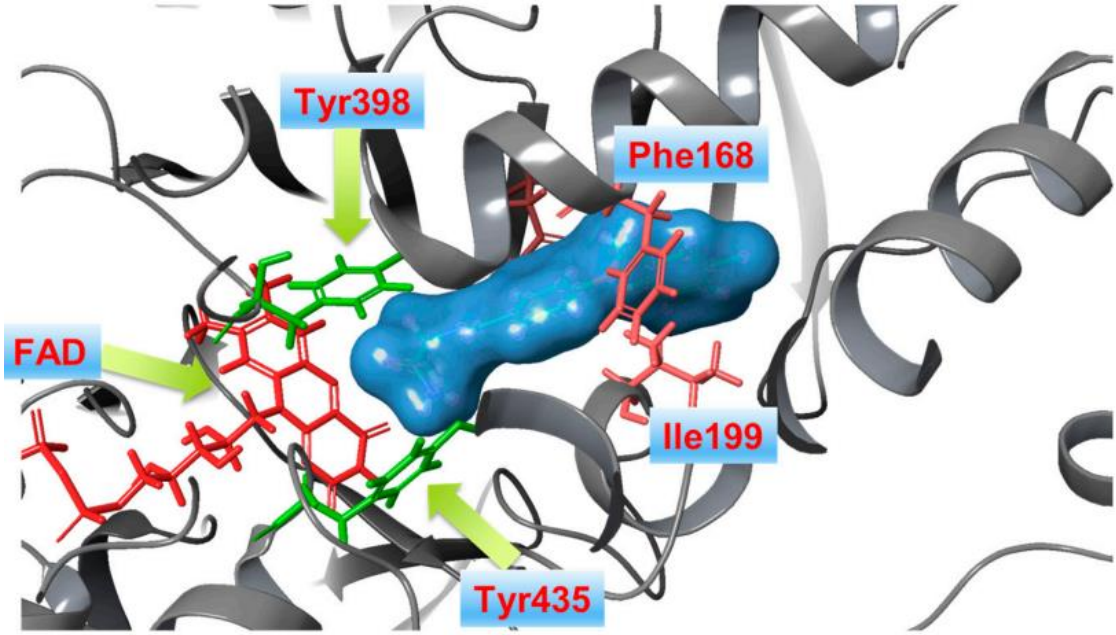


Fig 3.4: The blue mesh in the X-ray structure of *h*MAO-B in association with safinamide (PDB: 2V5Z). Red represents the coenzyme flavin-adenine dinucleotide (FAD). Tyr398 and Tyr435 amino acids, which together create the "aromatic cage," are shown in green. [7]

- The binding site of MAO-B is comprised of three distinctive functional regions known as the entrance cavity, substrate pocket, and the "aromatic cage." Among these regions, the entrance cavity (measuring 290 $Å^3$) and the substrate pocket which is a part of aromatic cage (measuring 490 $Å^3$) combine to form an active binding site **(Fig 3.5)** with a volume of approximately 700 $Å^3$ [8–12].

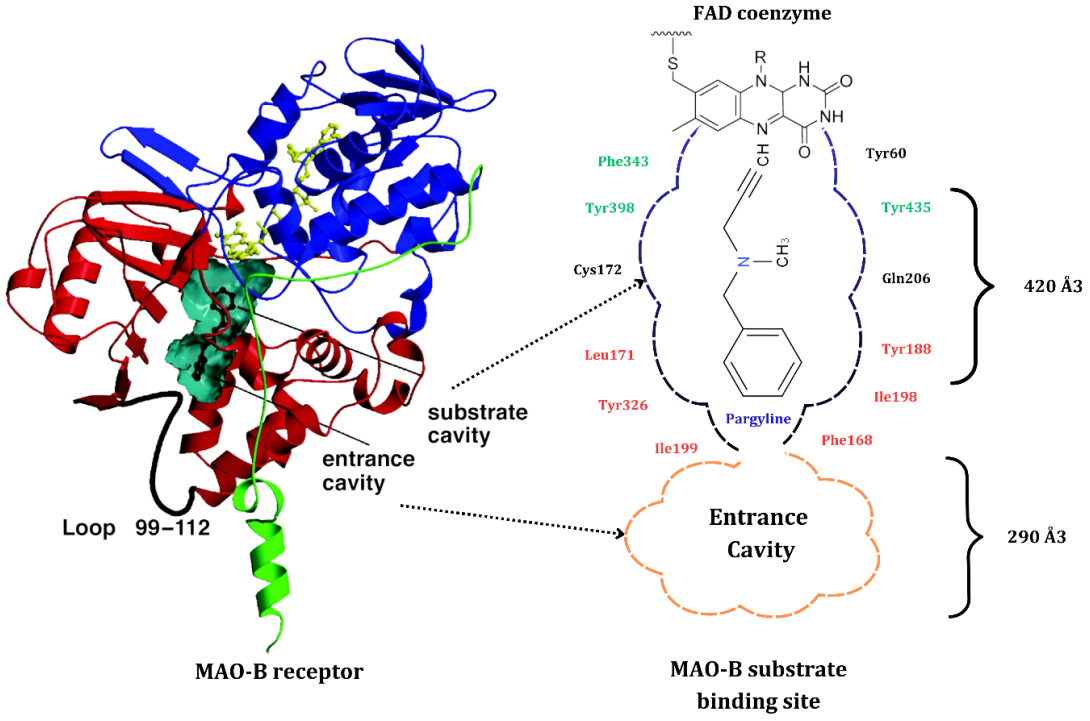


Fig 3.5.: Schematic diagram of active binding site of MAO-B.

- The separation of these two pockets is accomplished by a loop consisting of four amino acid residues: Phe168, Leu171, Ile199, and Tyr326. These residues play a crucial role in maintaining the stability of the complex and aiding the ligand in reaching the substrate pocket. Notably, Ile199 functions as the gate of the active site and can transition between an open and closed state depending on the presence of the ligand. In the closed state, in the absence of the ligand, Ile199 obstructs the space between the two cavities[12–15].
- Furthermore, Ile199 also contributes to the selectivity of MAO-B. The inhibitors of MAO-B also interact with Tyr326, which is a prerequisite for achieving selective inhibition. Positioned at the junction of both the substrate and aromatic gorge, Tyr326 acts as a barrier for the substrate cavity and imposes steric constraints in the active site of MAO-B. The aromatic cage of MAO-B consists of FAD, Tyr398, and Tyr435, all of which play a role in the catalytic effect of the protein[16–19].
- Here, co-crystallized ligand "safinamide" of 2V5Z into MAO-B (**Fig 3.6)** interacts with both entrance and substrate gorge where it interacts with Tyr326, Ile199 and Gln206 and FAD600, respectively.

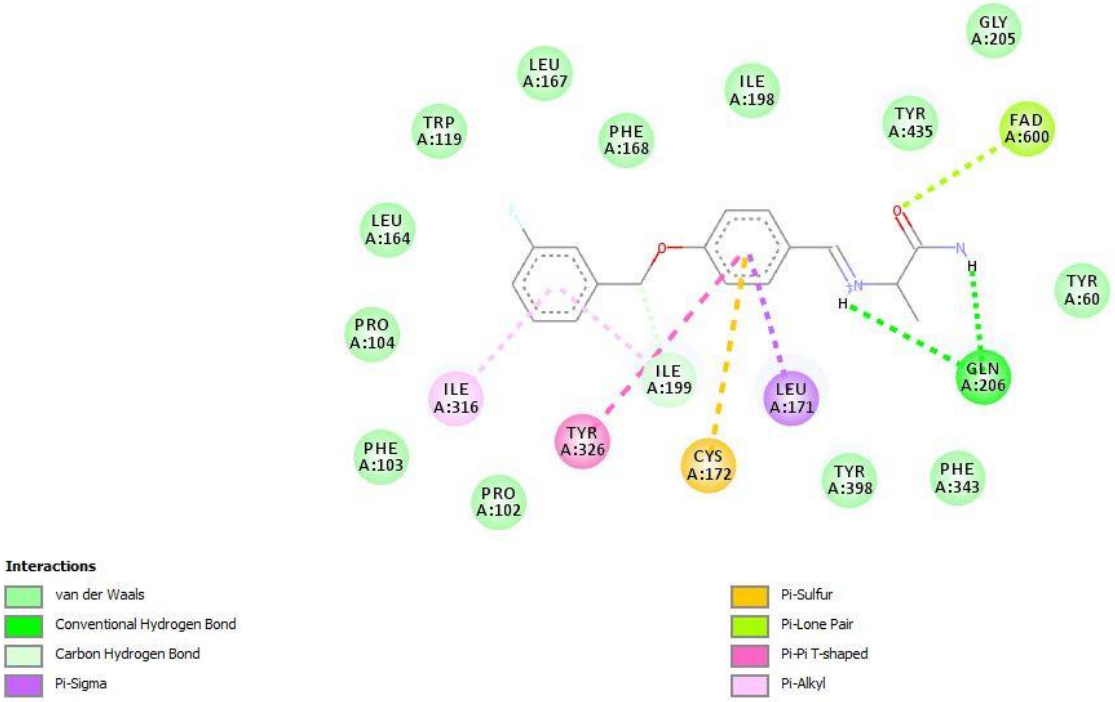


Fig 3.6: Interactions of co-crystalized ligand safinamide with MAO protein pdb: 2V5Z.

- In the literature survey[20-21], there are reports of Schiff base derivatives along with hydrazone having nanomolar activity and showing interactions with key amino acids of the active site of MAO-B receptor protein, therefore we decided to fuse

carbohydrazide with benzofuran to produce novel MAO-B inhibitor to treat AD as shown in **Fig 3.6.**

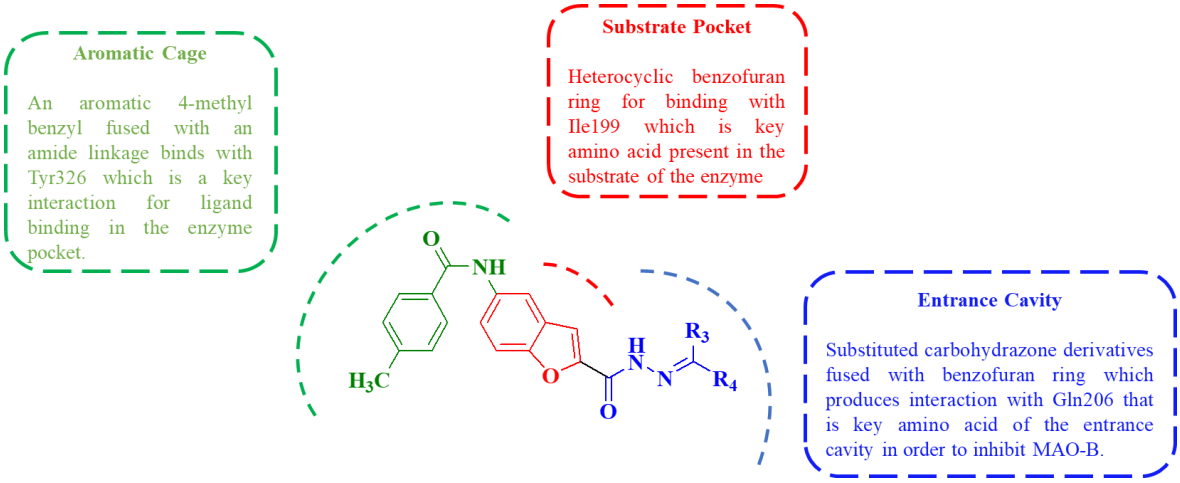


Fig 3.7: Designing of compound benzofuran based MAO-B inhibitors.

- On the basis of the enzyme discussed and literature survey conducted, the molecule was designed with the help molecular docking wherein, benzofuran was considered as central moiety that interacted with substrate pocket via Tyr398 and Tyr435 which was fused with a hydrophobic 4-methyl benzyl ring on one side via amide linkage that interacted strongly with aromatic cage via Try326, Cys172, Leu171 and Ile199 and on the other side carbohydrazide was fused which bonded at the entrance cavity by interacting with key amino acid Tyr435 which is shown **Fig 3.7.**

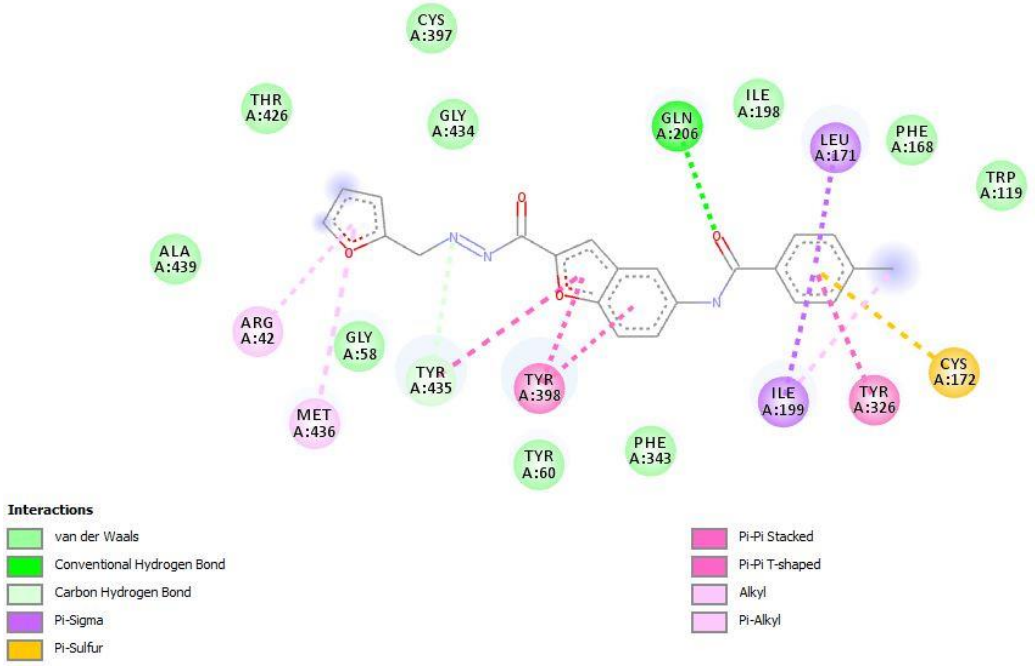
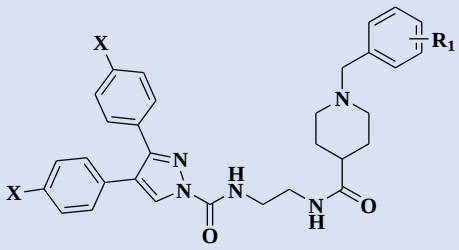
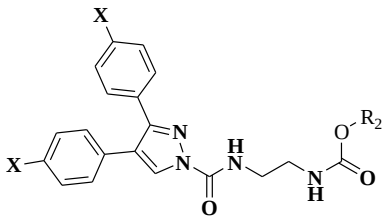
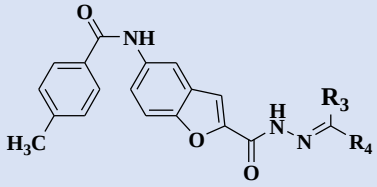


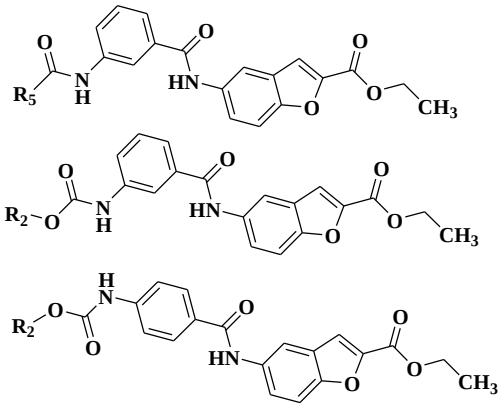
Fig 3.8: Interactions of proposed designed molecule with MAO protein pdb: 2V5Z

The aim of the present research was to synthesize novel molecules (**Table 3.1.**) as potential anti-Alzheimer's agents. The main objectives of the experiment were as follows:

- Design and synthesis of derivatives (**I-IV**) mentioned in **Table 3.1**.
- To establish the method for the synthesis of intermediates and proposed compounds.
- To confirm the structures of the synthesized compounds by IR, ^{1}H-NMR, ^{13}C-NMR, HRMS and MS analysis.
- To evaluate the proposed compounds for various anti-Alzheimer's activity like MAO-B, and cholinesterase (AChE & BuChE) inhibitory effects along with neurotoxicity, metal chelation and antioxidant assay.

Table 3.1.: Proposed molecules for synthesis.

Derivative	Structure	Remark
Vicinal diaryl pyrazole fused benzyl piperidine derivatives **(I)**		**X**= H, OCH_3, Cl **R_1**= H, Cl, diCl, CF_3, OCH_3, CH_3, NO_2
Vicinal diaryl pyrazole fused carbamate derivatives **(II)**		**X**= H, OCH_3, Cl **R_2** = Phenyl, Isobutyl, Ethyl, Methyl, n-Pentyl, Benzyl, 2,2,2-Trichloroethyl, 9*(H)* Fluorenyl methyl
Benzofuran fused carbohydrazide derivatives **(III)**		**R_3**= H, CH_3, Benzyl **R_4** = Aromatic, Aliphatic, Heterocyclic.

Benzofuran fused carbamate and amide derivatives **(IV)**		**R_2** = Phenyl, Isobutyl, Ethyl, Methyl, n-Pentyl, Benzyl, 2,2,2-Trichloroethyl, 9*(H)* fluorenyl methyl **R_5** = Benzyl, Phenylacetyl, Isonicotinyl, Napthyl, Chloroacetyl.

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