Chapter 1. Introduction

1.1 Parkinson's disease

Parkinson's disease (PD) is a gradual progressive central neurodegenerative disorder that affects body movement and is characterized by symptoms such as muscle rigidity, resting tremors, loss of facial expression, hypophonia, diminished blinking, and akinesia.1,2 Parkinsonian like central nervous system (CNS) disorders usually involve the pigmented neuronal systems of the brainstem, particularly the zona compacta of the substantia nigra which gives rise to the dopaminergic nigrostriatal pathway and the locus ceruleus giving rise to a noradrenergic pathway.3 The motor disabilities characterizing PD are primarily due to the loss of dopaminergic neurons in the substantia nigra4,5 resulting in a dramatic decrease in the dopamine (DA, 8, section 1.3.4) levels in the brain.6 Once the DA neuronal cell death reaches the critical level of 85-90%, the neurological symptoms of PD appear.7 The current treatment for PD is the systematic administration of levodopa (L-DOPA), a precursor to DA which enters the brain via a carrier-mediated transport system where it is converted to DA by the enzyme L-aromatic amino acid decarboxylase (L-AAAD).8 Since the discovery in the 1960s that striatal dopamine is deficient in PD and it's replacement with high dosages of L-DOPA could ameliorate the symptoms of parkinsonism, research on PD has increased dramatically. Although this is still used to treat PD, several problems usually develop during the chronic use of L-DOPA.9 The most common and vexing problems are dyskinesias.10 Investigators have attempted to gain insight into
the mechanism of these troublesome side effects by studying the metabolism of L-DOPA in lesioned animal models. Many proposals have been put forth to account for these side effects but none have been definitively proven. Research efforts focusing on the treatment of PD is required because PD affects 1 in 500 of the general population and its incidence increases with advancing age to 1-2% of the population over 50. The etiology and possible means of prevention are unknown. However, there are three hypotheses regarding the etiology of Parkinson's disease, but none have been proven: genetic factors, aging of the CNS, and infections or toxic factors. One of the most explored hypotheses is that PD may be caused by exposure to environmental agents or endogenous toxins resulting in the acceleration of the normal age related decline in the number of substantia nigra DA containing neurons. Epidemiological studies have shown that the prevalence of Parkinson's disease is higher in highly industrialized countries. These results support the hypothesis that environmental or endogenous factors may play a role in the development of PD.

1.2. MPTP - A parkinsonian inducing agent

1.2.1. Discovery and background

Although there are many causes of parkinsonism, there is only one well defined pathological entity generally referred to as idiopathic Parkinson's disease. Motor deficiencies similar to those observed in idiopathic PD were reported in young drug abusers after self administration of an impure
preparation of 1-methyl-4-phenyl-4-propionoxypiperidine (MPPP, 2, Scheme 1) a meperidine (1, Scheme 1) analog which was contaminated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 3, Scheme 1).\textsuperscript{16-19} MPTP appears to destroy nigrostriatal dopaminergic cells in the substantia nigra, causing a parkinsonian state in humans,\textsuperscript{2,16,17} primates,\textsuperscript{20,21} and mice.\textsuperscript{22-25} MPTP administration in humans, like idiopathic PD, causes a permanent parkinsonian condition,\textsuperscript{26} distinguishing it from many forms of reversible drug-induced parkinsonism. Further paralleling idiopathic PD, MPTP exposed individuals exhibit the same chronic impairment of motor skills\textsuperscript{20,21} which is subsequently treated successfully with drugs (L-DOPA) normally administered to relieve symptoms in idiopathic PD.\textsuperscript{2,17,19} MPTP has been effective in producing a nonhuman model for PD\textsuperscript{20,21} and provides an opportunity for researchers to investigate the mechanism of action of drug induced parkinsonism.

\textbf{Scheme 1.} The Origin of MPTP from MPPP.
1.2.2 Proposed mechanism of MPTP neurotoxicity

Upon further investigation, MPTP was shown to produce a selective loss of dopaminergic neurons in several species. The intravenous (i.v.) administration of MPTP to rhesus monkeys in various doses produced visible symptoms of parkinsonism.\textsuperscript{20} The intraperitoneal (i.p.) administration of MPTP to squirrel monkeys produced essentially the same symptoms as observed in the rhesus monkey.\textsuperscript{21} Chiueh examined the neurotoxicity of MPTP in rats and guinea pigs. It was discovered that neither rats nor guinea pigs show permanent DA deficiencies in the striata or display movement disorders as in primates.\textsuperscript{27-30} The animal model studies were expanded to include beagle dogs.\textsuperscript{31} The dogs, like primates exhibited the degeneration of the nigrostriatal tract. Others reported that a higher dosage of MPTP administrated to mice, particularly the C57BL/6J strain, was toxic to this species.\textsuperscript{23,32,33} It is apparent that different species and tissues have different susceptibilities to the toxicity of MPTP. Primates are more susceptible than rodents. DA depletion resulting from MPTP administration through various routes [intraperitoneal, intravenous, subcutaneous (s.c.), and intranigral] in mice was noted but no rigidity or tremors were found as in the case of humans, monkeys, and dogs.\textsuperscript{29} It is known that much higher doses of MPTP are required to induce neurotoxicity in mice as compared to primates and dogs.\textsuperscript{34}

As studies on MPTP continued, it was found that MPTP itself was not neurotoxic, rather MPTP must be bioactivated to a species which mediates its neurotoxicity. The mechanism of neurotoxicity has been studied extensively
and it is known that the flavin containing enzyme monoamine oxidase (MAO),\textsuperscript{35-39} mainly type B and to a lesser degree type A,\textsuperscript{39} catalyzes the conversion of MPTP to the 2,3-dihydro-1-methyl-4-phenylpyridinium species MPDP\textsuperscript{+} (4, Scheme 2)\textsuperscript{40-42}. MPDP\textsuperscript{+} undergoes another two electron oxidation, by a pathway that may be enzyme mediated, autoxidation, or by a disproportionation mechanism, to form the neurotoxic 1-methyl-4-phenylpyridinium species MPP\textsuperscript{+} (5, Scheme 2)\textsuperscript{38,43,44}. It is reported that MPTP must be converted to MPP\textsuperscript{+} before it can elicit a neurotoxic effect\textsuperscript{44}. Further evidence to support the proposed MAO-B bioactivation of MPTP is the fact that MPTP neurotoxicity is blocked by the selective MAO-B inhibitors pargyline and (R)-deprenyl\textsuperscript{45}, but not the selective MAO-A inhibitor clorgyline\textsuperscript{35,38}. The MPP\textsuperscript{+} metabolite of MPTP is the major metabolite found in the brain of experimental animals\textsuperscript{43,46}. MPP\textsuperscript{+}, being a positively charged molecule, is unable to cross the blood-brain barrier and must therefore be formed in the brain. The currently accepted mechanism for the MAO-B mediated neurotoxicity of MPTP (Figure 1) is believed to begin with the partitioning and concentration of MPTP in the brain. Once in the brain, MPTP is converted extraneuronally in the astrocytes, cells rich in MAO-B, to MPDP\textsuperscript{+},\textsuperscript{21} which then is further oxidized to MPP\textsuperscript{+}. MPP\textsuperscript{+} leaks out of the glial cells and into the extracellular space where it is a substrate for the presynaptic DA uptake system (Figure 1), which results in its energy dependent concentration within the dopaminergic neurons\textsuperscript{47-50}. Some supporting evidence for the fact that MPP\textsuperscript{+} is a substrate for the dopamine transporter is that MPP\textsuperscript{+} toxicity can be blocked in rodents by dopamine reuptake blockers such as mazindol and nomifensine\textsuperscript{51}. However, there is some doubt concerning the efficiency of DA
uptake blockers to prevent MPTP toxicity in primates.\textsuperscript{52,53} This intraneuronal concentration of MPP\textsuperscript{+} may be enhanced further by the binding of MPP\textsuperscript{+} to neuromelanin. Melanized dopaminergic neurons have been shown to be more susceptible to neurodegeneration in Parkinson’s disease and MPTP neurotoxicity.\textsuperscript{54-56} MPP\textsuperscript{+} is concentrated in the matrix of the mitochondria,\textsuperscript{47} where it is a potent inhibitor of the mitochondrial oxidation and the electron transport chain at the complex I level\textsuperscript{57,58} by binding to NADH dehydrogenase (Figure 1).\textsuperscript{59-61} MPP\textsuperscript{+} combines with NADH dehydrogenase at a point distal to its iron-sulfur cluster but prior to the Q\textsubscript{10} ubiquinone combining site.\textsuperscript{36,37,59-69} This leads to cessation of oxidative phosphorylation, adenosine triphosphate (ATP) depletion,\textsuperscript{58} decrease in reduced glutathione (GSH),\textsuperscript{70} changes in intracellular calcium content and neuronal cell death.\textsuperscript{71}

Several species have been investigated to use as a model to study the metabolism and neurotoxic properties of MPTP and analogs of MPTP. Extensive in vitro and in vivo studies using MPP\textsuperscript{+} and various pyridinium analogs have been conducted to assess their neurotoxicity. From in vivo microdialysis studies\textsuperscript{72,73} and in vitro mitochondrial respiration and cell culture experiments,\textsuperscript{74-76} some of these compounds were determined to be potent, selective and irreversible neurotoxins for dopaminergic neurons. It has been established that C57BL/6 mice may be a useful animal model to study MPTP-induced neurotoxicity.\textsuperscript{77}
Scheme 2. MAO Catalyzed Oxidation of MPTP

3 → MAO-B → 4 → ? → 5
Figure 1. The Mechanism of MPTP Neurotoxicity.
1.2.3 Structure-activity relationships (SAR)

The discovery that MAO-B is responsible for the catalytic oxidation of MPTP is exciting because there are no other cyclic tertiary amines reported to display MAO substrate properties. The combination of the unique neurotoxic properties and structural features of MPTP has made it a molecule of interest to explore the mechanism of MAO catalysis, to model and explore idiopathic Parkinson's Disease, and to examine the structural features of the enzyme active sites. Several MPTP analogs have been prepared and tested for neurotoxic properties in order to determine the basic structural features responsible for causing neurotoxicity. Analysis of the available data has led to the following generalizations concerning the structural requirements and biological conditions for neurotoxicity of MPTP and analogs:

• The 4,5 double bond (Scheme 2) is essential for MAO substrate activity.

• The N-H, N-ethyl, N-propyl, N-β-hydroxyethyl analogs display less MAO activity than MPTP. In general, longer alkyl chains abolish MAO reactivity.

• The 4-phenyl group of MPTP is not essential for substrate activity. For example, replacement of the phenyl ring with a benzyl group or a phenoxy group enhances the enzyme activity. The 4-cyclohexyl analog has substrate properties comparable to those of MPTP. Hence, substitution at the 4 position of the tetrahydropyridine ring is the only versatile structural variable tolerated. Below is a three dimensional representation of MPTP, which illustrates some of the unique structural features such as the considerable flattening of the piperidine ring by the 4-5 double bond and the dihedral angle...
between the tetrahydropyridine ring and the phenyl ring which is approximately 32°.

Many researchers are exploiting these properties by structurally modifying MPTP at the C4 position to investigate the active sites of MAO-A and MAO-B by evaluating the influence of the C4 substituent on enzyme activity and selectivity.

1.3. MAO-A and MAO-B.

1.3.1. Enzyme characteristics of MAO-A and B

The enzyme responsible for the deamination of certain biogenic amines was identified by Mary Hare in 1928, who termed the enzyme, which was found in rabbit tissue extracts, tyramine oxidase.\textsuperscript{96} It was later renamed monoamine oxidase (EC 1.4.3.4) by Zeller\textsuperscript{97} to reflect more accurately the substrate selectivity and also to distinguish the enzyme from diamine oxidases (DAOs)\textsuperscript{98} and polyamine oxidases (PAOs) according to their ability to catalyze the oxidation of monoamines, diamines, and polyamines.\textsuperscript{99}

Monoamine oxidase (MAO) is an integral protein of the mitochondrial
outer membrane of neuronal, glial, and other cells in the human body such as platelets, liver, heart, lung and placenta. The flavin adenine dinucleotide (FAD) containing enzyme catalyzes the oxidation of amines to the corresponding aldehyde and ammonia as illustrated in the following reaction:

\[
RCH_2NH_2 + FAD + O_2 + H_2O \rightarrow RCHO + FADH_2 + HOOH + NH_3
\]

The actual mechanism of enzyme catalysis is under debate and will be discussed later. The major role of MAO is the regulation of neurotransmitters in the brain and peripheral tissues. The classification of MAO into two forms, MAO-A and MAO-B, was first suggested by Johnson in 1968 on the basis of substrate preference and inhibitor selectivity. MAO-B is selectively inhibited by nanomolar concentrations of (R)-deprenyl (6) while MAO-A is inhibited by nanomolar concentrations of clorgyline (7).

It was Roth and Pierce who provided unequivocal evidence for the presence of two forms of MAO. It was demonstrated that both the A and the B forms of human brain MAO are separable with retention of enzyme activity by chromatography. Additional evidence was provided for the existence of both
MAO A and B when monoclonal antibodies were produced against each enzyme form indicating that the two major enzyme sub-types are different proteins\textsuperscript{105,106} and that MAO-A is located in catecholaminergic cells while MAO-B is located in serotonergic regions in the glial cells.\textsuperscript{107-109} Although substrates of MAO-A and MAO-B do not show absolute specificity, $\beta$-phenylethylamine (PE)\textsuperscript{102} shows MAO-B selectivity while serotonin is mainly a MAO-A substrate.\textsuperscript{97} Other supporting evidence for the two MAO isozymes was reported by Hsu who isolated the cDNA clones that encode MAO-A and MAO-B which allowed for the determination of the individual amino acid sequences.\textsuperscript{110} The MAO sub-types show 70% amino acid homology. As shown below, both MAO forms contain the peptide sequence Ser-Gly-Gly-Cys-Tyr in the active site in which the cofactor FAD is covalently bonded to the cysteine residue through a thio ether linkage.\textsuperscript{111}

![Chemical Structure](image)

Problems solubilizing proteins from mitochondrial membranes, combined with aggregation and possible interspecies differences have made it difficult to estimate the molecular weight of the MAOs. The molecular weights of the human MAO-A and MAO-B subunits have been estimated to be 59.7 KDa and 58.8 KDa, respectively.\textsuperscript{112} From the estimates, it was determined that in the human MAO enzymes there is a ratio of one FAD per 63 KDa for MAO-A
and 57 KDa for MAO-B.

The semi-purified enzymes used in the experiments to be discussed employ MAO-B isolated from bovine liver which has a molecular weight of 100 KDa based on flavin content and MAO-A isolated from human placenta which has been reported by Cawthon to be 63KDa. Although the amino acid sequences of the enzymes are known, the structure of the enzyme active sites have not yet been characterized due to the lack of x-ray structural data.

1.3.2 Distribution of MAO-A and MAO-B in the CNS

In mammals, MAO has been identified in all cell types with the exception of erythrocytes. In the human MAO-A and MAO-B appear to co-exist and this co-existence may occur on the level of a single cell. In humans, the highest levels of MAO-A are found in the liver, lung, and intestines with the spleen and cerebral microvessels having the lowest activity. The MAO-B enzyme activity is expressed in highest levels in the brain and liver, with very low levels present in the pancreas, spleen, lung, cerebral microvessels and cultured skin fibroblasts. It is known that the peripheral organs are rich in MAO. For example, the skeletal muscles have MAO-A levels comparable to that of the liver and MAO-B levels twice that found in the liver and the stomach is very rich in both forms of MAO.

The tissue distribution of MAO in the brain varies among species. In whole human brain, it is believed that there is approximately twice as much MAO-B as MAO-A. The MAO values are determined by titrating the two
isozymes with the selective inhibitors clorgyline or (R)-deprenyl and measuring the remaining activity towards a variety of MAO-A selective, MAO-B selective, and mixed substrates. The brain as a whole is one of the organs containing high MAO-A and MAO-B activity in humans. However, within the brain, the distribution of MAO-A and MAO-B is not uniform; there are regional and cellular variations. MAO-B is active in the cortex, hippocampus, brain stem, and substantia nigra region. MAO-B activity has been reported in all brain regions with the exception of the cerebellum, occipital cortex and white matter. MAO-A activity has been reported to be high in the limbic regions of the nucleus accumbens, hypothalamus, and mammilary complex and the brain stem nuclei (locus coeruleus and substantia nigra). The lowest MAO-A activity is found in the basal ganglia and cortical structures. Based on the observed rate of dopamine metabolism, there is a 3:1 greater prevalence of MAO-B activity to MAO-A activity in the human striatum. In general, both mitochondrial membrane bound isozymes of MAO are present in the central nervous system (CNS) but their entire function is not fully known.

On the cellular level in the CNS, MAO-A is present in the cell bodies of all catecholaminergic neuronal populations. MAO-A has also been demonstrated to be in the noradrenergic cell bodies of the locus coeruleus, sub-coeruleus complex, and the lateral tegmentum. MAO-A is the sole isozyme present in neuronal cell bodies of the dopaminergic projections from the substantia nigra. In contrast, MAO-B appears in serotonergic regions of the hypothalamus and in the astrocytes. In general, the distribution of MAO-A and MAO-B in human brain cells appears to be identical to that reported in primates and rodents although the role of MAO
differs from species to species.

1.3.3. Roles of MAO-A and MAO-B in the CNS

One of the primary roles of monoamine oxidase A and B is to regulate the levels of biogenic amines in the brain and other tissues by catalyzing the oxidative deamination of the amine neurotransmitters. The studies on the contribution of MAO-A and MAO-B to the metabolism of neurotransmitters has mainly concentrated on DA and serotonin (5-HT). Obviously, the in vivo metabolism by MAO in humans is not easily studied, thus the majority of the available literature reports experimental animal studies. Although under in vitro conditions DA is a selective MAO-B substrate, the enzyme selectivity of DA in vivo has not been adequately resolved. In rats, using tissue homogenates as well as microdialysis studies, it was discovered that MAO-A is the isozyme responsible for 85% of the DA metabolism. Similar findings by Fagervall and Ross as well as Liccione and Azzaro indicated that in rats MAO-A plays the major role in DA metabolism (95%). It was also observed that intrasynaptosomal deamination of DA occurred solely by MAO-A and the extrasynaptosomal metabolism was carried out by MAO-B. The metabolism of DA by MAO-B only becomes important when the dopamine reuptake pathway is inhibited. Further support for the important role of MAO-A in rats is that the blockage of MAO-B with the selective inhibitor (R)-deprenyl does not effect greatly the levels of DA or its metabolites 3,4-dihydroxyphenyl acetic acid (DOPAC) and homovanillic acid (HVA).

The role of MAO-A in rats does not appear to be readily transferable to
the human. DA has been regarded as a preferential MAO-B substrate in the human brain. It was reported that 75% of DA metabolism in human brain homogenates was catalyzed by MAO-B.\textsuperscript{130,139} An examination of the metabolism of DA by MAO-A and MAO-B in different human brain regions revealed regional differences. Experiments designed to examine the contribution of intra- and extraneuronal MAO with respect to DA metabolism in humans revealed that the metabolism of DA occurs mainly via the extraneuronal (glial) route by MAO-B.\textsuperscript{134,140} Like the human, studies in the guinea-pig\textsuperscript{130}, macaque\textsuperscript{141}, and pig\textsuperscript{140} indicate DA metabolism is greatly dependent on MAO-B. In humans, MAO-A is responsible for some of the metabolism of DA which has been transported back into the neuronal cells, however, MAO-A catalyzed metabolism of DA only becomes important when MAO-B is inhibited. In contrast, the metabolism of the neurotransmitter (R)-norepinephrine is predominately mediated by MAO-A upon re-uptake into the neurons.\textsuperscript{109,117}

The metabolism of 5-HT appears to have additional factors which make it unique. As stated previously, 5-HT is a selective MAO-A substrate in vitro. However, serotonin cell bodies have been demonstrated to contain only MAO-B and not MAO-A. In the nerve terminals, from which 5-HT is released and where uptake occurs, evidence suggests that only MAO-A is present.\textsuperscript{135,137,138} Thus, serotonin metabolism in humans appears to be only MAO-A mediated. Why MAO-B only appears within 5-HT cell bodies is not presently not understood.

The most selective MAO-B neurotransmitter β-phenylethylamine (PE) holds true to its selectivity in humans. PE is metabolized by MAO-B and the site of metabolism is almost exclusively in the glial cells where the enzyme is
localized. The presence of two isozymes of MAO in the CNS has been suggested to be a system of checks and balances in which one form of MAO plays a principal role in transmitter metabolism while, in the event of inhibition, the other isozyme can play a more dominant role.

1.3.4. Endogenous substrates (catecholamines and aminoalkyl indoles)

The major endogenous substrates for MAO are divided into two categories, the catecholamines, which includes DA (8), tyramine (T, 9) epinephrine (E, 10), and norepinephrine (NE, 11) and the aminoalkylindole 5-hydroxytryptamine (serotonin, 5-HT, 12). The only MAO-B selective neurotransmitter, β-phenylethylamine (PE, 13), is not included in either of these divisions. All neurotransmitters with the exception of PE are hydroxyl containing water soluble amines. These neurotransmitters are derived from the action of specific enzyme systems on amino acid precursors.
The endogenous substrates show selectivity for MAO-A or MAO-B as illustrated by the selectivity coefficients in Table 1. DA and T are MAO-B selective while serotonin is MAO-A selective.

**Table 1. Selectivities of the Endogenous Substrates with MAO-A and MAO-B**

<table>
<thead>
<tr>
<th>Endogenous MAO Substrate</th>
<th>SC$_{A/B}$</th>
<th>SC$_{B/A}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine (8)</td>
<td>0.23</td>
<td>4.4</td>
</tr>
<tr>
<td>Serotonin (12)</td>
<td>120</td>
<td>0.008</td>
</tr>
<tr>
<td>Tyramine (9)</td>
<td>0.125</td>
<td>8.0</td>
</tr>
<tr>
<td>(R)-Epinephrine (10)</td>
<td>0.86</td>
<td>1.2</td>
</tr>
<tr>
<td>(R)-Norepinephrine (11)</td>
<td>1.5</td>
<td>0.65</td>
</tr>
</tbody>
</table>

SC$_{A/B}$ = selectivity coefficient = ratio of MAO-A to MAO-B activity
SC$_{B/A}$ = selectivity coefficient = ratio of MAO-B to MAO-A activity

The life cycle of these biogenic amines in the brain was reported as follows: 145-147

- The appropriate amino acid precursor is transported across the blood-brain barrier and the neuronal cell membrane by transporters.
- Synthesis of the amine neurotransmitter takes place in the neuron.
- The amine formed is taken up by membrane-bound vesicles and stored in an inactive form. These vesicles are transported from the nerve cell bodies to the nerve terminals.
- In response to a stimulus, the neurotransmitter is released into the synaptic cleft in the active form.
- Dissociation of the neurotransmitter from the receptor site takes place with the termination of the stimulus. The neurotransmitter either undergoes metabolic degradation or is taken up by the nerve terminals.
1.3.5 MAO substrates

In addition to the neurotransmitters, MAO catalyzes the oxidation of other primary and secondary amines as well as some tertiary amines. MAO, unlike most enzymes, has a wide substrate selectivity. Selectivity, however, is observed between the substrates processed by MAO-A relative to MAO-B. As mentioned earlier, MAO-A preferentially metabolizes 5-HT\(^{102}\) while MAO-B shows a preference for PE\(^{144}\). The selectivity between the two forms of MAO is also observed in numerous substances of exogenous origin such as benzylamine (14),\(^{148}\) which is a preferred MAO-B substrate, and kynuramine (15),\(^{149}\) which is a preferential MAO-A substrate.

![Molecule 14](image14.png)
![Molecule 15](image15.png)

The major class of tertiary amines to display good MAO substrate activity are cyclic tetrahydropyridines. These MAO substrates clearly show enzyme-substrate selectivity seen with MAO-A and MAO-B. The MAO-A vs MAO-B selectivity is known to be dependent on the nature of the C-4 substituent. For example, the \(V_{\text{max}}/K_{\text{m}}\) ratio for the oxidation of MPTP (3) favors MAO-B by a factor of almost 4\(^{150}\) while the corresponding ratio for the oxidation of the C-4 2-isopropylphenyl tetrahydropyridine analog (38, table 2, section 2.1) is 20 times in favor of MAO-A.\(^{150}\)

The results obtained from the examination of a series of flexible 1-
methyl-4-aryl methyl and 4-arylethyl MPTP analogs suggest that MAO-A can accommodate bulky substituents more readily than MAO-B. Additional data from a systematic structure-substrate activity relationship study employing a series of 1-methyl-1,2,3,6-tetrahydropyridine derivatives bearing a 4-naphthoxy or 4-phenoxy group substituted in the para, meta, and ortho positions with chloro, methoxy, methyl, phenyl, and nitro groups provide supporting evidence for the greater flexibility of the MAO-A active site compared to the active site of MAO-B. Heterocyclic analogs (indolyl, pyrrolyl, furanyl, and isoindolyl) have yielded additional information about the types of substrates favored by MAO. Little is known regarding the structural features of the active sites which lead to the selectivities observed with various substrates.

1.3.6 MAO Inhibitors

Substances that decrease the rates of enzyme catalyzed reactions when present in the reaction mixture are inhibitors. MAO has been studied with a wide variety of inhibitors. One class of reversible MAO inhibitor, the α-methylmonamines, was described as early as 1937. Other classes of MAO reversible, competitive inhibitors are the harmala and vinca alkaloids, tetrahydro-β-carbolines, oxazolidinone derivatives, and xanthones. There are three basic classes of irreversible MAO inhibitors which are hydrazines, propargylamines, and cyclopropylamines.

The early monoamine oxidase inhibitors were used therapeutically to treat depression in the 1950s and early 60s. It was the recognition of an important clinical side effect referred to as the "cheese effect" that brought about
increased interest in MAO inhibitors. The majority of drugs which inhibit MAO such as, iproniazid (16), a specific and potent inhibitor of MAO once used to treat tuberculosis, have been removed from the market. Some of the MAO inhibitors currently in clinical use for the treatment of depression include phenelzine (17), tranylcypromine (18), pargyline (19) and isocarboxazid (20).

The emergence of selective MAO inhibitors such as clorgyline and (R)-deprenyl as well as the need for MAO inhibitors in the treatment of neurodegenerative diseases has sparked a renewed interest in therapeutic MAO inhibitors.

1.4. Proposed mechanisms of monoamine oxidase catalysis

1.4.1. Single electron transfer

The mechanism of MAO catalysis is not fully understood. Mechanistic studies concerning the catalytic pathway have led to three main proposals: the single electron transfer (SET) pathway, the hydrogen atom transfer pathway
(HAT) and a polar addition-elimination pathway. The SET pathway proposed by Silverman and colleagues\textsuperscript{173} (Scheme 3, pathway a) is based on studies with mechanism based inactivators. A mechanism based inactivator is a compound that is unreactive initially but is converted by the enzyme to a reactive species. From these studies, the SET pathway is believed to proceed via a one-electron transfer from the amine lone pair to the oxidized flavin (FAD) which gives the amine radical cation (22) and the flavin semiquinone (FADH\textsuperscript{+}). Loss of a proton gives the \(\alpha\)-amino radical (23) which can transfer the second electron to the flavin semiquinone to give the reduced flavin (FADH\textsubscript{2}) and the iminium product (24).

**Scheme 3.** Proposed Radical Mechanism for MAO Catalysis

\[
\begin{align*}
\text{FAD} & \quad \text{FADH}^+ \\
\text{RCH}_2\text{NH}_2 & \quad \text{RCH}_2\text{NH}_2 & \quad \text{RCHNH}_2 & \quad \text{RCH}=\text{NH}_2 \\
21 & \quad 22 & \quad 23 & \quad 24
\end{align*}
\]

Many cyclopropyl-containing substrate analogs have been examined as potential mechanism-based inactivators of MAO\textsuperscript{174-178} such as N-benzylcyclopropylamine (25). Upon incubation of MAO with each of these compounds, the enzyme became inactivated. When processed via the SET pathway, the resulting aminium radical cation 26 is thought to undergo rapid cyclopropyl ring opening to give a reactive primary radical (27) (Scheme 4).
Covalent attachment of the primary radical to either the flavin semiquinone or an amino acid radical is thought to mediate the inactivation.

**Scheme 4.** Proposed MAO Inactivation Pathway for Cyclopropylamines

More recent evidence for this type of enzyme mediated inactivation was reported by Zhong and Silverman. Using matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry, Silverman was able to identify the active site residue which is covalently modified via the cyclopropyl containing inactivators below. He incubated these inactivators with MAO-B, isolated the adducts, and digested the protein on which he performed MALDI-TOF mass spectrometry. The data indicate that it is a cysteine amino acid residue that is modified.
1.4.2. Hydrogen atom transfer

In 1967, Hull$^{180}$ performed studies on primary amine oxidations by ClO$_2$ and demonstrated that in addition to the SET pathway, there was also an operative HAT pathway that generated a carbon centered radical by hydrogen atom abstraction from the $\alpha$-carbon of amines like 21 (scheme 3). Later Edmondson $et \ al.$$^{181}$ based on work with substituted benzylamines have proposed an alternative pathway for MAO catalysis which involves hydrogen atom abstraction. The HAT pathway (Scheme 3, pathway b) generates the $\alpha$-carbon radical 23 directly and therefore bypasses the aminium radical cation 22. Loss of an electron from 23 yields 24. Edmondson has examined the binding properties of meta- and para-substituted benzylamine analogs to MAO-B as well as the isotope effect on MAO catalysis. He found that para- and meta-substituted benzylamines showed large deuterium kinetic isotope effects on the rate of the MAO catalyzed oxidation and that the magnitude of the effect is independent of the nature of the substituent, indicating that a H is involved in the rate determining step of the catalytic reaction. Model system studies of aminium cation radical deprotonation have also displayed reasonably large deuterium isotope effects with the magnitude dependent on the nature of the abstracting base. Edmondson also concludes that one electron oxidations of amines by MAO flavin are unlikely based on redox considerations, that is, the SET pathway is thermodynamically unfavored.
1.4.3 Polar addition-elimination pathway.

The flavin analog 3-methyl-5-ethylumiflavinium perchlorate (28) was used by Mariano$^{182,183}$ as a chemical model to study three major MAO inactivators: cyclopropylamines, α-silylamines and hydrazines. From the results of these studies, Mariano proposed that the MAO catalysis could proceed via an addition-elimination mechanism (Scheme 5). The amine 21 acts as a nucleophile to attack the activated flavin to form an amine-flavin adduct 29. In a concerted step that follows, the amine-flavin adduct cleaves to release the imine 24 and the reduced flavin. Further hydrolysis of the imine leads to the aldehyde 30 and ammonia. Mariano demonstrated that activated flavins such as the lumiflavin 28 promote ground state oxidative deamination reactions of primary and secondary amines. Tertiary amines have not been examined experimentally. Although these results point out the possibility of other existing catalytic pathways, there is more convincing evidence to support either the SET or HAT pathways.

![Chemical structure of 3-methyl-5-ethylumiflavinium perchlorate (28)]
Scheme 5. Proposed Polar Addition-Elimination Mechanism

MAO-Flavin + RCH₂NH₂ → MAO-Flavin-H₂+ RCH=NH

H₂O₂ → RCHO + NH₃

H-acid

Base: