Chapter 2

Literature Review
2.1 REVIEW OF LITERATURE

Among the family of five membered nitrogen containing heterocycles, 2-pyrazolines are recognized as a promising scaffold to inhibit monoamine oxidases (MAO). These are considered as a suitable pharmacophore, among diverse heterocyclic chemotypes integrating two nitrogen atoms, for synthesizing selective MAO inhibitors as these are prepared by the cyclization of various hydrazine derivatives. Suitably substituted pyrazolines are considered as valuable lead molecules endowed with ample number of biological activities. In the recent past, substitution at 1\textsuperscript{st}, 3\textsuperscript{rd} and 5\textsuperscript{th} position of the pyrazoline scaffold has displayed remarkable activities towards MAO isoforms. However, selectivity of pyrazoline nucleus towards MAO isoenzyme depends upon the bulkiness of the ring at 1\textsuperscript{st} and 3\textsuperscript{rd} place of the scaffold. Therefore, majority of them were evaluated as dual inhibitors against diverse group of enzymes in a synergistic manner (Cyclooxygenase, Acetylcholinesterase, Butyrylcholinesterase). Moreover, because of direct involvement of this scaffold in MAO inhibition, biological activities such as antidepressant, anti-anxiety and anticonvulsant properties in animal models are pertinent to them.\textsuperscript{206, 207}

- Nayak et al. (2015) synthesized some new pyrazoline carbamate derivatives 56 and evaluated them as MAO inhibitors. All the synthesized derivatives showed selective MAO-A inhibition and the phenyl carbamate analogs were more potent than ethyl carbamates, displaying excellent selectivity index. The most potential candidate of the series with the $K_{i_{MAO-A}}$: 4.96 ± 0.21 nM exhibited almost equal potency to the standard drug, Moclobemide ($K_{i_{MAO-A}}$: 5.01 ± 0.13 nM) with a superior selectivity index ($8.86 \times 10^{-5}$). Molecular docking simulations with $R$ and $S$ conformers of the most active derivative revealed the $S$-enantiomer to be better than the $R$-enantiomer, as reported earlier.\textsuperscript{208}
Mertens et al. (2014) synthesized alkynyl-coumarinyl ethers through hydroxyl coumarins, and tested for their hMAO-B inhibitory activity. The position of the alkynyloxy chain and the residue at 3-position of the pyran-2H-one part played a major role to optimize the inhibitory activity. The 3-methoxycarbonyl derivative was found to possess dual inhibition of MAO-A and MAO-B.

A series of 1-[2-((5-chloro/methyl)-2-benzoazolinone-3-yl)acetyl]-3,5-diaryl-2-pyrazoline analogs were synthesized and characterized by Salgin-Goksen et al. (2013). All the derivatives were examined for their selective MAO inhibitory activity using in vitro tests. MAO inhibitory activity of the prepared derivatives was comparable with that of the standard drugs, moclobemide and selegiline, acting in a competitive and reversible manner. Also, the binding paradigm of N-substituted pyrazoline derivatives as novel MAO-A inhibitory agents were investigated using docking calculation and quantum chemical modeling tools, by Erdem et al. (2013).

MAO-B is a validated target for many neurodegenerative diseases. Recently, Mishra et al. (2012 and 2011) performed structure based virtual screening against an in-house library to screen out some potential hits, 57.

They synthesized and tested these compounds against both MAO-A and B inhibitory activities. All the compounds were selectively active towards MAO-B enzymes in the nM range (100 folds higher potency than selegeline). The most potent derivative with $K_i \leq 0.31\text{nM}$ and MAO-B/A selectivity index $\geq 100$ was selected for preclinical studies, showing maximal liver and brain MAO-B activity at 7 mg/kg, i.p., dose, which was reversed after 8 h of administration. The stereotype behavior was also markedly potentiated by this compound, showing protection against reserpine induced glutathione oxidation and partial reversal of reserpine induced dyskinesia in cerebrum without any symptoms of hypertensive crisis on tyramine co-treatment.
Jagrat et al. (2011) synthesized twenty two 2-pyrazoline derivatives 58-60 and tested them for their hMAO inhibitory activity. Presence of ring at N-1 position increased its potency and the selectivity index (SI) towards hMAO-A.180

Sahoo et al. (2010) synthesized some novel 3,5-diaryl pyrazoline derivatives 61-63 and investigated them for their MAO inhibitory activity. The synthesized derivatives resulted into selective and reversible inhibitors against either of the MAO isoforms. Docking simulations were utilized for the theoretical evaluation of the probable interactions with the target protein.53

Kaplancikli et al. (2010) constructed a series of triazole substituted-pyrazoline analogs 64 to explore their antidepressant effect, using modified FST and TST, when compared with fluoxetine. Rota-rod test was used to examine the probable neurological discrepancies of the test compounds.162
Rossella et al. (2010) synthesized a series of N-substituted-3-[(20-hydroxy-40-prenyloxy)-phenyl]-5-phenyl-4,5-dihydro-(1H)-pyrazolines 65 and tested them on hMAO-A and B isoforms. Structure–activity relationship and modeling studies showed that benzyloxy or chlorine substitutions provided the best interactions with active site of hMAO-B.\(^{174}\)

![Chemical structure of 65](image)

\[X = \text{COCH}_3, \text{CSNH}_2\]

A series of N-substituted-3-[(2-hydroxy-4-prenyloxy)-phenyl]-5-phenyl-4,5-dihydro-(1H)-pyrazolines 66 was prepared and tested on hMAO-A and MAO-B isoforms by Fioravanti et al. (2010). SARs and modeling studies showed that benzyloxy or chlorine substitutions improved their interactions with the active site of hMAO-B enzyme, while methyl and methoxy groups at the same place gave a significant decrease or loss in the inhibition. Beside this, it was observed that different behavior of the derivatives was probably due to the substituents on N1 as well.\(^{210}\)

![Chemical structure of 66](image)

Where, x may be; COCH\(_3\) or CSNH\(_2\)

In turn to reduce the steric hindrance in the catalytic site, and strengthen the interaction with the isosalloxazine nucleus of the cofactor flavin adenine dinucleotide (FAD), Chimenti et al. (2010) synthesized quite a few novel compounds with (hetero) aromatic moieties in the 3\(^{\text{rd}}\) and 5\(^{\text{th}}\) place of the above pharmacophore and examined the modulation hMAO inhibitory activity. 5-(4-Fluorophenyl)-3-(furan-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide 67 emerged as the most potent compound of the series, with an IC\(_{50}\) value of 2.75±0.81 mM and selectivity ratio of 25.\(^8\)
Karuppasamy et al. (2010) synthesized 3,5-diaryl pyrazoline analogs 68 and evaluated them for inhibition of MAO enzymes. The synthesized derivatives have come into sight as selective and reversible MAO-A inhibitors with a selectivity index (SI) of magnitude 103–105. The docking simulations established further structural details about their binding mode and feasible interactions with MAO-A protein. Interestingly, the theoretical inhibitory constant (Ki) values obtained by molecular docking studies were in congruence with their experimental (Ki) values.\(^{181}\)

Maccioni et al. (2010) synthesized some diversely substituted 3-aryl-2pyrazoline-1-carbothioamides 69 intending to investigate their MAOI activity. The structural confirmations were attained through IR, 1H-NMR, 13C-NMR spectroscopic and elemental data. Despite the substitution on the heterocyclic nucleus, each and every compound showed MAO-B selectivity. To rationalize the inhibition mechanism of most active/selective analogs, docking simulations were accomplished.

Where, R & R’ are either H or CH$_3$;
Ar= 4-F-C$_6$H$_4$, 4-CH$_3$-C$_6$H$_4$, 3-CH$_3$-C$_6$H$_4$,
2-CH$_3$-C$_6$H$_4$, 4-OMe-C$_6$H$_4$, 3-OMe-C$_6$H$_4$,
2,4-Cl-C$_6$H$_3$, fur$^2$-yl etc.
Boppana et al. (2010) investigated the interaction of known ligands with the MAO-B protein, by the combined use of structure and pharmacophore based modeling approaches. Results of the study clearly suggested a good agreement between both the models used to identify potential selective MAO-B inhibitors. The best pharmacophore model Hypo2, consisting of donarHB, acceptHB and ring aromatic features was used to retrieve 5500 compounds by screening of an in-house library. Finally, after docking simulations, 15 selective MAO-B inhibitors were shortlisted on the basis of structural novelty and selectivity and were suggested for further synthesis and pharmacological screening in wet lab.

Some new 3,5-diaryl 2-pyrazoline analogs 70 were generated and tested for their MAO inhibitory property by Sahoo et al (2010). These compounds showed reversible and selective inhibition of either one of the MAO isoforms. Docking simulations established a fair interaction between the ligand and MAO proteins. The theoretical values were in congruence with their experimental data.

Where, R may be: H, COCH3, CSCH3, C(NH)NH2OH, SO2C3H7CH3; R1 & R2 may be; H or OH

Two novel series of 2-thiocarbamoyl substituted hexahydro-1H and 2H-indazole analogs 71, 72 were synthesized and tested for their ability to inhibit MAO-A and B isoforms by Gokhan-Kelekci et al. (2009). The synthesized derivatives were identified and characterized on the basis of physicochemical and spectroscopic data (IR, 1H-NMR, 13C-NMR, 2D-NMR, DEPT, EI-MASS and elemental analysis). The synthesized compounds were highly active against both the MAO-A and B enzymes. The influence of structure on the biological activity suggested that compounds bearing no substitution and/or N-methyl/ethyl substitution possess increased activity and selectivity towards MAO-B protein. However, compounds bearing N-allyl and N-phenyl substitutions were found selective towards MAO-A enzyme. A competitive and reversible inhibition was noticed for all the compounds.
Gökhan-Kelekci et al. (2009) prepared a novel series of 2-pyrazoline derivatives 73 from quinazolinone ring, and evaluated them for MAO inhibitory and antidepressant activities using in-vivo and in-vitro tests. These compounds evidenced for high activity against both the MAO isoforms. However, few of the derivatives showed good antidepressant potential. The computational methods were used to establish the correlation between H-bonding/hydrophobic parameters of ligands and their biological activity data. It was evident from the results that potential edge-to-face hydrophobic interactions, in association with π-π stacking interactions, were keys to success of such type of 2-pyrazoline derivatives in these experiments.177

Chimenti et al. (2008) demonstrated the MAO inhibitory potential of different reversible MAOIs, among which N1-acetyl and N1-thiocarbamoyl substituted 2-pyrazolines 74 disclosed good selectivity and high potency. For better understanding of the role of the N1 substituent on inhibitory mechanism, another series of N1-propanoyl analogs were prepared without any changes in the A and B rings. This molecular manipulation yielded some selective MAO-A inhibitors effective in micromolar ranges. In addition, computational outcomes highlighted the most relevant interactions in the mechanistic recognition at MAO-A and B enzyme active sites.6
Jayaprakash et al. (2008) synthesized a pilot library of thirty two N-carbothioamide substituted 3,5-diaryl 2-pyrazoline derivatives as mycobactin analogs 75-77, prompted by clinical complications raised due to MAO inhibitory activity of Linezolid, a newly introduced antibiotic. The spectrum of activity, from selective to nonselective, and also as competitive, reversible to non-competitive, irreversible against two rat live MAO isoforms was analyzed. It was interesting to note that quite a few anti-tubercular agents were also found to selectively inhibit MAO-B isoforms of rat liver. The same set of compounds was also evaluated for AChE inhibitory activity, seeking for MAO-B/AChE dual inhibitors, having potential therapeutic utility in AD.

Ozdemir et al. (2007) synthesized 1-phenyl-, 1-thiocarbamoyl- and 1-N-substituted thiocarbamoyl-3-(2-furyl)-5-phenyl/ (2-furyl)-2-pyrazoline derivatives 78 and investigated them for their antidepressant potential using Porsolt’s behavioural despair (forced swimming) test on albino mice.
A recent approach used in the treatment of Alzheimer’s disease was by combining MAO-B inhibitors with anti-inflammatory agents. Considering the fact that pyrazoles have been promising compounds for anti-inflammatory analgesic activity and MAO-B inhibition, it was decided by Gokhan-Kelekci et al. (2007) to create a new series of 1-thiocarbamoyl-3-substituted-phenyl-5(2-pyrrolyl)-4,5-dihydro-(1H)-pyrazole derivatives and screen them. Most of the derivatives were found to be effective in this assay. Additionally, analgesic, anti-inflammatory and ulcerative potential of these compounds was also evaluated and it was concluded that such type of compounds demonstrated anti-inflammatory activity analogous to that of indomethacin, without ulcerogenic effects.

Chimenti et al., (2005) synthesized a new series of 1-thiocarbamoyl-3,5-diaryl-4,5-dihydro-(1H)-pyrazole derivatives and investigated them for their ability to selectively inhibit monoamine oxidase- A and B enzymes. All the synthesized derivatives showed high effectiveness against both the isoforms, with \( K_i \) values ranging between 27-4 nM (for MAO-A) and 50-1.5 nM (for MAO-B) respectively, except a few showing activity in micromolar range.

Twelve 1-N-substituted thiocarbamoyl-substituted-2-pyrazoline derivatives were synthesized by Ucar et al. (2005). The biological interactions of these compounds with human plasma, erythrocyte AChE/BuChE enzymes were assessed suggesting a selective, non-competitive and reversible inhibition by interacting with a region near
the peripheral sites of the enzyme which shifted the proper positioning of the catalytic center. Some compounds in the series potentially inhibited both, AChE and BuChE enzymes, but a higher extent for BuChE. These outcomes hold promise of being utilized in the treatment of AD and PD.\textsuperscript{215}

\begin{equation}
\text{R} \begin{array}{c}
\text{N} \\
\text{S} \\
\text{N} \\
\text{S} \\
\text{NHR'} \\
\text{S}
\end{array}
\text{R'}
\end{equation}

Where, R may be; CH\textsubscript{3}, Cl, OCH\textsubscript{3}; and R'= CH\textsubscript{3}, C\textsubscript{2}H\textsubscript{5}, C\textsubscript{3}H\textsubscript{5}, C\textsubscript{6}H\textsubscript{5},

\textbullet\hspace{1em} A new series of 1-acetyl-3-(4-hydroxy- and 2,4-dihydroxyphenyl)-5-phenyl-4,5-dihydro-(1\textit{H}) pyrazole derivatives \textsuperscript{83} was prepared and evaluated against MAO-A and MAO-B enzymes by Chimenti \textit{et al.} (2004). The synthesized derivatives showed a better selectivity index for MAO-A. Some of the enantiomers were found to show very good \(K_i\) values, with a high selectivity index.\textsuperscript{168}

\begin{equation}
\text{R} \begin{array}{c}
\text{N} \\
\text{R} \\
\text{O} \\
\text{R}
\end{array}
\text{O}
\end{equation}

Where, R= 4-OH, 2,4-OH; R'= 2-Cl, 3-Cl, 4-Cl, 3-CH\textsubscript{3}, 4-CH\textsubscript{3}, 2-OCH\textsubscript{3}, 4-OCH\textsubscript{3}, 2,4-OCH\textsubscript{3}

\textbullet\hspace{1em} A novel series of 1-acetyl-3,5-diphenyl-4,5-dihydro-(1\textit{H})-pyrazole derivatives \textsuperscript{84} was synthesized and investigated by Manna \textit{et al.} (2002) for the ability to selectively inhibit MAOs, bovine serum amine and swine kidney oxidases. These analogs were found to be non-competitive and reversible inhibitors against all the four types of amine oxidases. Among the synthesized derivatives, 1-acetyl-3-(2,4-dihydroxyphenyl)-5-(3-methylphenyl)-4,5-dihydro-(1\textit{H})-pyrazole was found to be the most potent MAOI, with a \(K_i\) of about 10\textsuperscript{-8} M. The probable interactions were studied with the help of computational approaches.\textsuperscript{67}
2.2. RESEARCH ENVISAGED

The synthesis of pyrazolines and their derivatives has engrossed substantial attention for many years. Increasing evidence suggests that pyrazoline is an important scaffold since it is known to be associated with multiple biological activities such as; tranquilizer, muscle relaxant, antidepressant, anticonvulsant, psychoanaleptic and MAO inhibitory. Pyrazolines are also well established pharmacophores for activities other than nervous system; such as anti-amoebic, anti-cancer, anti-viral, anti-malarial, anti-inflammatory-analgesic, anti-microbial etc.

Nowadays, the therapeutic interest in MAO inhibitors falls into two major categories. MAO-A inhibitors have been used mostly in the treatment of mental disorders, in particular depression and anxiety, while MAO-B inhibitors could be used in the treatment of Parkinson’s disease and Alzheimer’s disease. Efforts have been oriented toward the discovery of reversible and selective inhibitors of MAO-A/MAO-B leading to a new generation of compounds to gain a complete understanding of the pharmacophoric requirements necessary for the rational design of new inhibitors.

Studies by different workers demonstrated that 1,3,5-trisubstituted-2-pyrazolines have MAO inhibitory properties showing reversible and selective inhibition, belonging to the third generation of MAO inhibitors. Results of the study by Chimenti et al. indicated the influence of the para-substituted hydroxyl group on the aromatic ring bonded to C3 of the pyrazoline ring, as well as the ortho-substituted methoxy group on the aromatic ring bonded to C5 of the pyrazoline nucleus. Also, Sahoo et al. concluded that, the presence of sulphonyl group at N1 position of pyrazoline nucleus establishes a hydrogen bonding interaction between the sulphonyl oxygen of the ligands and hydroxyl hydrogen of either TYR444 or TYR407. Due to these Hydrogen bonding interactions, substituted phenyl ring at C5 and sulphonyl ring at N1 position of pyrazoline nucleus, such type of ligands are well placed in the aromatic cage (FAD, TYR407 and TYR444, Pocket1). It has been observed that one of the aromatic substitutions of previously mentioned compounds at these positions is involved in hydrophobic and probably aromatic interaction. Therefore, it is envisaged to substitute the phenolic moiety with other aromatic/heteroaromatic hydrophobic functional groups.
In view of these observations, it has been decided to synthesize and characterize a novel series of 1,3,5-trisubstituted-4,5-dihydro-(1H)-pyrazoles with different sulphonyl substitution at 1<sup>st</sup> position and aromatic/hetero-aromatic substitutions at 3<sup>rd</sup> and 5<sup>th</sup> positions respectively, and to evaluate them as MAO inhibitors using suitable models. The molecular modeling studies shall also be done.

Novelty of the proposed derivatives has been ascertained through exhaustive literature survey by conventional methods as well as using modern chemical databases such as; SciFinder, ChemSpider, ChemBank, ACD, ZINC and ASINEX databases.
2.3. PLAN OF WORK

Part A: Synthesis and purification

1. Synthesis

**Step I:** Synthesis of chalcone derivatives (1a-1t).

\[
\text{Ketone} + \text{Aldehyde} \rightarrow \begin{align*}
\text{R}_2\text{C} & \quad \text{H} \\
\text{O}_\text{C} & \quad \text{C} \\
\text{CH}_3 & \quad \text{R}_3
\end{align*}
\]

Claisen-Schmidt condensation

\[\text{C}_2\text{H}_5\text{OH}, \text{aq. NaOH, stir, 4-48 hrs, 10-20^0C.} \]

Where, \( \text{R}_2 = \)

\[
\begin{array}{c}
\text{OH} \\
\text{Cl}
\end{array}
\]

and \( \text{R}_3 = \)

\[
\begin{array}{c}
\text{OCH}_3 \\
\text{Cl}
\end{array}
\]

**Step II:** Synthesis of 3,5-aryl/heteroaryl substituted-4,5-dihydro-(1H)-pyrazole derivatives (2a-2t).

\[
\text{R}_2\text{C} = \text{C} \rightarrow \begin{align*}
\text{H} & \quad \text{R}_3 \\
\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O}
\end{align*}
\]

Hydrazine hydrate (excess)

\[\text{C}_2\text{H}_5\text{OH}, \text{Reflux, 3-6 hrs} \]

**Step IIIA:** Synthesis of 1-p-nitrobenzenesulphonyl-3,5-aryl/heteroaryl substituted-4,5-dihydro-(1H)-pyrazole derivatives (3a-3t).

\[
\text{O}_\text{2N}-\text{SO}_2\text{Cl} \rightarrow \begin{align*}
\text{R}_2 & \quad \text{N} \\
\text{O} & \quad \text{O}
\end{align*}
\]

THF, stir, 0.5-4h

**Step IIIB:** Synthesis of 1-p-methoxybenzenesulphonyl-3,5-aryl/heteroaryl substituted-4,5-dihydro-(1H)-pyrazole derivatives (4a-4t).
Step IIIC: Synthesis of 1-\(p\)-chlorobenzenesulphonyl-3,5-aryl/heteroaryl substituted-4,5-dihydro-(1\(H\))-pyrazole derivatives (5a-5t).

2. Purification
   a. Recrystallization
   b. Column chromatography

Part B: Characterization

1. Physicochemical analysis
   a. Solubility profile
   b. Melting range
   c. \(R_f\) value
   d. Elemental analysis

2. Spectral analysis
   a. IR Spectroscopy
   b. Mass Spectrometry
   c. NMR Spectroscopy
Part C: Biological evaluations

1. *In-vivo screening*
   a. *Antidepressant activity*
      - Despair swim test /forced swim test (FST) in mice
      - Tail suspension test (TST) in mice
   b. *Anti-anxiety activity*
      - Elevated plus maze model test in mice
   c. *Neurotoxicity studies in mice*
      - Neuromuscular coordination studies using rota rod test
      - Locomotor activity using actophotometer test
   d. *Acute toxicity studies in mice* [most potent derivative(s) only]

Part D: Molecular modeling studies

1. Selection of best MAO crystal structure and validation of docking protocol
2. Molecular docking simulations of synthesized compounds
3. *In-silico* ADME prediction of synthesized compounds
4. *In-silico* toxicity prediction of synthesized compounds