Chapter 1

Introduction
The homeostasis of bigenic amines, such as 5-hydroxytryptamine (5-HT) or serotonin, dopamine (DA) and noradrenalin (NE), in the brain is maintained by its elimination from the synaptic cleft through a reuptake mechanism and the oxidation by monoamine oxidases (MAOs). MAO [E.C. 1.4.3.4] is a flavin-containing key enzyme situated on the outer membrane of mitochondria bound via a C-terminal transmembrane polypeptide segment and inserted in the membrane through ubiquitin with energy provided by ATP, in neuronal, glial, and other cells, which regulates monoaminergic homeostasis and neurotransmission. The action is achieved via deamination of neuroactive and vasoactive biogenic molecules in the central nervous system (CNS). These include: Indoleamines such as serotonin and tryptamine; catecholamines, such as DA, NE and epinephrine; trace amines, such as $\beta$-phenylethylamine ($\beta$-PEA), tyramine and octopamine. The quick degradation of brain monoamines, such as 5-HT, NE and DA is essential for the correct functioning of synaptic neurotransmission. Modulation of mood/emotion, the control of motor, perceptual and cognitive functions is maintained by monoaminergic signaling mechanisms. In addition, it is also responsible for the biotransformation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) into 1-methyl-4-phenylpyridinium (MPP$^+$), a Parkinsonian producing neurotoxin.

MAO inhibitors (MAOIs) have been used as therapeutic agents for many years, but their use has been dwindled because of some potential side effects, food and drug interactions, and the introduction of other classes of drugs. However, curiosity in MAO inhibitors is reviving and the recent progress to a new-fangled generation of highly selective, reversible MAO inhibitors, has elicited to a renovated interest in the therapeutic prospective of these compounds.

### 1.1 Tissue distribution of MAOs

MAO-A and MAO-B enzymes are firmly united to mitochondrial outer membrane, with a minute microsomal fraction. During development, MAO-A appears before MAO-B. However, after birth the level of MAO-B dramatically increases in the brain. MAOs are present in a majority of the mammalian tissues, but their proportions vary from tissue to tissue. Histochemical studies have revealed that MAOs are confined...
to a small area in the endothelial cells of the endoneurial vessels, in Schwann cells and in
the unmyelinated axons of rat peripheral nervous system (PNS). Microvessels in the
blood–brain barrier (BBB) are rich in MAO-B. There are regional differences in MAO
activity in human brain as; highest level of activity is shown in striatum (basal ganglia)
and hypothalamus, whereas cerebellum and neocortex showed minimum activity. In vivo
distribution of MAO-A and MAO-B in human brain is monitored by Positron
Emission Tomography (PET). The two isoenzymes are not evenly distributed in the
human brain. Immunohistochemical studies have shown that basal ganglia, serotonergic
neurons (for example, cells of the dorsal raphe nucleus) and astrocytes predominantly
contain MAO-B, whereas catecholaminergic neurons (substantia nigra, oculs coeruleus
and the periventricular regions of the hypothalamus) contain mainly MAO-A. Specific
uptake inhibition mechanisms showed that caudatal dopaminergic nerve endings
contained only MAO-A, whereas serotonergic nerve terminals contained only small
amounts of this isoenzyme. After selective MAO-A inhibition, 5-HT levels increase in
glial cells. Percent of total MAO-A and B activity reported in different human tissues
has been presented in Table 1.1.

Table 1.1: Percentage of total MAO-A and B activity in selected human tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>MAO-A (%)</th>
<th>MAO-B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>&lt;20</td>
<td>&gt;80</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>&gt;80</td>
<td>&lt;20</td>
</tr>
<tr>
<td>Kidney</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>Lung</td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td>Platelets</td>
<td>&lt;5</td>
<td>&gt;95</td>
</tr>
</tbody>
</table>

1.2 Genes for MAOs

Genes for MAO-A and MAO-B are located on the X chromosome, at Xp11.23–11.4. hMAO-A and B genes were isolated from X chromosome genomic libraries. It consists of fifteen exons and have an identical exon-intron organization. Out of these, exon-12 coding the covalent flavin adenine dinucleotide (FAD) binding site is the most
conserved exon, showing 93.9% amino acid identity between MAO-A and B. These results suggest that MAO-A and B were resulting from the duplication of a common ancestral gene.\textsuperscript{24} The coding sequence in the normal male human MAO-A gene were found to be highly conserved.\textsuperscript{25} The maximal promoter activity for MAO-A and B was found in a 0.14 and 0.15 kb fragment, respectively, of the 5'-flanking sequence.\textsuperscript{26} Both fragments are GC-rich, contain potential specificity protein (Sp1) binding sites, and share approximately 60% sequence identity. The MAO-A 0.14 kb fragment lacks a TATA (Goldberg-Hogness) box, contain three Sp1 elements, and exhibits bi-directional promoter activity.\textsuperscript{27} The MAO-B promoter 0.15 kb fragment consists of two clusters of overlapping Sp1 sites separated by a β-globin CAACCC element.\textsuperscript{26} An upstream 5'-sequence of the human MAO-A gene down-regulates the MAO-A promoter in the presence or absence of an initiator-like protein.\textsuperscript{28} This difference in promoter organization of MAO-A and B genes may underlie their different tissue- and cell-type-specific expression, especially in catecholaminergic neurons and serotonergic neurons in the brain.\textsuperscript{29}

1.3 Structure and binding site analysis of MAOs

The two MAO isoforms (MAO-A and MAO-B) are composed of 527 and 520 amino acid identities with molecular weights of 59,700 and 58,800, respectively.\textsuperscript{30} Both the isoforms are distinctly different as; the MAOs containing flavin adenin dinucleotide-amine oxidases (FAD-AOs) as cofactor and the semicarbazide sensitive AOs (ssAOS) containing Copper-II and 2,4,5-trihydroxyphenylalanine quinine (TPQ-Cu AOs) as cofactors. Both the isoenzymes were found to possess 70% identity and a pentapeptidic primary sequence (Ser-Gly-Gly-Cys-Tyr), binding through a thioester covalent linkage of cysteine\textsuperscript{30-32} to the cofactor FAD via 8α-methyl group of the isoalloxazine ring (Figure 1.1, and 1.2). The deduced primary sequence is decidedly significant for the differences between MAO-A and MAO-B catalytic activity. Mutagenesis studies have demonstrated that the internal segment (between aminoacids 152 and 366) confers inhibitor and substrate specificities.\textsuperscript{33} The secondary structures of human MAO-A and bovine MAO-B have been explored by Fourier Transform Attenuated Total Reflection Spectroscopy
(FTIR ATR). The experimental results indicated a distinct folding and molecular specificity of the two MAO subtypes.\(^3\)\(^4\) Four extremely conserved regions in the MAO isozymes have been identified.\(^3\)\(^5\) These are; (1) an ADP-binding unit (residues 6-43); (2) a putative substrate binding domain (residues 178-221); (3) a site for the covalent attachment of FAD (residues 350-458); and (4) a C-terminus region (residues 491-511) predicted to form a transmembrane-associated R-helix.\(^3\)\(^1\)\(^,\)\(^3\)\(^4\)

![Structure of MAO isoforms showing various regions](image)

**Figure 1.1:** Structure of MAO isoforms showing various regions\(^1\)\(^7\),\(^3\)\(^6\),\(^3\)\(^7\)

A combination of affinity labeling experiments, using the pseudosubstrate inhibitor \(N-[2\text{-aminoethyl}]-5\text{-chloro-2-pyridinecarboxamide}\) (Lazabemide), and site-directed
mutagenesis (SDM) studies have provided evidence that amino acid residues His382 and Thr158 in MAO-B are essential for the catalysis whereas Phe208 in MAO-A and Ile199 in MAO-B are key determinants of substrate specificity. SDM studies also demonstrated that Cys397 is essential for the catalysis by of MAO-B, but not MAO-A, since the Cys406 in MAO-A mutant exhibits 40-60% catalytic activity binding through co-valent linkage to FAD. Besides this covalent binding site, two non-covalent flavin binding regions have been identified in MAO-B (residues 6-34 and 39-46). Experiments have shown that Glu34, Arg42, Tyr44, and Tyr45 are important amino acid residues that engage in initial non-covalent interactions with FAD and generation of catalytic activity.

Sablin and Ramsay have shown that both isozymes hold a redox-active disulfide in the catalytic active site suggesting that the MAOs may represent a novel type of a disulfide oxidoreductase. Some recent studies have revealed that both isozymes possess an imidazoline-binding site (I2-BS) that recognizes imidazolines, guanidiniums, and related structural analogues.

![Figure 1.2: Partial amino acid sequence of MAO containing flavin moiety](image)

The structure explication of rat and human MAOs disclosed that substrate binding and oxidation take place in elongated cavities extending from the flavin site at the core of the enzyme to the protein surface on the opposite side of the FAD adenosine ring. Although in both the enzymes cavities were generally hydrophobic, details of the active site architectures demonstrated disparities in their structural properties which accounts for their discrete substrate and inhibitor specificities. The recent portrayal of the two human MAO (MAO-A and MAO-B) isoforms crystal structure provided information to clarify the mechanism underlying. This allowed investigation of the selective interactions between MAO proteins and their ligands to gain a complete perception of the pharmacophoric requirements necessary for the rational design of new inhibitors. The structural comparison of human MAO-A and MAO-B revealed a significant
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Conformational variation in the cavity-shaping loop of MAO-A (from residues 210–216) and MAO-B (residues 201–207). Due to this, the aromatic cage of MAO-A becomes wider than that of MAO-B and can accommodate comparatively bulkier aromatic groups. Additionally, docking studies\textsuperscript{52, 53} clarified the importance of ring substitution at 1\textsuperscript{st} position of the pyrazoline nucleus, which was found to be positioned in the aromatic cage (FAD, TYR407 and TYR444, Pocket1). These observations set a logical ground to design potent and selective MAO-A inhibitors.\textsuperscript{54} The recently reported crystal structure of human MAO-A (2.2 Å) in complex with harmine\textsuperscript{55} allows a more detailed assessment of the active sites of both enzymes. The volume of the hMAO-A cavity is ~400 Å\textsuperscript{3}, whereas that of the combined two cavities of hMAO-B is ~700 Å\textsuperscript{3}. The cavity in MAO-B protein is bipartite, comprising of two separate spaces, the entrance cavity (~300 Å\textsuperscript{3}), and the substrate cavity (~400 Å\textsuperscript{3}) with the former facing the solvent due to movement of loop 99–110. The conformation of Ile199 determines catalytic site plasticity of MAO-B protein as a large single cavity or a bipartite cavity. The side chain of this “gating” residue may depend on the nature of the bound ligand, which adopts two different conformations (“closed” and “open”)\textsuperscript{56}. The corresponding residue in human and in rat MAO-A is Phe208, but it does not function as a gating residue. Mutagenesis experiments were performed on human MAO-B Ile199, since the well-studied bovine enzyme contains a Phe at 199 position rather than Ile.\textsuperscript{57}

Structural and inhibitor binding experiments demonstrated that the bulky Phe side chain impedes such conformational flexibility by reducing the space of the entrance cavity and interferes with the binding of specific MAO-B inhibitors.\textsuperscript{57} These findings provide a structural rationale for differential binding affinities of bovine and human MAO-B preparations as reported in the literature \textsuperscript{58} and present a warning on pitfalls that can be encountered on the use of differing MAO sources for development of inhibitors of human MAO\textsuperscript{59}. Another difference between active site cavity structures is the presence of Tyr326 side chain in MAO-B. Although it was not found to be directly involved in the partitioning of the two cavities, but it produce a restriction which is less pronounced in hMAO-A where Ile335 occupies the same position. The active site residues, Tyr pair of the “aromatic sandwich” (at the re face of the flavin coenzyme isoalloxazine ring), and a
Lys residue B-bonded through a water molecule to the N(5) position of the flavin (Lys305 in MAO-A and Lys296 in MAO-B) remain conserved in both the enzymes. However, other non-conserved residues in the active sites include Asn181, Ile180 in MAO-A and Cys172, Leu171 in MAO-B, respectively; although, these do not significantly affect the shapes of the cavities. Therefore, Phe208, Ile335 resided of MAO-A and the Ile199, Tyr326 pairs of MAO-B appeared to be major determinants in dictating the differential substrate and inhibitor specificities of the two enzymes.

An intriguing issue in MAO enzymology is to understand where and how the substrates (or inhibitors) are admitted to the active sites. In hMAO-B, the cavity is extended and substrate binding is likely to occur in proximity to the outer mitochondrial membrane surface region with the entrance loop (residues 99–110) involved in the access. On the other hand, the active site cavity of both rat and human MAO-A is more compact. The site directed mutagenesis studies by Son et al.\(^55\) have recently demonstrated that the conserved Gly110 residue is important for ensuring flexibility of the entrance loop to provide access for the substrate.

Consequently, structural data suggested that MAO-A and MAO-B ligands chase same pathways for binding with the target protein. Furthermore, it was also disclosed that the cavity-shaping loop (residues 210–216 in MAO-A) assumes a helical conformation, which was conserved in all structures except for hMAO-A in complex with Clorgyline. The inhibitor was solely responsible for this as, Clorgyline adopts different conformations when complexed with rat MAO-A, retaining the folded conformation of loop 210–216. This observation suggested that this loop may be more susceptible to structural flexibility in MAO-A than in MAO-B. The elongated cavity of human MAO-B is typically hydrophobic having petite hydrophilic regions in front of the re face of the flavin cofactor\(^56\) that is occupied by highly conserved water molecules. Different sized ligands binds with hMAO-B, and many them react with the flavin cofactor to form a covalent adduct (an example of mechanism-based inhibitors). Generally, all inhibitors possess an aromatic moiety (as expected considering the aromatic nature of the neurotransmitter substrates), however, the cavity also showed a substantial affinity for aliphatic ligands like- trans,trans-farnesol. Recently, the common laboratory plasticwares
including; oleamide, slip agent, and a biocide, di(2-hydroxyethyl) methyldecylammonium ion were reported to inhibit MAO-B. This inhibitory versatility is related to the plasticity of the hMAO-B active site cavity. As described above, the cavity can hold small inhibitors such as isatin and tranylcypromine, or cavity-filling ligands such as safinamide, *trans-trans*-farnesol and 1,4-diphenyl-2-butene (a component of polystyrene plasticware) depending on the conformation of the gating residue Ile199. In the former case, the active site is restricted to a small cavity separated from an entrance cavity space that opens to the exterior like a funnel, whereas in the latter the cavity shape has a compact ellipsoidal entity. Binding of inhibitors such as Rasagiline, N-(2-aminoethyl)-p-chlorobenzamide, and Deprenyl induce a mid-span type of cavity because they are large enough to push the gating residue Ile199 into the open conformation. The shape of the resulting cavity has a more bipartite character than that induced by the cavity-filling inhibitors with Rasagiline type of inhibitors. It is imperative to notice that the positions of the active site residues remain highly conserved in all these MAO-B-inhibitor complexes, apart from the gating residue Ile199, and this cavity plasticity is probably determined by subtle conformational changes.

From a pharmacological viewpoint, this inhibitory versatility has important implications. Small compounds such as isatin and tranylcypromine showed similar binding affinities for both MAO-A and MAO-B, whereas cavity-filling ligands are highly specific MAO-B inhibitors. In case of Rasagiline, it is pretty surprising that it occupies only one half of the entire cavity but is highly specific for MAO-B. Even more astonishing is the evidence that a rasagiline analog methylated on the amino moiety of the propargyllyne chain, connecting the inhibitor to the flavin, loses this characteristic specificity for MAO-B.

1.4 MAO substrates and catalysis

Before the molecular characterization, difference between the two MAO isoforms was defined on account of their substrate and inhibitor sensitivity. It was suggested that MAO-A and MAO-B have remarkably different roles in monoamine metabolism. MAO-A preferentially oxidizes serotonin (5-HT) and noradrenaline (NE)
whereas MAO-B preferentially oxidizes benzylamine (BA) and phenylethylamine (PEA). However, both the isoforms contribute in the metabolism of dopamine (DA) and other monoamines (such as tryptamine and tyramine) (Figure 1.3). Various exogenous substances are also oxidatively metabolized by MAOs, in addition to the endogenous MAO substrates. Benzylamine is a preferred substrate of MAO-B, however kynuramine is favored by MAO-A. Recently, it has been reported that Ile-335 in MAO-A and Tyr-326 in MAO-B have a decisive role in determining substrate and inhibitor specificities. The two isoenzymes are best distinguished by pharmacological criteria; as MAO-A is selectively inhibited by Clorgyline and Moclobemide, whereas MAO-B is blocked by Lazabemide and Deprenyl (Selegiline) in low doses irrespective of tissue and substrate differences in substrate specificity.

Both the isoforms of MAO, in coordination with FAD molecule, catalyse the oxidation of primary, secondary and tertiary amines (having two hydrogens on α-carbon). FAD plays a key responsibility in the oxidative deamination of amine substrate, and is involved in the transfer of electrons from amine nitrogen to oxygen. The MAO catalyzed chemical reaction consists of degrading monoamines into the corresponding aldehydes,
which are then oxidized into acids by aldehyde dehydrogenase (ALDH) or into alcohols/glycols by aldehyde reductase (ALR). During these reactions, several potentially neurotoxic species (such as hydrogen peroxide and ammonia) are produced as byproducts. In particular, hydrogen peroxide can trigger the production of ROS and induce mitochondrial damage and neuronal apoptosis. The catalysis by MAOs has been proposed in three different ways, one by radical mechanism with the iminium ion intermediate (SET pathway), second by the hydrogen atom transfer (HAT) pathway and the third by polar or nucleophilic mechanism (Figure 1.4).
Figure 1.4: Oxidative deamination of monoamines catalyzed by MAOs

(a) General reaction; (b) General mechanism; (c) Radical (SET) mechanism;
(d) Hydrogen atom transfer (HAT) mechanism; (e) Polar or nucleophilic mechanism

1.5 Functions of MAOs

MAOs protect the body through oxidation of amines in blood or by ending their entry into the circulation in peripheral tissues (such as the intestine, liver, lungs and placenta). MAO-B in the microvessels of the BBB presumably has a similar protective function, acting as a metabolic barrier. Also, it has been suggested that intraneuronal MAO-A and MAO-B protect neurons from exogenous amines by terminating the amine neurotransmitter actions regulating their intracellular stores in the PNS and CNS. Although, in terminating the actions of neurotransmitters and dietary amines by MAO-A and B were studied extensively, there was little awareness towards the functions of MAO products activity. During this reaction, the produced hydrogen peroxide might have essential metabolic and signalling functions in the brain. However, the aldehydes obtained from the deamination of 5-HT and noradrenaline should have been involved in the sleep regulation. Nevertheless, the products ammonia and hydrogen peroxide are toxic at higher concentrations.

In Parkinson’s disease patients, the levels of aldehyde dehydrogenase is highly decreased and hence aldehydes concentration is highly raised in substantia nigra which appear to get accumulated in the healthy brain and produces cytotoxic effects
(neurodegeneration). A toxic adduct (formed by reacting aldehydes with amines) tetrahydropapaveroline, was found to be present in patients of parkinsonism and alcohol-related abnormalities. The neurotransmitter metabolism and behaviour of MAO-A and MAO-B knockout mice was found entirely different showing enhanced reactivity to stress, similar to that observed after administration of non-selective MAOIs. Gene deletion studies together with a rare Norrie disease put a point forward that MAOs are not essential for survival, however, it may be considerable during development. The compulsive aggressive phenotype resulting from lack of MAO-A function in humans and mice reflects the consequences of 5-HT levels during development, and can be mimicked by administering MAO-A inhibitor Clorgyline, during the early postnatal period. Also, there is a momentous concern between platelet MAO activity and behavior/personality, voluntary alcohol intake and biochemical measures of CNS serotonergic activity. The regulation of platelet MAO activity is attributed to transcription factors such as activator-protein-2 (AP-2) which also regulate central monoaminergic activity. Thus, it can be concluded that, MAOs have vital roles in brain development and functioning.

1.6 Estimation of MAOs

Monoamine oxidase (MAO) activity in tissues is measured by using various continuous and discontinuous techniques, such as; manometric, spectrophotometric, fluorimetric and radiometric assays. These methods vary in their selectivity, specificity, sensitivity and tissue requirements. All of these highly sensitive methods involve a fair amount of sample manipulation, i.e. extraction, washing, etc. before spectrophotometry or fluorimetry or scintillation counting can be applied.

The simplest and most rapid method to determine MAO activity is spectrophotometric method by Weissbach et al. This assay method utilizes a direct spectrophotometric technique which allows quick and accurate determination of the disappearance of kynuramine (360 m ), or of the appearance of hydroxyquinoline (310 to 335 m ). The assay is also applicable to crude extracts of tissues and preferred for the localization and purification of MAO. This method is appropriate for highly active tissues such as that of guinea pig or rabbit liver, but often difficult to apply for less active tissues.
Another continuous spectrophotometric method described by Duley et al.\textsuperscript{88} was found suitable for the estimation of peroxisomal oxidases in rat tissue homogenates through continuous appraisal of the reaction product-\textit{H}_2\textit{O}_2 by combining it to the reduction of a chromogen, \textit{o}-dianisidine, with horseradish peroxidase. Catalase interference was overcome using azide to inhibit its activity and a \textit{H}_2\textit{O}_2 standard curve was used to quantify oxidase activity in terms of microkatalts per milliliter of enzyme.

A well adopted continuous peroxidase-linked spectrophotometric assay was described by Holt et al.\textsuperscript{89} which is appropriate for measuring monoamine, diamine oxidase and semicarbazide-sensitive amine oxidase activities in tissue homogenates. This assay is based on the oxidation followed by condensation of, 4-aminoantipyrine with vanillic acid into a red coloured quinoneimine dye. The amount of hydrogen peroxide liberated in the amine oxidase reaction was proportionally equal to the absorbance at 498 nm. The molar absorptivity of dye at pH 7.6 was 4654 M\textsuperscript{-1}cm\textsuperscript{-1}. The method can be properly used for any amine oxidase substrate which has a higher oxidation–reduction potential than that of 4-aminoantipyrine. Following pre-incubation of rat liver homogenates with selective monoamine oxidase (MAO)-A and -B inhibitors, kinetic constants were obtained for metabolism of the mixed substrate, \textit{p}-tyramine. The inhibition of MAO in rat liver homogenates could also be measured after phenelzine administration. This inexpensive assay employs least toxic reagents and hence can also be utilized to determine the degree of MAO inhibition produced by potential anti-parkinsonian and antidepressant drugs. This method is also useful in screening of different drugs, their metabolites and various environmental toxins as probable substrates or inhibitors of amine oxidases, along with the determination of their kinetic constants for turnover of novel substrates.

A fluorimetric adaptation of the Weissbach method was developed by Krajl et al.\textsuperscript{90} This provides a highly sensitive, rapid and convenient assay of MAO activity. Instead of determining the disappearance of kynuramine, one can readily monitor the appearance of 4-hydroxyquinoline (4HOQ) fluorimetrically. This fluorimetric adaptation was applied to diverse tissues, e.g. guinea pig atria, rat brain and cat ganglia. Matsumoto et al.\textsuperscript{91} developed a sensitive fluorometric assay for serum MAO with kynuramine as the
substrate. Since this method is much simpler and more sensitive than other methods conventionally used, it is recommended for routine clinical investigations. In addition to this, a one-step fluorimetric method for the determination of MAO activity in 96-well microplates, with 10-fold higher sensitivity than the conventional spectrophotometric methods, was developed by Zhou et al. The detection of $\text{H}_2\text{O}_2$ in horseradish peroxidase is based on the coupling reaction using a highly sensitive and stable probe, $\text{N}$-acetyl-3,7-dihydroxyphenoxazine (Amplex Red). This assay is valuable for both continuous and end-point measurements of MAO activity with a single sample. Moreover, this method is capable of detecting very low MAO-B activity using commercial enzymes, and selectively distinguishes both MAO-A and MAO-B activities in normal and diseased tissues, blood samples, and other biological fluids.\textsuperscript{92}

1.7. Monoamine oxidase inhibitors (MAOIs)

The development of MAOIs started with the serendipitous finding of antidepressant effects in patients treated with Iproniazid (a hydrazine-based antitubercular agent structurally similar to Isoniazid). During the early clinical testing of iproniazid as an antituberculous agent in 1951, the progress of euphoria in patients was noted as side effect of the drug\textsuperscript{93}. In 1952, Zeller and co-workers\textsuperscript{94} found that iproniazid was an inhibitor of MAO \textit{in vivo} and \textit{in vitro} and suggested the potential antidepressant utility of such a compound, which could result from potentiation of amines whose metabolism was normally catalyzed by the MAO enzyme in the CNS. This possibility was dramatized later with a manifestation that rabbits and mice pretreated with Iproniazid and then injected with reserpine showed extreme “amphetamine-like” stimulation. Clinical interest in MAO inhibitors \textit{per se} as therapeutic agents had been awakened. This discovery, in association with the MAO inhibition capabilities of Iproniazid\textsuperscript{94}, resulted into a blueprint to develop other MAO inhibitors such as Phenelzine. Such types of hydrazine-based MAO inhibitors were devoid of serious side effects, such as liver toxicity and “cheese reaction”,\textsuperscript{95} which was hypothesized to be related with nonselective and irreversible MAO inhibition. This finding shifted the entire research paradigm towards some novel categories of antidepressants with diverse mechanisms (such as tricyclic antidepressants
and serotonin selective reuptake inhibitors). However, the quest for MAO inhibitors devoid of untoward effects prompted research to characterize selective MAO-A and MAO-B inhibitors.

MAOIs have been used for decades in the treatment of depression. In fact, they were the first antidepressant drugs described and are still used today with great success, especially in patients who are resistant to other treatments. The first generation of irreversible and nonselective MAOIs (i.e., Phenelzine, Tranylcypromine) induced some serious side effects and these liabilities led to the search for a second generation of MAOIs (i.e. Clorgyline) that exhibited an irreversible and selective profile, but undesirable hypertensive crises persisted. Afterwards, selective and reversible MAO-A inhibitors, having a much better safety profile than irreversible inhibitors, have been developed.

Almost all initially known therapeutically used MAO inhibitors act irreversibly, however a reversible inhibitor was the need of the hour from pharmacokinetic standpoint. Among the drugs with some specificity for MAO-A vs MAO-B activity, clorgyline, L-51641, harmine, harmaline, α-ethyltryptamine and PCO [5-phenyl- 3-(N-cyclopropyl)-ethylamine, 1,2,4-oxadiazole] inhibit MAO-A selectively, while deprenyl and pargyline were preferential inhibitors of MAO-B as originally reported by Johnston in his classification of MAO into A and B forms. Other irreversibly acting MAO inhibitors, e.g., pheniprazine and nialamide, were equally active on both types.

The importance of MAO in controlling neurotransmitters level had prompted interest in learning the mechanism of substrate oxidation and the nature of the active site as well as in understanding the molecular mechanisms of MAO inhibition by a wide variety of chemical compounds, all of which exhibited a pharmacological profile including potent antidepressant activity. Some of the known MAO inhibitors include; arylalkylhydrazines, aryl hydrazides, arylpropargylamines, arylcyclopropylamines, aryloxycyclopropylamines, N-cyclopropylaryloxyethylamines, β-carbolines, and α-methylated arylalkylamines.
1.8. Classification of MAOIs

Broadly, MAOIs are divided into non-selective and selective reversible (competitive and slow, tight-binding) or irreversible (affinity labeling agents or mechanism-based inactivators) inhibitors. A detailed classification of MAOIs has been presented in the Table 2.

Compounds belonging to the first generation of MAOIs were mechanism-based inactivators and act via formation of reactive electrophilic intermediates that covalently modify the protein. Nowadays, development of newer, selective and reversible MAOIs has fortified this area of research. Selective, irreversible MAOIs involve the inclusion of substituents on the nitrogen atom of isozyme selective substrates, which undergo MAO-catalyzed conversion to electrophilic intermediates that alkylate the enzyme’s active site. N-Substituents that are capable of imparting irreversible inhibition properties to MAO substrates include amino (hydrazines), allyl, propargyl, cyclopropyl, cyclobutyl, trialkylsilanyl, oxazolidinonyl, and furanoyl groups. Furthermore, extending the distance between this electron rich center and the aromatic ring, or incorporating a bulky group on the aromatic ring of irreversible MAO-B selective inhibitors, affords irreversible MAO-A selective inhibitors. This is apparent from structural differences between (R)-deprenyl (MAO-B selective) and clorgyline (MAO-A selective).

The recognition of two forms of MAO (A and B) and their selective irreversible inhibitors resolved some of the problems (cheese reaction) associated with the non-selective inhibitors. However, irreversible MAO-A inhibitors carry on to persuade a cheese reaction, whereas MAO-B inhibitors at their selective dosage were not found to do so. Considering the fact about selective inhibititon of MAO-A and B by clorgyline and deprenyl, several attempts have been made to establish SAR with the acetylenic inhibitors.\textsuperscript{100-102} This was difficult to describe the structural features of selectivity and reversibility. However, it has been proposed that the distance between the N- terminal and the phenolic group may be crucial for selectivity.\textsuperscript{103} Thus, clorgyline, N-methyl- N-propyl-3-(3,4-dichlorothiophenyl) ethylamine HCl (M & B 9303) and N-methyl-N-propynyl-quinine HCl (Abbott 1671) are selective inhibitors of the MAO-A species having a distance equivalent to 3 to 4 carbon units between these chemical groups.
Compounds with 1-2 carbon units in the same position, e.g. Pargyline, Deprenyl, N-Methyl-N-propynyl-indanamine HCl (AGN 1135) N-Desmethyl-N-propynyl-indanamine HCl (AGN 1133), N-Desmethylpargyline and N-Methyl-N-propynyl-dihydrofuran-isopropylamine HCl (U 1424) showed structural resemblance with phenylethylamine, benzylamine and are selective inhibitors of the MAO-B species. However, substitutions on the phenolic group of the N-terminal may also be important, since introduction of halogens or alkyl groups modifies the selectivity and inhibitory activity. On the other hand, this SAR is not apparent in other chemical types of inhibitors. What is noticeable and well established is that both clorgyline and deprenyl, and other acetylenic inhibitors, react irreversibly (covalently) and stoichiometrically with the enzyme cofactor FAD at its active centre. This binding take place at the N-5 of the FAD isoalloxazine moiety. Hence, selectivity of these compounds is because of diverse substrate or inhibitor recognition sites near the active centre and not due to another mode of irreversible binding to the FAD. Whether these recognition sites are owing to different protein structures or lipid microenvironment, or to the binding of MAO to other positions on the outer mitochondrial membrane, has not been established.

Reversible MAOIs can be subdivided further into competitive and slow, tight-binding inhibitors that can be distinguished by the kinetic model. Accordingly, the first step in the competitive inhibition process involves the formation of a rapidly reversible enzyme- inhibitor complex. The second step, usually observed with slow, tight-binding inhibitors, involves the slow, time-dependent conversion of the enzyme-inhibitor complex to an activated complex in which the inhibitor is bound more tightly to the enzyme resulting in a conformational change in the enzyme’s three-dimensional structure. Development of reversible inhibitors of MAO-A (RIMA), moclobemide (i.e. p-chloro-N-[2-morpho-linoethyl] benzamide) and Brofaromin, as antidepressants and possible anti-Parkinson’s agent with limited tyramine potentiation was based on the displacement of inhibitor by amine from the binding site of enzyme. Chemically, the reversible MAO-A inhibitors were initially the derivatives of morpholine (e.g. moclobemide), benzofuranylpiperidine (e.g. brofaromine), 2-aminoethylcarboxamide (e.g. Ro41-1049) and oxazolidinone (e.g. toloxatone, cimoxatone) etc. There are some
other compounds of diverse chemical structures having MAO-A inhibitory effect, which are presently undergoing pre-clinical investigations. Moclobemide, a prototype of reversible inhibitors of MAO-A,\textsuperscript{106} was found to produce an inhibition of up to 80\% in the brain and liver 30 minutes after drug treatment, and enzyme activity recovered almost 100\% after 16 hours of washing period in \emph{ex vivo} experiments.\textsuperscript{107} Extrapolating from these results, the carryover effect caused by moclobemide inhibition is short-lasting, in contrast to another irreversible inhibitor, tranylcypromine.\textsuperscript{108} For brofaromine, the time-course of reversibility was between 24 and 48 hours, whereas for cimoxatone, it was approximately 24 hours.\textsuperscript{109} In contrast, for the irreversible inhibitor tranylcypromine, there was only a partial recovery of the MAO-A activity (approximately 50\%), which took approximately 48 hours. Complete recovery of MAO-A activity took much longer, causing long-lasting carryover effect.\textsuperscript{108} Lazabemide is amongst the few examples of reversible MAO-B inhibitors, while others (Pargyline, Seligiline, Rasigiline, Mofegiline etc.) are selective but irreversible type. It always been attempted to achieve a bigger pharmacological advantage by inhibiting both forms of the enzymes to catch the full functional activities of amine neurotransmitters, without inducing a “cheese reaction”. This was not possible till the development of the novel cholinesterase-brain selective MAO-AB inhibitor, TV3326 (\textit{N}-propargyl-(3\textit{R})-aminoindan-5-yl-ethyl methylcarbamate hemitartrate), a carbamte derivative of Rasagiline. Pharmacologically, it is analogous to moclobemide and being a MAO-AB inhibitor it has antidepressant, anti-Parkinson and anti-Alzheimer activities without tyramine potentiation in respective models\textsuperscript{105}
### Table 1.2: Classification of MAOIs

<table>
<thead>
<tr>
<th>Group</th>
<th>Class</th>
<th>Drugs/ research molecules</th>
<th>MAO selectivity</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First generation MOAIs (Non-selective)</strong></td>
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<tr>
<td>Hydrozines</td>
<td></td>
<td>Iproniazid (Marsilid, Iprozid, Iprond) 9</td>
<td>A and B</td>
<td>Antidepressant</td>
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<tr>
<td></td>
<td></td>
<td>Isocarboxazid (Marplan) 10</td>
<td>A and B</td>
<td>Antidepressant, Anxiolytic</td>
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<tr>
<td></td>
<td></td>
<td>Isoniazid (Laniazid, Nydrazid) 11</td>
<td>A and B</td>
<td>Antitubercular, Antidepressant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phenelzine (Nardil, Nardelzine) 12</td>
<td>A and B</td>
<td>Antidepressant, Anxiolytic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hydralazine (Apresoline) 13</td>
<td>A and B</td>
<td>Antidepressant</td>
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<tr>
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<td></td>
<td>Nialamide (Niamid) 14</td>
<td>A and B</td>
<td>Antidepressant, Anxiolytic</td>
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<tr>
<td></td>
<td></td>
<td>Benmoxin (Nerusil, Neuralex) 15</td>
<td>A and B</td>
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<td>Octamoxin (Ximaol, Nimaol) 16</td>
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<td>Antidepressant</td>
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<td></td>
<td>Iproclozide (Sursum) 17</td>
<td>A and B</td>
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<td></td>
<td>Mebanazine (Actomol) 18</td>
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<td>Phenoxypropazine (Drazine) 19</td>
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<td>Pivalylbenzhydrazine (Tersavid) 20</td>
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<td>Procarbazine (Matulane, Indicarb) 21</td>
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<td>Antineoplasitc, Antidepressant</td>
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<td></td>
<td></td>
<td>Saffraine (Safra) 22</td>
<td>A and B</td>
<td>Antidepressant</td>
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<tr>
<td></td>
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<td>Metfendrazine 23</td>
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<td><strong>Non-hydrozines:</strong></td>
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<td>Benoxazinacetamide</td>
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<td>Caroxzone (Surodil, Timostenil) 24</td>
<td>A and B</td>
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<td>Quinolinamine</td>
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<td>Echinopsidine (Adepren) 25</td>
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<td>Nitrofuran</td>
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<td>Furazolidone (Furoxone, Dependal-M) 26</td>
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<td>Amphetamine derivatives</td>
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<td>Tranylcypromine (Parnate, Jatrosom) 27</td>
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<td>Antidepressant, Anxiolytic</td>
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<td>Oxazolidinones (Antibiotic)</td>
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<td>Linezolid (Zyvox, Zyvoxam, Zyvoxid) 28</td>
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<td>Antibacterial, Antidepressant</td>
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<tr>
<td>Curcuminoid</td>
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<td>Curcumin (Desmethoxycurcumin) 29</td>
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<td>Antidepressant</td>
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<tr>
<td>Flavonoids</td>
<td>Anthocyanins</td>
<td>A and B</td>
<td>Antidepressant</td>
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<tr>
<td>Flavonoid glycosides, Terpenoids</td>
<td>Myricetin 31 and Quercetin 32 Ginkgolides 33 and Bilobalides 34</td>
<td>A and B</td>
<td>In dementia, Anti-Alzheimer</td>
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<tr>
<td>Second generation MAOIs (Selective-irreversible)</td>
<td>Propargylamines</td>
<td>Clorgyline 35</td>
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<td>Pargyline (Eutonyl) 36</td>
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<td>Anti-Parkinson</td>
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<td>Selegiline (L-deprenyl, Emsam) 37</td>
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<td>Anti-Parkinson</td>
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<td></td>
<td></td>
<td>Rasagiline (Azilect) 38</td>
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<td>Anti-Parkinson</td>
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<tr>
<td></td>
<td>Butanamines</td>
<td>Mofegiline (MDL-72974) 39</td>
<td>B</td>
<td>Anti-Parkinson</td>
</tr>
<tr>
<td>Third generation MAOIs (Selective-reversible)</td>
<td>Oxazolidinones</td>
<td>Befloxatone (MD370503) 40</td>
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<td>Antidepressant</td>
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<td></td>
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<td>Cimoxatone 41</td>
<td>A</td>
<td>Antidepressant</td>
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<tr>
<td></td>
<td>Piperidylbenzofurans</td>
<td>Brofaromine (Consonar) 42</td>
<td>A</td>
<td>Antidepressant</td>
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<tr>
<td></td>
<td>Morpholinobenzamide</td>
<td>Moclobemide (Aurorix, Manerix) 43</td>
<td>A</td>
<td>Antidepressant</td>
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<tr>
<td></td>
<td>Indole derivative</td>
<td>Prilindole (Lifril) 44</td>
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<td>Antidepressant</td>
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<tr>
<td></td>
<td>Phenylethylamine</td>
<td>Amiflamine (FLA-336) 45</td>
<td>A</td>
<td>Antidepressant</td>
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<td>Coumarin derivative</td>
<td>Esuprone 46</td>
<td>A</td>
<td>Antidepressant</td>
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<td>Stilbenoid</td>
<td>Resveratrol 47</td>
<td>A</td>
<td>Antidepressant</td>
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<td></td>
<td>Harmala alkaloids</td>
<td>Harmine 48, Harmaline 49</td>
<td>A</td>
<td>Antidepressant</td>
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<td></td>
<td>Pyridinecarboxamide</td>
<td>Lazabemide (Pakio, Tempium) 50</td>
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<td>Antiparkinson</td>
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<tr>
<td></td>
<td>Flavonoids (Flavanol)</td>
<td>Catechin 51</td>
<td>B</td>
<td>Antiparkinson</td>
</tr>
<tr>
<td>Mixed MAO-ChE inhibitors</td>
<td>Propylamines</td>
<td>Ladostigil (TV-3326) 53</td>
<td>A and B</td>
<td>Antidepressant, Antiparkinson, Anti-Alzheimer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Brain selective)</td>
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</table>

![Chemical structures](image_url)
Chapter 1

Introduction
**Figure 1.5:** Structure of some MAO inhibitors including drugs, clinical candidates and biologically active research molecules.

1.9. **Therapeutic potential of MAOIs**

In recent years, there has been a resurgence of interest in MAOIs, because of their therapeutic value in diverse conditions such as; recognition of glia-neuron interactions, stroke and ageing, neuroprotective, neurodegenerative and neurorescue consequences of several of drugs in various *in vivo* and *in vitro* toxicological situations. The new generation molecules are capable of selectively inhibiting MAO isoforms and this tendency has generated a renewed interest in the therapeutic possibility of these compounds.\(^{110,111}\) These days, the therapeutic concerns of MAOIs falls into two major groups. MAO-A inhibitors are being used in the management of depression and anxiety disorders.\(^{112}\) However, MAO-B inhibitors have shown their therapeutic value in a variety neurodegenerative and other diseases\(^{17,113}\) such as Parkinson’s\(^{110}\) and Alzheimer’s.\(^{114,115}\) An extensive assortments of reversible and irreversible MAO inhibitors are now available and their therapeutic uses have been mentioned in **Table 2**.

1.9.1. **Depression**

MAOIs were the first generation antidepressants used for decades in the treatment for patients with atypical depression\(^{116}\), anergic bipolar depression and treatment resistant depression\(^{117}\), high level of anxiety, specific phobias, post-traumatic stress disorder and migraine headaches resistant to other therapies.\(^{118}\) Phenelzine (Nardil) and
Tranylcypromine (Parnate) are non-selective irreversible inhibitors used with reversible MAOIs- Moclobemide, Befloxatone and Toloxatone. Reversible MAO-A inhibitor, Moclobemide, increases the concentrations of serotonin and noradrenaline in brain but significantly interacts with both selective SSRI and tricyclic antidepressants. When used sequentially, there should sufficient time variation between different types of drugs. MAOIs such as Marplan (isocarboxazide), Nardil (phenelzine), Parnate (tranylcypromine), and Eldepryl (selegiline) are used in the treatment of Parkinson’s disease rather than depression. Nardil, Parnate and Marplan are non-selective and irreversibly inhibit MAOs but Eldepryl is more selective to inhibit MAO-B. Phenelzine increases corticosterone levels associated with hypothalamic-pituitary-adrenocortical axis activity, is hyper-active in major depressive conditions but hypoactive in atypical depression. The use of L-Deprenyl and Lazabemide is already established in smoking cessation and continued abstinence.

1.9.2. Anxiety disorders

MAO inhibitors have been used for decades in the management of patients with various neurological disorders. Moclobemide and other RIMA agents are short acting, specific, reversible inhibitors of MAO-A, used in the treatment of anxiety disorders, and an optimal choice for post-traumatic stress and panic disorder. Marplan, Nardil and Parnate balance certain brain chemicals resulting in reduced anxiety symptoms. MAOIs are the treatment of choice in case of anxiety or depression with unusual features, like reactive mood and sensitivity to rejection. MAOIs have shown the highest efficacy in obsessive-compulsive disorder, especially when accompanied by phobic anxiety.

1.9.3. Parkinson’s disease (PD)

PD causes dysfunction in dopaminergic, noradrenergic and serotonergic neurotransmission. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which is a toxins/neurological insults, along with ROS damage the basal ganglia and/or the substantia nigra resulting into neurological disorders including Parkinsonian features. MAO-B catalyzes the oxidation of MPTP into MPP⁺, and that increased MAO-B activity in the astrocyte causing parkinsonian manifestation. Then, the formed MPP⁺ reaches
the extracellular fluid and transported to the nerve terminals by dopamine (DA) transporter producing parkinsonian symptoms. Inhibition of either MAO-B or DA transporter protects against MPTP generated MPP⁺ toxicity. MAO-A and B oxidatively deaminate dopamine equally and therefore, used effectively to take care of Parkinson's disease. At present, Selegiline is most commonly used MAO-B inhibitor in Parkinson's disease, although it has poor bioavailability and metabolized to potentially harmful neurotoxic metabolites, L-Methamphetamine and L-Amphetamine. Another MAOI is Rasagiline, whose neurotoxic metabolites are unknown but it is effective in monotherapy in early Parkinson's disease, as well as adjunctive therapy to levodopa in advanced disease. Moclobemide is a selective, prototypical reversible inhibitor of MAO-A which also has antiparkinsonian effects.

1.9.4. Alzheimer's disease (AD)

AD is the most common cause of dementia in late life and recognized as a major threat to the ageing population. It is characterized by progressive memory loss, decline in language skills and other cognitive impairments. Oxidative stress, excessive metal ions and reduced acetylcholine level play significant role in the pathogenesis of AD. The level of the monoamines (dopamine, norepinephrine, and serotonin) is decreased in the brain of AD patients as a result of loss of cholinergic neurons. Activity of MAO-B is linked to production of ROS that contribute to neurodegeneration. The oxidative stress could be reduced by blocking MAO-B activity and this may slow the progression of AD. l-Deprenyl inhibits MAO-B prominently which enhances central monoaminergic system. The major efficacy of L-deprenyl is due to its conversion to l-Amphetamine and l-Methamphetamine type of stimulants. This also enhances NO-mediated mechanism in vascular and neural tissues, which is partly accountable for the therapeutic efficacy of the drug. Rasagiline is another selective and irreversible inhibitor of MAO-B, which is structurally very similar to Selegiline and is ten times more active as compared to L-Deprenyl. Ladostigil combinedly inhibits AChE/MAO and possesses neuroprotective ability. It is formed after combining active components from Rasagiline (MAO inhibitor) and Rivastigmine (AChE inhibitor).
1.9.5. Other neurodegenerative diseases

Oxidative stress, iron accumulation, excitotoxicity, inflammatory process and the misfolding of toxic protein are the pathological features of the Huntington’s disease and amyotrophic lateral sclerosis (ALS) type of neurodegenerative diseases. L-Deprenyl, in combination with SSRI (fluoxetine), showed beneficial effects in Huntington’s disease but not in ALS. However, rasagiline and CGP 3466 were found to be effective in mouse models of ALS.

1.9.6. Attention deficit hyperactivity disorder (ADHD) and Tourette’s syndrome

Underactivity of dopaminergic or phenethylaminergic (PEA) pathway is correlated with ADHD. Both are wrecked by MAO and therefore, in ADHD, inhibition of MAO-B may increase the level of PEA, which is assumed to act as an endogenous amphetamine. Selegiline and Pargyline are specific MAO-B inhibitors tested in ADHD associated with Tourette’s syndrome and Pargyline showed promising results in this. Subsequent trials on Deprenyl showed that it reduces ADHD symptoms, particularly auxiliary impairments associated with the disease.

1.9.7. Cerebral ischaemia

Cerebral ischaemia is a major neurological problem triggered by ROS production, mitochondrial damage and neuronal apoptosis. l-Deprenyl protects the tissue damage that results from cardiac failure, by decreasing the generation of H₂O₂ together with an increase in the ratio of B-cell leukaemia/lymphoma2 (BCL2) to BCL associated protein X along with functionalization and transport of the anti-apoptotic protein kinases. Phase 2 clinical trial results confirm that l-Deprenyl can enhance recovery after cerebral infraction.

1.9.8. Neuroprotection and neurorescue

MAO-B, which is present in astrocytes and serotonergic neurons, metabolizes synthetic dopaminergic proneurotoxin, MPTP into the neurotoxin MPP⁺, which ultimately causes PD in human/non-human primates. Consequently, an elevated intracellular Ca²⁺activates the Ca²⁺ dependent enzyme (protein kinase and calpains),
which lead to various neurodegenerative diseases. A clinical study shows the l-Deprenyl decreases the progression of nigro-striatal neurodegeneration followed by substantial depletion of striatal dopamine. Proven neuroprotective/neurorescue ability of l-Deprenyl\(^{17,148-150}\) in PD initiated the development of other MAO-B inhibitors, such as p-fluoro deprenyl\(^{151}\), Lazabemide\(^{152}\), PF 9601N, Rasagiline\(^{153}\) and the aliphatic propargylamines, as potential antiparkinsonian drugs.\(^{154}\) l-Deprenyl effects cellular protective mechanism into three different ways, i.e. by neuroprotection, neurorescue and neurorestoration. Its neuroprotective effects are exerted by inhibiting the degradation of DA or other MPTP-like neurotoxins, attenuation of the neurotoxic actions of the endogenous toxins β-carbolines and tetrahydroisoquinolines. The neurorescuing tendency of deprenyl protect dopaminergic neurons after the administration of MPTP, at a time when most of it is metabolized to toxic MPP\(^{+}\), and the neurorestorative mechanism is explained by the increased production of neurotrophic factors.\(^{155}\) MAO-A inhibitor, Moclobemide, may also provide cellular protection by inhibition of MAO-A-derived hydrogen peroxide generation. For the first time, Deprenyl and α-tocopherol were clinically assessed as potential neuroprotective agents in the treatment of Parkinson's disease. The neuroprotective effect of α-Tocopherol may be exerted by scavenging free radicals and, thus, inhibiting cytotoxic processes such as lipid peroxidation. In cultured astrocytes, l-Deprenyl and Selegilne were found to fuel the production of the growth factors (nerve growth factor (NGF), brain derived neurotrophic factor (BDNF) and glial cell line derived neurotrophic factor (GDNF)). l-Deprenyl supresses astrogliosis and protects nerve cell death resulting from oxidative stress, glutathione depletion, peroxynitrite and nitric oxide, N-methyl-R-salsolinol, 6-hydroxy dopamine and physical damage caused by crushing or axotomy.\(^{156}\)

1.9.9. Cancer

Some recent reports underlined that MAO-A produces an enzyme called amine oxidase, which is known to affect carcinogenesis. Clorgyline, an MAO-A inhibitor, prevented apoptosis in melanoma cells, \textit{in vitro}.\(^{157}\) It has been observed that certain type of carcinomas (such as Cholangiocarcinoma) suppress expression of MAO-A enzyme.
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Introduction

The patients with over expressed MAO-A showed less likelihood to adjacent organ invasion along with better prognosis and survival. MAO-A was found to be a new candidate therapeutic target for advanced prostate cancer, and found to be over expressed in grade 4/5 cancers in comparison to grade 3 cancers. It is also expressed by normal basal cells and in vitro studies entailed that its function is to limit secretory differentiation.

1.10. Side effects/ADRs associated with MAOIs and challenges to newer agents

The MAOIs are associated with several side effects that often limit their usefulness and tolerability. First generation, non-selective and irreversible MAOIs were devoid of some severe side-effects such as; orthostatic hypotension, hepatotoxicity and most importantly hypertensive crisis (characterized by occipital headache that may radiate frontally, neck stiffness or soreness, palpitation, sweating, nausea, vomiting, dilated pupil, photophobia, tachycardia or bradycardia that can be associated with constricting chest pain; collectively these are termed as cheese effect or cheese reaction). This can occur owing to the ingestion of tyramine rich foods (e.g. aged cheeses) along with MAO inhibitors.

The Mechanism of “Cheese” Reaction

![The Mechanism of “Cheese” Reaction](image)

**Figure 1.6:** The mechanism of “Cheese” reaction
Some other familiar side effects associated with MAOIs are daytime sedation, dizziness, dry mouth, nervousness, muscle aches, insomnia, weight gain, sexual dysfunction, and urinary difficulty. Sexual effects persuaded by MAOIs include anorgasmia (inability to achieve orgasm) and impotency, which are apparently more frequent with Nardil (Phenelgine) than Parnate (Tranylcypromine). Some patients may be bothered by muscle aches and paresthesia (pricking or tingling sensation). Perhaps this may takes place due to the interference of MAOI’s with the metabolism of vitamin B₆ (pyridoxine). When Selegiline is introduced in combination with levodopa, it may result in dry mouth, anorexia/nausea, dyskinesia, and orthostatic hypotension in patients suffering from Parkinson’s disease.¹¹⁵ The most frequently reported adverse effects of Moclobemide (reversible inhibitor of MAO-A (RIMA)) are sleep disturbances, increased anxiety, restlessness, and headache. Regarding tolerability, Moclobemide, exhibited satisfactory results and possesses limited potential to elicit hypertensive crisis, which could be attributed to its selectivity and reversible competitive binding to MAO-A. This signifies that Moclobemide is easily dislocated from the enzyme’s binding site by other substrates. The combination of other medications boosting serotonin concentration (such as SSRIs), with MAOIs could precipitate a potentially perilous condition called serotonin syndrome (a reaction caused by excessive serotonin stimulation in the brain). The initial signs of serotonin syndrome include—restlessness, confusion, tremors, flushing, excessive sweating, and involuntary muscle jerks. Following this if medications are not stopped, the individual may develop more life threatening complications.

1.11. Pyrazoles and pyrazolines as MAOIs

The pyrazoles and their hydrogenated congeners pyrazolines have engrossed substantial attention for many years. Rising evidence put forward 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\textsuperscript{185-188}, anti-cancer\textsuperscript{172, 189-192}, anti-viral\textsuperscript{193-195}, anti-malarial\textsuperscript{185, 196}, anti-inflammatory-analgesic\textsuperscript{173, 175, 197, 198}, anti-microbial\textsuperscript{199-203} etc.

Recently, the augmentation of third generation MAOIs engendered a renewed interest towards design, synthesis, and study of reversible and selective compounds\textsuperscript{204}. Colibus et al.\textsuperscript{63} scrupulously portrayed the structural comparison of human MAO-A and MAO-B and demonstrated that binding of 1,3,5-trisubstituted pyrazoline ligands to either MAO-A or MAO-B enzymes revealed the importance of substitution in N1 position. The slight variation in this position greatly influenced both, the potency as well as selectivity. Modeling studies hypothesized that replacing the substituted phenyl ring at N1 by an acetyl group reduces the bulkiness of the compound and increases the positive charge on N1 position of the heterocycle and hence, reinforce the charge-transfer bonding interactions (postulated as one of the major interactions with MAOs of reversible inhibitors) with the isoalloxazine ring\textsuperscript{31, 54}. A preliminary computational analysis was performed on 1-acetyl-3-(2-hydroxyphenyl)-5-(3-methylphenyl)-4,5-dihydro-(1H)-pyrazole to verify this hypothesis, which demonstrated another favorable interaction of this compound with isoalloxazine ring system with respect to old-generation compounds\textsuperscript{183}. Furthermore, the acetyl group at N1 position of pyrazoline derivative was replaced with an unsubstituted thioamide moiety and inferred that the C=S group was more polarizable than the C=O group because of the larger kernel of electrons in the S atom\textsuperscript{171}. Quite a few studies reported in the literature have contributed to a better understanding of the factors responsible for MAOI activity and MAO-A/B selectivity\textsuperscript{54, 205}. Such studies argued that the factors affecting selectivity were fundamentally different. To inhibit the active site of MAO-B, molecule lipophilicity was fundamental, while to inhibit the MAO-A site, a fundamental element is the N5 charge transfer bond of the isoalloxazine nucleus of FAD. Besides these, other equally essential factors were the hydrogen bonds that stabilized the enzyme-inhibitor complex between the polar groups on the inhibitor, the amino acid residues of the active site of the enzyme, and finally the planar structure of the inhibitor that has been best adapted to the receptor pocket\textsuperscript{168}.

On the basis of experiments, various substitutions in the aryl rings on C3 and C5 positions, as well as N1 substituents, have been tried which showed that N1-acetyl and
N1-thiocarbamoyl 2-pyrazolines showed high potency together with good selectivity and, because of their synthetic accessibility, permitted numerous chemical changes. Also, the inhibitory action was correlated with the existence of the halogen atoms on A and/or B rings (aryl rings on C3 and C5) and also with N1 substituent. In fact, the elongation of the N1 chain decreased the activity against MAO-B because of formation of an unstable complex.

SAR studies as depicted in structure 54 revealed that α-methyl or α-methoxy replacement in ring C abolished selectivity towards isoforms, irrespective of substitutions at ring A and B. α-Hydroxy substitution in ring A, had no role in selectivity but α, p-dihydroxy substitution favored selectivity for MAO-A, when ring C was unsubstituted. In ring B, α- or p- substitution with -OH, -OCH₃ or -Cl groups favored selectivity for MAO-A when ring C was unsubstituted. Also, the ortho substitution with -Cl or -OH groups in ring B was found superior, but not significant, than the p-substitution. However, on substitution with p-methoxy group, a significant difference was found in selectivity along with potency. Replacement of ring B with thiophene or furan ring retained MAO-A selectivity with a little increase in the potenity, but not superior than the p-methoxy phenyl substitution. The spectrum of selectivity shifted towards MAO-A in the presence of five membered heterocyclic ring B and substitution at 1N position. Moreover, α-methoxy, p-hydroxy di-substitution in ring B also favored selectivity against MAO-A, when ring C was unsubstituted. Selectivity towards MAO-B was favored in absence of ring C, however in its presence, p-methyl or p-methoxy substitution favored selectivity towards MAO-B.

![Figure 1.7: A brief SAR of 2-pyrazolines as MAO inhibitor](image-url)
Docking studies stated that complex of a potent compound with MAO-B showed H-bond interaction between ligand’s amino hydrogen with hydroxyl oxygen of Tyr398 (this also allowed the ring B positioning in an aromatic cage comprised of FAD, Tyr398 and Tyr435) and hydroxyl hydrogen of ring A with hydroxyl oxygen of Tyr326. Relatively, a narrow active site cavity promotes the positioning of ring B in aromatic cage of MAO-B. Hydroxyl group in para position of ring B further favored hydrophobic interaction of its phenyl ring with phenyl rings of Tyr398 and Tyr435. In some compounds, two unsubstituted rings B and C were conveniently positioned in aromatic cage of MAO-B, while a ring with substitution drastically reduced approximated binding free energy. In presence of all three ring substitutions, only one ring get accommodated forcing the other two rings to a narrow cavity which lead to a poor estimated binding free energy. In other words, these factors makes them selective towards MAO-A, which has a comparatively a wider cavity than MAO-B.

The crystal complex of compound 55 with MAO-A enzyme showed H-bond interaction between the sulphonyl oxygen of the ligands and hydroxyl hydrogen of either Tyr444 or Tyr407. Because of this hydrogen bonding interaction, ring B/C were situated in the aromatic cage (Pocket1: Tyr407, Tyr444 and FAD). In ring C, the p-methyl substitution considerably changed the ligand’s orientation at the active site, that resulted in significant increase in potency and selectivity indices towards MAO-A. Remaining two rings were well placed in pocket2 (Ile180, Ile335, Leu337, Met350, Phe352) and pocket3 (due to cavity-shaping loop, Gly74, Arg206, Ile207, Phe208, Glu216, Trp441).

![Structure of a 1,3,5-trisubstituted 2-pyrazoliene derivative as a potential MAO inhibitor](image)

**Figure 1.8:** Structure of a 1,3,5-trisubstituted 2-pyrazoliene derivative as a potential MAO inhibitor
However, the crystal complex of compound 55 with MAO-B depicted the H-bond interaction of pyrazoline ring with hydroxyl oxygen of Tyr435 and carbonyl oxygen of Ile199. Because of this interaction, the ring A and ring B get oriented in the aromatic cage (pocket 1) and in the narrow cavity (pocket 2 and 3 were shrunked and joined together to form a narrow cavity in MAO-B owing to assorted positioning of the cavity forming loop) respectively. Therefore, structures with an additional ring C feel a momentous steric hindrance in this cavity, that explains poor interaction of these compounds with MAO-B\textsuperscript{53}. 