

Chapter 2. Research proposal and background

2.1. Steric parameters that influence enzyme selectivity

Monoamine oxidase A and B, as previously stated, display substrate and inhibitor selectivity and the factors that influence this selectivity are not fully understood. Although the primary structures of these enzymes have been elucidated from their corresponding gene sequences,^{111,184} relatively little is known regarding the structural features of the active sites which lead to the selectivities observed with various substrates¹⁸⁵ and inhibitors.¹⁸⁶⁻¹⁸⁸ Understanding the parameters which lead to selectivity could prove important in the design of specific inhibitors for the treatment of neurodegenerative diseases. The excellent MAO-A and/or B substrate and inhibitor properties of various 1,4-disubstituted-1,2,3,6-tetrahydropyridinyl derivatives offer an interesting opportunity to probe the active sites of these enzymes.^{95,153,189}

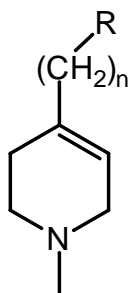
In an effort to understand the factors that influence enzyme selectivity and to characterize the active sites of these important enzymes, many experiments have been pursued to evaluate MAO-A and MAO-B activity with C4 substituted MPTP analogs. The results have lead to some general conclusions. The first class of MPTP analogs to be explored were analogs in which small groups were substituted about the phenyl ring in the *ortho*, *meta*, or *para* position (**31-49**, Table 2).¹⁹⁰⁻¹⁹² MPTP is a good MAO-B substrate [at 30 °C, $V_{\max}/K_m = 523 \text{ (min}\cdot\text{mM)}^{-1}$], and a modest MAO-A [at 30 °C, $V_{\max}/K_m = 143 \text{ (min}\cdot\text{mM)}^{-1}$] substrate. MPTP analogs bearing halogen or alkyl groups (**39-44**) at the *meta* position of the phenyl ring show enhanced selectivity for MAO-B.

MAO-B substrate activities are higher than for MPTP itself as reflected by the V_{\max}/K_m values which range from 650 to 2,036 $\text{min}^{-1}\text{mM}^{-1}$. In general, substitution at the *meta* position within this series enhances MAO-B selectivity. On the other hand, substitution at the *ortho* position of the phenyl ring (**31-38**) shows a marked increase in the efficiency of MAO-A catalyzed oxidation and MAO-A selectivity. Lengthening the alkyl chain in the *ortho* position beyond methyl progressively decreases MAO-B activity and increases MAO-A selectivity.

In order to expand upon the structural features that may effect MAO-A and MAO-B enzyme selectivity, another class of more flexible MPTP analogs was investigated by Krueger in 1992¹⁴⁹ and Efang in 1993.¹⁴⁸ Instead of the direct linked 4-aryl tetrahydropyridinyl analogs, a methylene or ethylene linker was inserted between the phenyl group and the tetrahydropyridinyl moieties of MPTP (Chart 1). The flexible analog **54**, showed enhanced MAO-A activity indicating that small electron withdrawing groups like fluorine in the *ortho* position are favored consistent with the above results, but, with the fluorine in the *meta* position (**55**) enhanced MAO-B activity is observed. Another general conclusion from this structure activity relationship (SAR) study is that lipophilicity enhances MAO-A activity as seen with the flexible naphthyl derivatives **60-65**. In contrast, the flexible 2 and 3 substituted thienyl derivatives of MPTP **66-68** and **69-71** (Chart 1) are better MAO-B substrates than MAO-A, which may be attributed to unfavorable polar interactions in the MAO-A active site as thienyl, unlike benzene, has a net dipole moment. Molecular size, however, appears to play a significant role in determining selectivity. In general, it appears that large, bulky, lipophilic analogs are

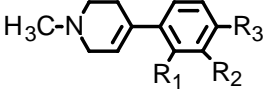
oxidized preferentially by MAO-A while those analogs which contain smaller C4 groups are preferentially oxidized by MAO-B. MAO-B is more sensitive to substrate size and it was shown that with these flexible analogs that molecular size is the major determinant factor in substrate selectivity.

Chart 1. Series of Flexible MPTP Analogs



- n = 1,2; R = phenyl (**51, 52**)
- n = 1, R = *o*-methoxyphenyl (**53**)
- n = 1, R = *o*-, *m*-, *p*-fluorophenyl (**54 - 56**)
- n = 1, R = *o*-, *m*-, *p*-methylphenyl (**57 - 59**)
- n = 0,1,2; R = α -naphthyl (**60 - 62**)
- n = 0,1,2; R = β -naphthyl (**63 - 65**)
- n = 0,1,2; R = 2'-thienyl (**66- 68**)
- n = 0,1,2; R = 3'-thienyl (**69- 71**)

Table 2. MAO-A and B Activity of Various MPTP Derivatives.

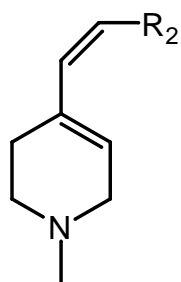
	V_{max}/K_m $\text{min}^{-1}\text{mM}^{-1}$	V_{max}/K_m $\text{min}^{-1}\text{mM}^{-1}$	$SC_{A/B}$
Substrates	MAO-A	MAO-B	
Kynuramine (15)	1,283		--
Benzylamine (14)		1,064	--
MPTP (3)	143	523	0.27
R ₁ -CH ₃ (31)	593	1,275	0.47
R ₁ -CH ₂ CH ₃ (32)	688	295	2.33
R ₁ -n-propyl (33)	658	86	7.65
R ₁ -OCH ₃ (34)	511	233	2.19
R ₁ -CF ₃ (35)	169	520	0.33
R ₁ -F (36)	100	1,054	0.09
R ₁ -Cl (37)	400	1,353	0.30
R ₁ -isopropyl (38)	1,131	51	22.18
R ₂ -CH ₃ (39)	76	650	0.12
R ₂ -F (40)	391	900	0.43
R ₂ -Cl (41)	567	1,132	0.50
R ₂ -Br (42)	300	2,036	0.15
R ₂ -OCH ₃ (43)	----	944	--
R ₂ -CF ₃ (44)	214	514	0.42
R ₃ -CH ₃ (45)	58	345	0.17
R ₃ -F (46)	----	423	--
R ₃ -Cl (47)	69	595	0.12
R ₃ -NH ₂ (48)	12	54	0.22
R ₃ -NO ₂ (49)	185	16	11.56

Additional evidence to support the above generalizations about the steric influence on MAO enzyme selectivity was provided by a study reported by Sablin *et al*^{193,194} in which a series of *cis*- and *trans*-tetrahydrostilbazoles **72-87** (Chart 2) were examined for activity with MAO-A and MAO-B. In general these analogs showed selective MAO-A activity. It was concluded that MAO-B is much more sensitive than MAO-A to substrate geometry. An SAR study published in 1993 by Kalgutkar *et al*¹⁵² examined the aryloxy derivatives **88-94** (Chart 3, panel A). All analogs displayed good MAO-A and MAO-B activity with the exception of the 9-phenanthroxy analog **94** which displayed only MAO-A activity. Thus bulky C4 aryloxy derivatives in general showed MAO-A selectivity which again identified steric features as a contribution to substrate selectivity. However, the possibility that the electronic nature of the C4 substituent may also be playing a role in substrate selectivity must be considered with this oxygen linked series.

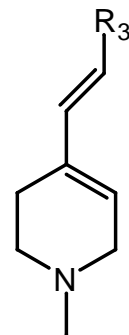
A more systematic evaluation of *o*-, *m*-, and *p*-substituted phenoxy tetrahydropyridinyl derivatives (**95-109**, Chart 3, panel B) was performed to look more carefully at the possible influence of electronic effects on MAO substrate selectivity.⁹⁵ Compounds **95-109** all display good MAO-A and MAO-B substrate activity. No clear electronic relationship to enzyme selectivity could be established. The possibility of electronic effects of the C4 substituent on the rate and mechanism of MAO catalysis and enzyme selectivity are being explored more carefully with other polar heterocyclic analogs of MPTP. Of particular interest in this phenoxy series is the selectivity of the *m*chlorophenoxy (**102**) and the *m*-phenylphenoxy analogs (**96**) which display 6 and 8.7 times greater selectivity for MAO-A than MAO-B, respectively. As previously stated

meta substituents about the phenyl ring of MPTP favor MAO-B activity and the *ortho* substituents favor MAO-A activity. In the phenoxy series, due to the flexibility and geometry of these derivatives, *meta* substitution is favored by MAO-A and not MAO-B. It seems clear, that the phenoxy substrates, which in general display greater MAO-A selectivity, provide additional support for the recognized role of steric interactions on MAO enzyme substrate selectivity. One general conclusion emerges from the studies discussed: The molecular geometry of the substrate is a deciding factor in MAO-A and -B substrate selectivities.

Chart 2. A Series of Cis and Trans 1-Methyl-4-substituted-1,2,3,6-tetrahydropyridines.



Cis alkene



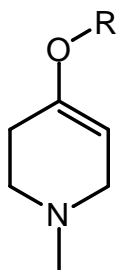
Trans alkene

R₂ = phenyl (**72**)
 R₂ = *o*-methoxyphenyl (**73**)
 R₂ = *o*-, *m*-, *p*-bromophenyl (**74 - 76**)
 R₂ = α -naphthyl (**77**)

R₃ = phenyl (**78**)
 R₃ = *o*-, *p*-methoxyphenyl (**79, 80**)
 R₃ = *p*-methoxyphenyl (**81**)
 R₃ = *o*-, *m*-, *p*-bromophenyl (**82 - 84**)
 R₃ = α -naphthyl (**85**)
 R₃ = *p*-methylphenyl (**86**)
 R₃ = *p*-fluorophenyl (**87**)

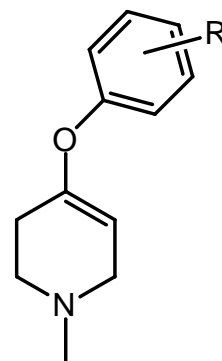
Chart 3. A Series of 4-Aryloxy (panel A) and 4-Substituted-phenoxy (panel B)

Tetrahydropyridines



Panel A

- R = phenyl (**88**)
- R = α -naphthyl (**89**)
- R = β -naphthyl (**90**)
- R = *tert*-butyl (**91**)
- R = *n*-butyl (**92**)
- R = 2,4-dichlorophenyl (**93**)
- R = 9-phenanthryl (**94**)



Panel B

- R = *o*-, *m*-, *p*- Ph (**95 - 97**)
- R = *o*-, *m*-, *p*-OCH₃ (**98 - 100**)
- R = *o*-, *m*-, *p*-Cl (**101 - 103**)
- R = *o*-, *m*-, *p*-CH₃ (**104 - 106**)
- R = *o*-, *m*-, *p*-NO₂ (**107 - 109**)

2.2 Molecular modeling of the active sites of MAO-A and MAO-B and the relationship of these models to endogenous MAO substrates

Since there are no x-ray crystal structures of the MAO-A and MAO-B active sites, SAR and quantitative SAR (QSAR) remain the best tools to investigate the structure of the enzyme active sites. These important FAD containing enzymes play a major role in neurotransmitter metabolism in humans. Understanding the types of substrates and inhibitors that are selective for one or the other form of MAO could prove useful in the design of drug candidates to target these enzyme systems. The good substrate properties of several semirigid MPTP analogs have provided an opportunity to generate models of the MAO-A and MAO-B active sites.

In 1990 Maret and colleagues^{7,195} reported a QSAR study on 33 MPTP analogs employing different statistical methods (principal component analysis, multiple linear regression analysis), as well as comparative molecular field analysis (CoMFA). CoMFA represents molecules by their steric and electronic fields sampled at the intersection of a three dimensional (3D) lattice. These workers used a series of phenyl ring substituted MPTP analogs to generate contour maps of the MAO-A and MAO-B active sites. With these contour maps the regions in the active sites where steric interactions increase and decrease MAO activity were identified. From this study Maret concluded that *para*-substitution on the phenyl ring of MPTP reduces MAO activity relative to MPTP. Whereas, *ortho*- and *meta*-substitution lead to favorable interactions with MAO. From the CoMFA analysis it was concluded that the N-methyl group of MPTP

has the ideal size and elicits ideal interactions within the MAO active pocket while smaller and larger groups are less favorable.

A later QSAR analysis of MPTP derivatives by Altomare and coworkers¹⁹⁶ employs CoMFA to evaluate the individual features of the MAO-A and MAO-B active sites that elicit reactivity and selectivity. This study attempted to relate the lipophilicity, electronic factors, and steric parameters to the selectivity observed with MAO-A and MAO-B as well as generating maps of the active sites. They concluded that steric effects exerted by bulky substituents in the *ortho* position of MPTP effect MAO-B activity negatively. With regards to MAO-A, these workers concluded that lipophilicity does not play a major role in MPTP analog activity. They define the areas and volumes of favorable and unfavorable lipophilic and steric effects as generated in 3D-QSAR models of the active sites. The results of this QSAR study clearly establish on a quantitative level that the MAO-A and MAO-B catalytic sites differ in their hydrophobic, steric, and stereo electronic requirements.

In 1991 Efange and Boudreau¹⁵¹ reported results obtained with nineteen MPTP analogs used to probe the structural parameters that influence MAO catalysis. The models of the active sites were generated by superimposing the available MPTP analogs. The tetrahydropyridinyl substrates that displayed selectivity for MAO-A were superimposed to yield the MAO-A active site model (Figure 2, panel 1) while the MAO-B selective MPTP substrates were superimposed to generate an MAO-B active site model (Figure 2, panel 2). From this analysis Efange and coworkers have divided the substrate binding site into two regions: (1) the amine binding pocket (for the tetrahydropyridine moiety) and (2) a bulky substituent region (for the phenyl

group and its substituents). These models define the size of the regions. It is clear from the superimposition of the MAO-A and -B active site models (Figure 2, panel 3) that the size and topography of the substrate pocket play important roles in determining substrate selectivity.

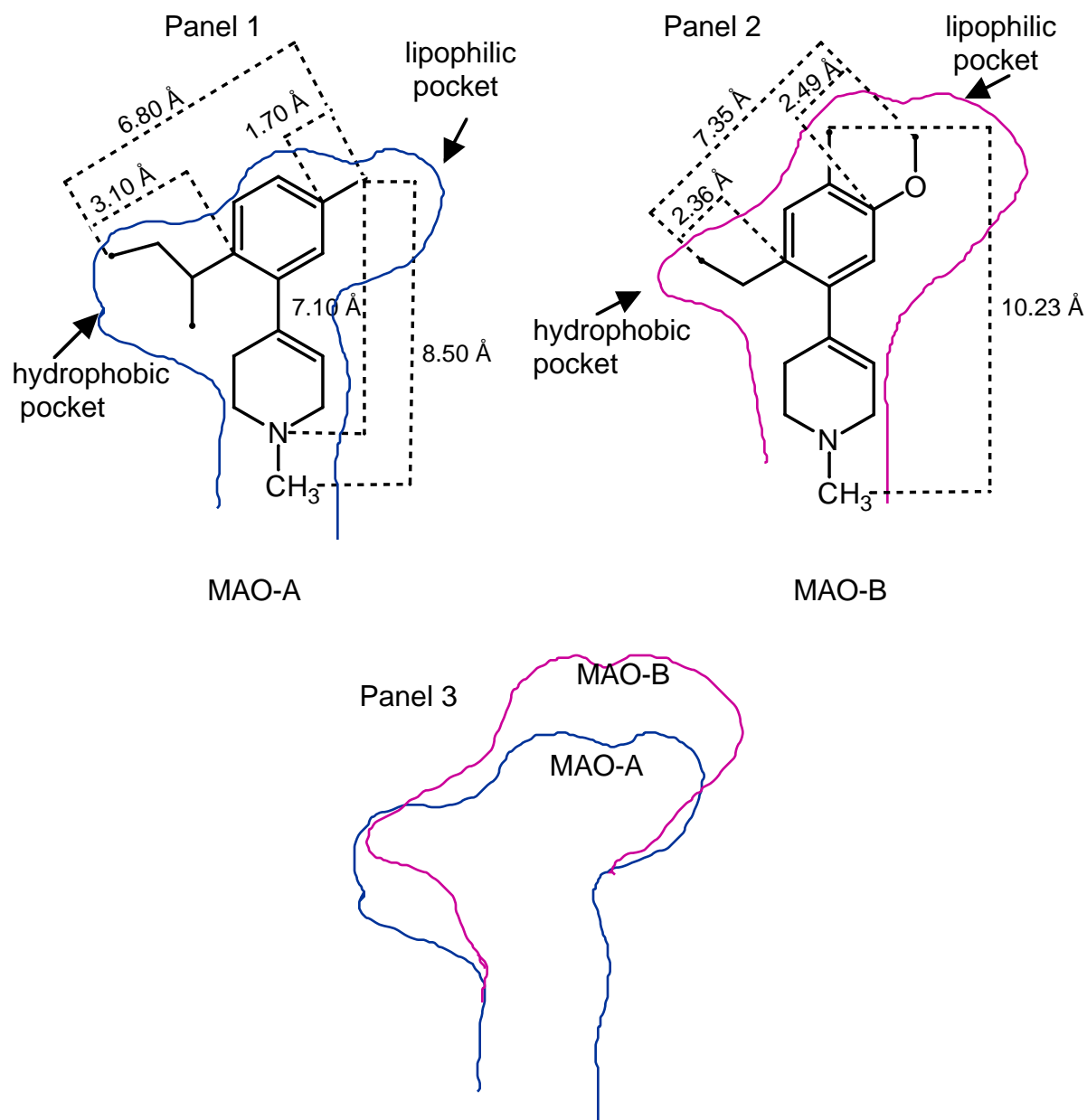


Figure 2. The MAO-A and MAO-B active sites models proposed by Efange and Boudreau.¹⁵¹

Another SAR analysis reported in 1996 by Mabic *et al*¹⁹⁷ has also led to insights into factors that may determine MAO enzyme selectivity. The principal findings are as follows:

- It was suggested that the dihedral angle between the 4-5 position in the tetrahydropyridine and the C4 substituent may be an important geometric factor which influences substrate selectivity.
- With MPTP derivatives the length of the molecule along the x-y (N-C4) axis is a defining factor in substrate activity.
- The dipole moment and polarity of the molecule also may play important roles in the substrate activity.

With the availability of many more MPTP analogs, an effort is underway to define and revise the MAO-A and MAO-B active site models. It is noteworthy, however, that all of the current models of the MAO-A and MAO-B active sites are generated using MPTP and other tetrahydropyridinyl analogs and have not attempted to evaluate the active site models relative to the endogenous neurotransmitters.

2.3. Investigation of MAO-A mediated neurotoxicity

The highly selective MAO-B substrate MPTP, which induces a parkinsonian syndrome, has been used to establish a mechanism for the observed selective nigrostriatal dopaminergic neuronal neurotoxicity in humans,¹⁷ primates,²⁰ and rodents.^{22,46} The details of the mechanism proposed to explain the MAO-B mediated neurotoxicity can be found in section 1.2.2. MPTP itself is bioactivated in the brain extraneuronally by MAO-B in the glial cells to MPP⁺, the actual neurotoxin. MPP⁺ must then be transported intraneuronally by the dopamine reuptake transporter to the site of toxicity in the mitochondrial cell membrane. As previously discussed MAO-A is located in the

mitochondria of dopaminergic neurons, the actual site of neurotoxicity. There has been speculation that exposure to exogenous compounds that may be similar to MPTP in nature could be the cause of idiopathic Parkinson's Disease. There is no reason that a parkinsonian inducing neurotoxin must rely on MAO-B rather than MAO-A for bioactivation. It has been established that MAO-B can mediate selective dopaminergic neurotoxicity,⁴⁴ but there has been much interest in evaluating the possible role of MAO-A in mediating neurotoxicity as well as determining if the MAO-A mediated neurotoxic pathway has any correlates with the pathway for MAO-B mediated MPTP neurotoxicity. In order to study MAO-A mediated neurotoxicity, a very selective MAO-A substrate is required.

The general model for neurotoxicity is the C57Bl/6 mice in which i.p. administration of MPTP elicits degeneration of nigrostriatal dopaminergic neurons as characterized by the loss in the striatal content of DA and its metabolites DOPAC and HVA.²³ In addition to MPTP, other selective MAO-B substrates with activity as good or better than MPTP (Table 2), such as 2-F-MPTP (**36**), 2-CF₃-MPTP (**35**), 2-CH₃-MPTP (**31**), have been shown in C57Bl/6 mice to be more neurotoxic than MPTP.¹⁹⁸ The neurotoxicity of MPTP and the described neurotoxic analogs of MPTP (**31,35, 36**) can be blocked (R)-deprenyl, a selective MAO-B inhibitor, as well as by DA reuptake inhibitors, but not the selective MAO-A inhibitor clorgyline. This supports the proposed pathway for MAO-B mediated neurotoxicity.

There is no obvious reason why MAO-A should not mediate neurotoxicity. To fully investigate the possibility that MAO-A may mediate the bioactivation of protoxins, an MAO-A specific or highly selective substrate must

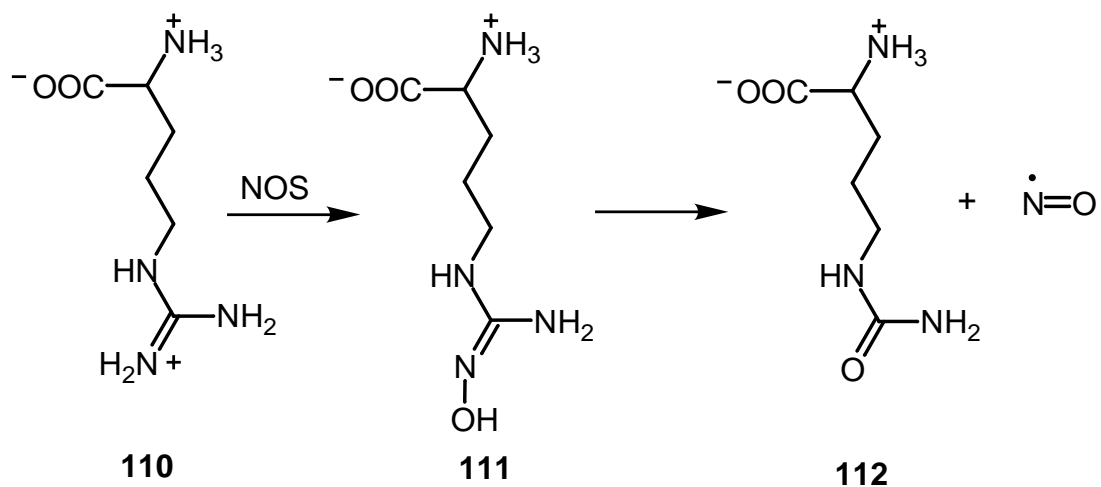
be available. A mixed A/B substrate has been used to explore MAO-A mediated neurotoxicity. The neurotoxicity, if mediated by MAO-A, should be blocked in vivo by clorgyline but not (R)-deprenyl or DA reuptake inhibitors.

In an attempt to evaluate the role of MAO-A in the bioactivation of the MAO-B selective MPTP analog 1-methyl-4-(2-methylphenyl)-1,2,3,6-tetrahydropyridine (**31**, $SC_{B/A} = 2.2$), Kindt and coworkers¹⁹⁸ carried out in vivo studies in mice. The neurotoxicity observed with this 2-methyl-MPTP analog was shown to be only partially inhibited by the MAO-B inhibitor (R)-deprenyl. The Kindt report stated that pretreatment of the mice with (R)-deprenyl and clorgyline, to inhibit both MAO-A and MAO-B, led to complete neuroprotection against 2-methyl-MPTP toxicity. These results provide the first evidence that MAO-A can play a role in the bioactivation of MPTP like analogs to neurotoxic pyridinium species. A similar study was performed by Heikkila¹⁹⁹ in which the neurotoxicity of an MAO-A selective substrate 1-methyl-4-(2-ethylphenyl)-1,2,3,6-tetrahydropyridine (**32**, $SC_{A/B} = 2.3$) was evaluated in mice. It was concluded that although both (R)-deprenyl and clorgyline are required to protect against 2-ethyl-MPTP neurotoxicity, the MAO-A inhibitor clorgyline alone cannot significantly protect against neurotoxicity. This study again confirms that MAO-A can play a role in MPTP type neurotoxicity. Nevertheless, a more selective MAO-A substrate should help to evaluate more definitively the MAO-A pathway leading to neurotoxicity.

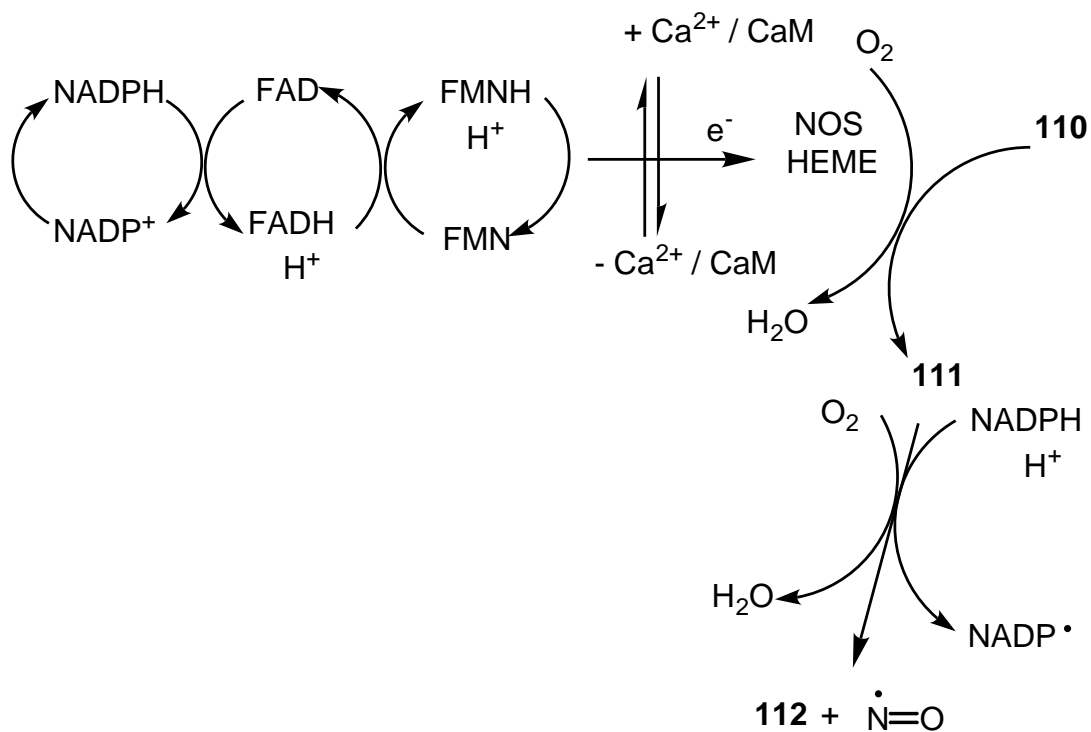
2.4 Investigation of the neuroprotection of 7-nitroindazole using the MPTP model of neurotoxicity

In the drug development arena, there has been for some time a growing interest in discovering compounds that may prove useful as neuroprotective agents to prevent neuronal cell death in the cases of Parkinson's Disease, Alzheimer's disease, and brain injury due to trauma, ischemia, or stroke. The enzyme, nitric oxide synthase (NOS), is a P₄₅₀-like heme protein that requires tetrahydrobiopterin (BH₄), flavin mononucleotide (FMN), calmodulin (CaM), and FAD to catalyze the nicotinamide adenine dinucleotide-3'-phosphate reduced form (NADPH) and oxygen dependent oxidation of L-arginine (**110**) to L-citrulline (**112**) and nitric oxide (NO)²⁰⁰⁻²⁰² (Scheme 6). The mechanism of the catalysis by NOS is very complex and not fully elucidated. The proposed mechanism of oxidation (Scheme 7) is believed to involve an initial build up of electrons from NADPH that feed into the flavins (FAD, FMN). Calcium must bind to calmodulin to trigger the transfer of electrons from the flavins to the NOS heme iron.²⁰³ The substrate L-arginine (**110**) binds to the enzyme and there is a rapid oxidation of NADPH in the presence of oxygen to yield the intermediate N-OH-L-arginine (**111**). The further oxidation of **111** yields L-citrulline (**112**) and nitric oxide (NO). The reduction of the iron heme of NOS has been associated with increased NADPH oxidation and the production of super oxide (O₂⁻) in the absence of the substrate L-arginine.

Scheme 6. NOS Catalyzed Oxidation of L-Arginine to L-Citrulline and Nitric Oxide.

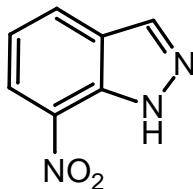


Scheme 7. The Proposed Mechanism of the NOS Catalyzed Oxidation of L-Arginine



There are three mammalian forms of NOS: neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS).^{201,204} Of most interest in neuroprotection is nNOS on which this section will focus.

NO generated by nNOS catalysis, is believed to be an important mediator of cell functions in several biological systems. Among the many functions of NO are the regulation of vascular tone, the cytotoxic action of macrophages, neuronal death, learning and memory and neurological signaling.^{200,205,206} Although NO plays a mediating role in many important biological processes, it may be neurotoxic under conditions of excessive formation. Excess NO formation, as a result of its reactive free radical properties, has been implicated in a wide variety of diseases.²⁰⁷ Over production of NO has been implicated in stroke,²⁰⁸ septic shock,²⁰⁹ seizures,²¹⁰ schizophrenia,²¹¹ migraine headaches,²¹² and Alzheimer's disease.²¹³ The search for selective inhibitors of nNOS as therapeutic agents has been active. There are several classes of NOS inhibitors. The inhibitors vary greatly in their structural nature and the target site of inhibition. Because NOS catalysis relies on many cofactors, there are many sites of inhibition. There are inhibitors that target NADPH, calmodulin, BH₄, and the flavin cofactors.²¹⁴ The classes of inhibitors include arginine analogs²¹⁵⁻²¹⁸, indazole derivatives,^{219,220} and imidazole derivatives.²²¹ The inhibitor 7-nitroindazole [7-NI (**113**)] has been reported to be a selective inhibitor of nNOS which could prove to be useful as therapeutic neuroprotective agent.^{219,222-224}



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Because of the experimental convenience and the fact that the neurotoxic mechanism has been extensively studied, the MPTP model of neurotoxicity is being used to evaluate potential neuroprotective agents. The neurotoxic properties of the parkinsonian inducing agent MPTP, are mediated by the 1-methyl-4-phenylpyridinium species MPP⁺ which is formed via the monoamine oxidase-B (MAO-B) generated 1-methyl-4-phenyl-2,3-dihydropyridinium intermediate MPDP⁺ ¹⁵⁰. MPP⁺ is transported into the nigrostriatal nerve terminals ²²⁵ where it localizes within the inner mitochondria membranes and inhibits complex I of the mitochondrial respiratory chain as previously described.^{59,226} The MAO-B catalyzed oxidation of MPTP is an essential step in the expression of MPTP's neurotoxicity and MAO-B inhibitors protect against this toxicity. It has been reported that treatment of both rodents^{227,228} and baboons²²⁹ with the neuronal nitric oxide synthase (nNOS) inhibitor 7-NI^{219,223} also protects against MPTP toxicity. These neuroprotective effects were reported to be due to decreased nitric oxide (NO) production and not MPP⁺ production since 7-NI did not inhibit the MAO-B catalyzed oxidation of benzylamine by mouse brain mitochondrial preparations.²²⁸ Furthermore, Przedborski *et al.*²²⁷ found that the striatal levels of MPP⁺ in MPTP treated mice were unaffected by neuroprotective doses of 7-NI.

Despite these observations, a consideration of the structural features of 7-NI and the extensive literature showing that a wide variety of planar, heterocyclic systems are competitive inhibitors of MAO-B²³⁰⁻²³³ prompted the further investigation of the inhibitory effects of 7-NI on MAO-B catalysis. The discovery that 7-NI displayed competitive inhibition properties with MAO-B prompted a study to evaluate the effect of MAO-B inhibition by 7-NI on the

metabolism of MPTP in vivo.²³⁴

2.5 Research rationale and proposals

It has been well established that the active sites of MAO-A and MAO-B differ in the factors that delegate enzyme selectivity for substrates and inhibitors (i.e. (R)-deprenyl and clorgyline). Mainly from studies on bulky, flexible C4 substituted MPTP analogs, emerged the conclusion that steric interactions in the active sites of MAO-A and MAO-B play an important role in distinguishing substrate selectivity (section 2.2). One of the goals of this thesis work is to design substrates and inhibitors with bulky, less flexible C4 phenyl substituents to examine the steric limits of the MAO-A and MAO-B active sites as well as to probe the relationships between substrate and inhibitor selectivities.

The many efforts to characterize and model the active sites using SAR and QSAR have led to MAO-A and MAO-B active site models as described in section 2.2. Our efforts to examine the steric effects in the active sites with semi rigid MPTP derivatives have led to additional information about the limits of substrate size. A topological analysis of the MAO-A and MAO-B active sites also has allowed for the further refining of the existing active site models.

We have examined several MPTP analogs to characterize their potential to produce selective dopaminergic neurotoxicity in mice. The role of MAO-A in mediating neurotoxicity has not been completely explored due to the lack of available MAO-A selective substrates (section 2.3). With the assistance of MAO active site models and SAR studies, we have designed an MAO-A selective substrate in an attempt to examine the potential for MAO-A mediated

neurotoxicity in mice.

The discovery that the reported nNOS selective inhibitor 7-NI is a competitive inhibitor of MAO-B in vitro has presented a challenging problem. It has been reported that 7-NI protects against MPTP neurotoxicity in mice. The question we must ask is whether or not the observed neuroprotection against MPTP toxicity is a result of nNOS inhibition or the inhibition of MAO-B. The MAO-B competitive inhibitor properties of 7-NI prompted us to examine the influence of 7-NI on the levels of MPP⁺ in the striata of MPTP treated C57BL/6 mice in an effort to begin to examine these questions.

The endogenous substrates of MAO-A and B display enzyme specificity and selectivity as seen in Table 1, section 1.3.4. All of the existing models of the MAO-A and MAO-B active sites have been generated using C4 substituted tetrahydropyridines. In an attempt to dock the endogenous substrates in the newly developed MAO-A and B active site models generated by Mabic *et al*,²³⁵ and to rationalize the observed substrate selectivities, we have examined the active sites of MAO-A and B with a series of hydroxylated MPTP derivatives, which are designed to overlay the corresponding phenolic groups present on the catecholamines and 5-HT.