

Chapter 4

Monoamine oxidase inhibitors in Parkinson's disease

4.1 Introduction

The monoamine oxidases (MAOs) are flavine adenine dinucleotide (FAD) dependent enzymes, bound to the outer membrane of mitochondria (Binda *et al.*, 2007; Son *et al.*, 2008). These enzymes are responsible for the oxidative deamination of neurotransmitters and dietary amines (Hubálek *et al.*, 2005).

The two isoforms of MAO, MAO-A and –B, are considered to be drug targets for the therapy of depression and neurodegenerative diseases, respectively (Yamada & Yasuhara, 2004; Son *et al.*, 2008). Even though these enzymes share a 70% sequence identity, each enzyme has a specific substrate and inhibitor preference (Binda *et al.*, 2007; Son *et al.*, 2008).

As mentioned before, PD is the second most common neurodegenerative disease, with incidence sharply increasing with age (Prediger *et al.*, 2012). The motor symptoms associated with PD are considered to be the result of the progressive loss of neuromelanin-containing dopaminergic neurons from the SNpc (Nagatsu & Sawada, 2006; Prediger *et al.*, 2012). MAO-B preferentially metabolizes dopamine in the human brain (Yamada & Yasuhara, 2004; Nagatsu & Sawada, 2006; Youdim *et al.*, 2006). Thus, it is not surprising that selective inhibitors of MAO-B enhance the levels of dopamine by reducing the metabolic degradation of central dopamine and, as a result improve the motor symptoms associated with PD (Fernandez & Chen, 2007; Youdim *et al.*, 2006). MAO-B inhibitors are frequently employed as adjunct therapy with L-DOPA (Binda *et al.*, 2007). Non-motor symptoms of depression (Youdim & Bakhle, 2006) and anxiety (Prediger *et al.*, 2012) are also underlying problems in PD. Since MAO-A inhibitors possess an antidepressant action, MAO-A inhibitors are a favourable treatment for PD associated depression (Youdim & Bakhle, 2006).

In this chapter, the rationale for the use of MAO inhibitors in the treatment for PD will be discussed (section 4.3). In addition an overview will be provided of the structures of MAO-A and MAO-B (section 4.2).

4.2 MAO-A and MAO-B

In humans, MAO is classified into two isoforms (Rybaczyk *et al.* 2008, Tazik *et al.* 2009, Hubálek *et al.*, 2005). These include MAO-A and MAO-B. As mentioned above, both isoforms are bound to the outer membrane of mitochondria, contain the FAD co-factor and require no

iron or copper ions for their activities (Youdim *et al.*, 2006). MAO-A consists of 527 amino acids, whereas MAO-B consists of 520 amino acids (Nagatsu, 2004). During development, MAO-A activity appears before MAO-B activity. However, the presence of MAO-B increases significantly in the brain after birth. MAO activity is found in different regions of the human brain, as indicated below (Youdim *et al.*, 2006):

- highest levels of activity –striatum and hypothalamus
- low levels of activity – cerebellum and neocortex

Even though both MAO-A and MAO-B may contribute to dopamine metabolism, MAO-B is the predominant form in the basal ganglia (striatum) and is responsible for the oxidative deamination of dopamine (Youdim *et al.*, 2006; Tazik *et al.*, 2009). The ratio of MAO-A to MAO-B in the human brain is 25:75 (Stahl & Felker, 2008).

The two isoforms can be distinguished by their substrate specificities and their sensitivities towards clorgyline inhibition (Foley *et al.*, 2000). MAO-A metabolizes serotonin (Son *et al.*, 2008) and the dopamine derivatives adrenaline and noradrenaline (Rybaczuk *et al.*, 2008), whereas MAO-B metabolizes mainly dopamine (Youdim *et al.*, 2006) and the dietary amine β -phenylethylamine (Rybaczuk *et al.*, 2008). In low concentrations, clorgyline acts as a selective and irreversible inhibitor of MAO-A, whereas MAO-B is relatively insensitive towards clorgyline inhibition (Foley *et al.*, 2000; Son *et al.*, 2008).

The MAO isoforms are of therapeutic importance and the need exists to develop selective MAO inhibitors, particularly MAO-B inhibitors, for the treatment of PD. In order to understand the differences between the two isoforms of MAO, a detailed discussion is to follow regarding the structures of MAO-A and MAO-B.

4.2.1 Structure of MAO-A

X-ray crystallography is used to determine how a pharmaceutical drug may interact with its protein target and what changes to the drug may improve activity. In order to understand the human MAO-A structure Son and co-workers (2008) determined the X-ray structure of human MAO-A in complex with harmine (a reversible inhibitor of MAO-A) at 2.2-Å resolution.

Human MAO-A crystallizes as a monomer, while human MAO-B crystallizes as a dimer (Youdim *et al.*, 2006). The structure of human MAO-A may be divided into two domains, namely an extra-membrane domain and a membrane binding domain. The extra-membrane domain may be further divided into a FAD binding region (Figure 4.1, indicated in yellow) and a substrate/inhibitor binding region (Figure 4.1, indicated in red).

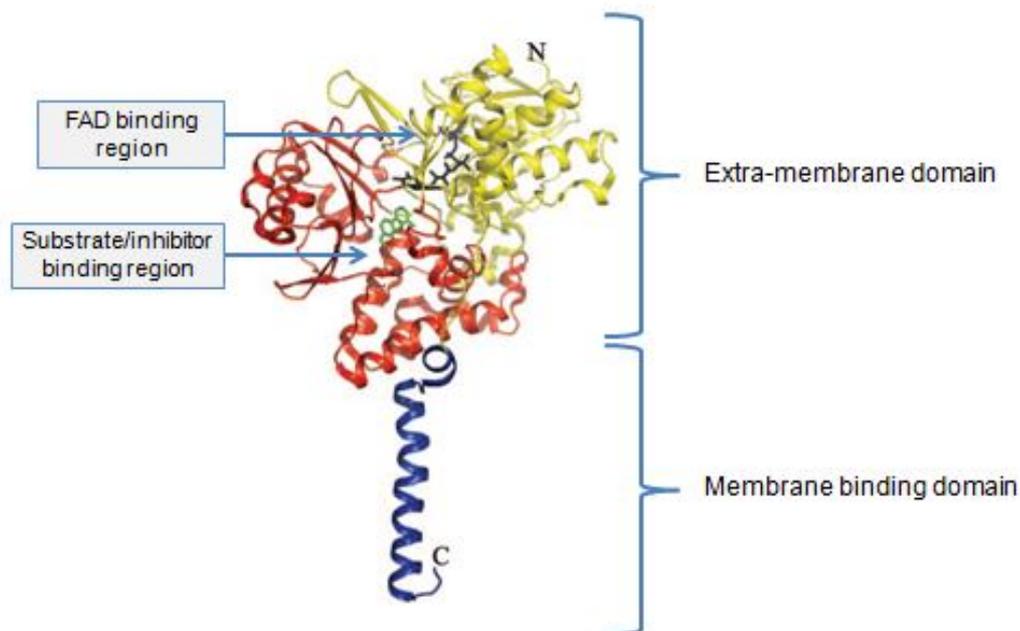


Figure 4.1: The structure of human MAO-A, as a monomer, in complex with harmine. The overall structure is drawn in ribbon mode. The N-terminus and C-terminus are indicated. The FAD (black) and harmine (green) are represented as stick models in the extra-membrane domain (Son *et al.*, 2008).

The active site cavity of human MAO-A has an approximate volume of 550 \AA^3 and is formed by a cavity-shaping loop consisting of residues 210-216 (Youdim *et al.*, 2006). Loop 108-118 is thought to regulate access to the active site. The width of the entrance is too narrow for compounds such as harmine to pass through during the steady-state phase. The flexibility of loop 108-118 together with anchoring of the enzyme into the membrane seems to be essential for substrate/inhibitor access to the active site (Son *et al.*, 2008).

The substrate/inhibitor cavity of human MAO-A is hydrophobic (Youdim *et al.*, 2006). The active centre cavity of human MAO-A is depicted in Figure 4.2 where harmine interacts with the following residues: Tyr-69, Asn-181, Phe-208, Val-210, Gln-215, Cys-323, Ile-325, Ile-335, Leu-337, Phe-352, Tyr-407, Tyr-444 and FAD (Son *et al.*, 2008). The space between the inhibitor and the aforementioned residues is occupied by seven water molecules. Residue Gln-215 of human MAO-A undergo π - π interactions with coplanar aromatic rings such as those in the harmine structure (Son *et al.*, 2008).

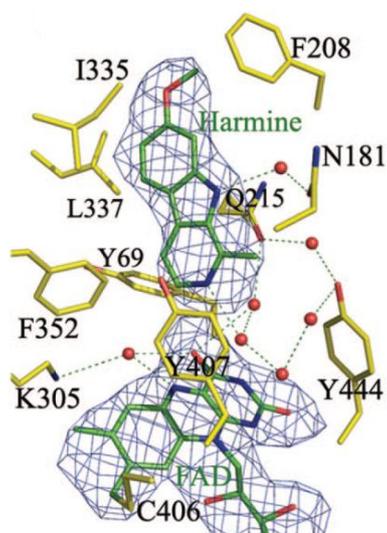


Figure 4.2: The substrate/inhibitor binding site of human MAO-A, generated at 2.2 Å resolution for the inhibitor (harmine) and FAD. Harmine and FAD are indicated in green. Amino acid residues interacting with harmine are indicated in yellow. The dotted lines indicate hydrogen bonds (Son *et al.*, 2008).

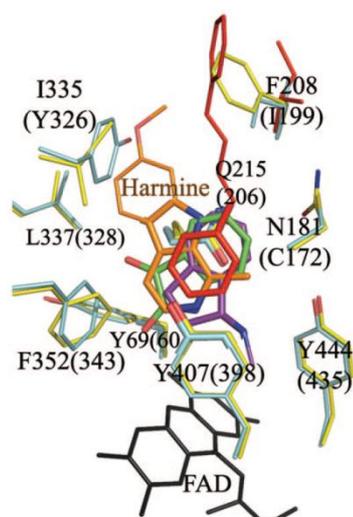


Figure 4.3: The substrate/inhibitor binding site in human MAO-A and MAO-B complexed with the following specific inhibitors: harmine (orange), isatin (green), an analogue of rasagiline (purple) and 1,4-diphenyl-2-butene (red). The FAD (black) is also shown. Amino acid residues for MAO-A and MAO-B are indicated in yellow and light blue, respectively. The residues are numbered according to human MAO-A with the parentheses indicating the numbers for MAO-B (Son *et al.*, 2008).

From literature it is known that the structures of human MAO-A and MAO-B are very similar. However, substrate/inhibitor selectivity between human MAO-A and MAO-B may be attributed to residue Ile-335 and Phe-208 in MAO-A, which corresponds to Tyr-326 and Ile-199 of MAO-B

(Figure 4.3) (Son *et al.*, 2008). Considering human MAO-A, one of the most unique characteristics is residue Phe-208. This residue is situated at the analogous position of residue Ile-199 in MAO-B and consequently results in the elimination of an entrance cavity. Therefore, human MAO-A only consist of a single cavity (De Colibus *et al.*, 2005) that occupies a bigger volume compared to MAO-B.

4.2.2 Structure of MAO-B

The first elucidation of the crystal structure of human MAO-B to a resolution of 3.0 Å was essential for a better understanding of the interactions of MAO-B with inhibitors (Binda *et al.*, 2002). Advances were made and a 1.65 Å structure was published later (Binda *et al.*, 2004).

MAO-B consists of 520 amino acids (Youdim *et al.*, 2006; Binda *et al.*, 2002) and share a 70% sequence identity with MAO-A. As mentioned, both isoforms are bound to the outer membrane of the mitochondria (Binda *et al.*, 2002). In contrast to MAO-A, the MAO-B enzyme crystallizes as a dimer (Binda *et al.*, 2002) with a large surface contact area between the monomers (De Colibus *et al.*, 2005). Each of the monomers of MAO-B consists of a globular domain that is anchored to the outer mitochondrial membrane via a C-terminal helix (Figure 4.4) (Binda *et al.*, 2004; Binda *et al.*, 2007).

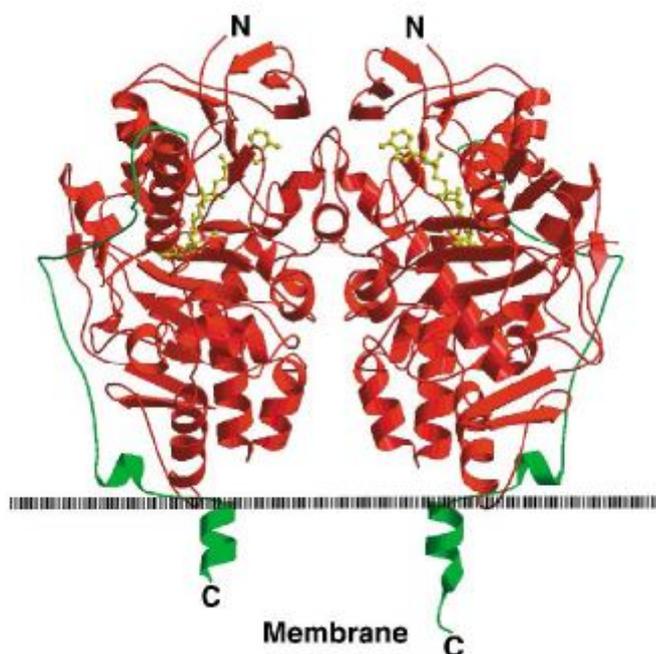


Figure 4.4: The crystallized dimer structure of human MAO-B depicted as a ribbon diagram. The N- and C-terminals are indicated with the letters “N” and “C”, respectively. The C-terminal is indicated in green. The FAD is depicted as yellow ball-and-stick. The thick dashed line represents the possible membrane region boundary (Binda *et al.*, 2002).

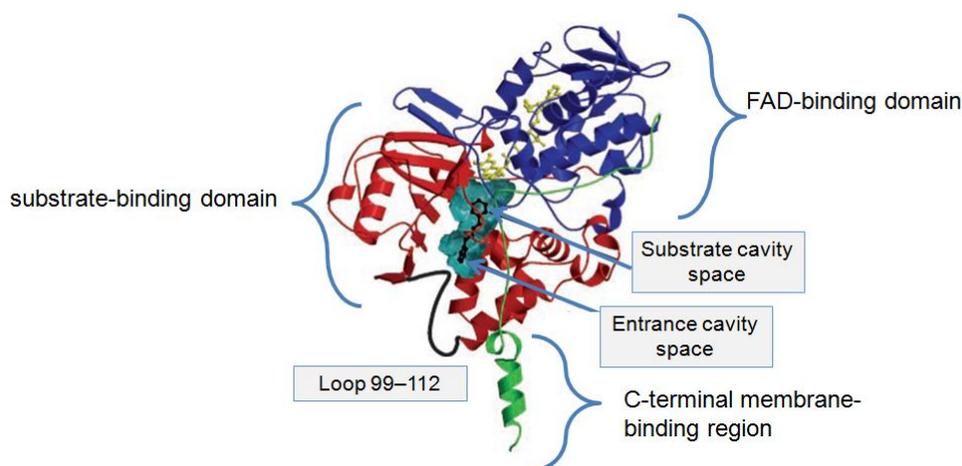


Figure 4.5: The structure of human MAO-B complexed with rasagiline (indicated in black ball-and-stick) with the FAD-cofactor depicted in yellow ball-and-stick. The binding domains are indicated as follow: substrate-binding domain is indicated in red, the C-terminal membrane-binding region in green and FAD-binding domain in blue (Binda *et al.*, 2003).

The active site of the MAO-B enzyme consists of two-cavities (Figure 4.5), known as the substrate cavity and the entrance cavity (Youdim *et al.*, 2006; Binda *et al.*, 2007). The substrate cavity is a flat hydrophobic cavity with a volume of 420 Å³ (Binda *et al.*, 2002). The hydrophobic environment may be attributed to the number of aromatic and aliphatic amino acids that line the substrate cavity (Binda *et al.*, 2002). Furthermore, the substrate cavity is separated from the entrance cavity with Tyr-326, which is located near the junction of the two cavities (Hubálek *et al.*, 2005). Residues Tyr-326, Ile-199, Leu-171 and Phe-168 form the boundary between the two cavities (Binda *et al.*, 2002; Son *et al.*, 2008) with loop 99–112 covering the entrance cavity of 290 Å (Binda *et al.*, 2002; Youdim *et al.*, 2006). The entrance cavity is lined by residues Phe-103, Pro-104, Trp-119, Leu-164, Leu-167, Phe-168, Leu-171, Ile-199, Ile-316 and Tyr-326 (Binda *et al.*, 2002). A substrate must negotiate access into the entrance cavity (Youdim *et al.*, 2006) and this requires movement of loop 99–112 (Binda *et al.*, 2002). Residue Ile-199 is thought to be the “gate” between the two cavities. The conformation of this residue determines if the substrate and entrance cavity are either fused or separated (Hubálek *et al.*, 2005). The conformation of Ile-199 side chain depends upon the nature of the substrate or inhibitor (Hubálek *et al.*, 2005). Fusion of the substrate and entrance cavity via residue Ile-199 results in the accommodation of larger ligands (Hubálek *et al.*, 2005; Legoabe *et al.*, 2012a). As mentioned before, substrate/inhibitor selectivity between human MAO-A and MAO-B may be attributed, in part, to residue Phe-208 in MAO-A which corresponds to Ile-199 in MAO-B (Son *et al.*, 2008). Binding of large MAO-B selective inhibitors may be blocked in MAO-A due to the

increased bulk of the phenyl side chain of the MAO-A residue Phe-208 (Edmondson *et al.*, 2009; Legoabe *et al.*, 2012a). Residue Tyr-326, found at the junction of the substrate and entrance cavity (Hubálek *et al.*, 2005), is also considered to play a role in the substrate/inhibitor selectivity of the two MAO isoforms (Son *et al.*, 2008; Legoabe *et al.*, 2012a). Tyr-326 may prevent the binding of MAO selective inhibitors to the MAO-B active site (Son *et al.*, 2008; Legoabe *et al.*, 2012a), thus aiding in selectivity towards MAO-A or MAO-B.

The biological significance of the C-terminal is still unclear (Son *et al.*, 2008). The C-terminal is a transmembrane α -helix that anchors the enzyme to the outer membrane of the mitochondrion, while the rest of the protein is exposed to the cytoplasm (Youdim *et al.*, 2006). It is thought that the transmembrane helix is 27 amino acids long and initiates at Val-489 (Binda *et al.*, 2002). At the end of loop 99–112, two residues (Pro-109 and Ile-110) also interact with the membrane (Hubálek *et al.*, 2005). Interestingly, truncation of the C-terminal may lead to a decrease in MAO-B catalytic activity but does not significantly change inhibitor specificity (Son *et al.*, 2008).

4.3 Therapeutic importance of MAO inhibitors in PD

The current symptomatic treatments of PD are focused on restoring dopaminergic functions. At present the dopamine precursor, L-DOPA, is used to alleviate the parkinsonian symptoms associated with a dopamine deficiency. Unfortunately, prolonged treatment with L-DOPA may induce motor related complications such as dyskinesia (involuntary movements) and motor fluctuations (Fernandez & Chen, 2007). Furthermore, evidence show that the non-motor symptoms (including depression and anxiety) related to PD does not respond to dopaminergic replacement therapy (Prediger *et al.*, 2012). Therefore, treatment with L-DOPA does not ameliorate non-motor symptoms (Fernandez & Chen, 2007).

Both the MAO-A and MAO-B isoforms are present in most mammalian tissues (Youdim *et al.*, 2006), with MAO-B as the predominant isoform in the human brain (Yamada & Yasura, 2004). Due to the therapeutic value of MAO inhibitors, many efforts have been made to develop novel compounds that exhibit MAO-A and/or MAO-B inhibition activities. The reversible or irreversible interactions of MAO inhibitors with the enzymes must also be taken into consideration when designing novel inhibitors of MAO-A and/or MAO-B.

4.3.1 Reversible and irreversible inhibitors of MAO

Generally, MAO inhibitors may be divided into two categories namely, reversible and irreversible inhibitors. This is based on the interactions of the MAO inhibitors with the enzymes.

Irreversible or “suicide” inhibitors of MAO-B bind to the enzyme reversibly at first, but after oxidation it forms a covalent interaction with the enzyme, rendering it permanently unavailable

for amine metabolism (Foley *et al.*, 2000). The above interaction may contribute to some of the disadvantages observed with irreversible inhibitors. For example, loss of isoform selectivity is observed with high drug concentrations or with repeated drug administration (Tipton *et al.*, 2004; Legoabe *et al.*, 2012b). Also after treatment with an irreversible inhibitor (Tipton *et al.*, 2004; Legoabe *et al.*, 2012b), several weeks may be required for enzyme activity to be recovered following termination of drug treatment (Legoabe *et al.*, 2012b). It is documented that MAO-B activity may take as much as 40 days to recover after termination of treatment with an irreversible inhibitor (Fowler *et al.*, 1994; Legoabe *et al.*, 2012b). These disadvantages may contribute to potential side-effects such as the “cheese reaction” (discussed in section 4.3.2) as irreversible inhibitors are often associated with the loss of isoform selectivity.

Reversible, competitive inhibitors bind non-covalently to the active site of the enzyme (Foley *et al.*, 2000). Thus, unlike the irreversible inhibitors, after withdrawal of the reversible inhibitor the enzyme activity is recovered with clearance of the inhibitors from the tissues. Furthermore, the potential risk of loss of isoform selectivity is reduced due to the shorter duration of action of reversible inhibitors (Legoabe *et al.*, 2012b). For these reasons, reversible inhibitors are thought to possess less side-effects compared to irreversible inhibitors.

4.3.2 MAO-A inhibitors

During the late 1950's and 1960's, the therapeutic importance of MAO-inhibitors was first recognized in the treatment of depressive illness with the use of iproniazid. Unfortunately, iproniazid with its hydrazine structure was associated with liver toxicity. The clinical use of the non-hydrazine MAO inhibitors that followed, were also compromised by serious adverse-effects, especially the “cheese reaction” (Figure 4.6) which is associated with the MAO-A isoform (Youdim & Backle, 2006).

The use of MAO inhibitors is associated with the potential of developing a hypertensive crisis after the consumption of high quantities of dietary tyramine (Stahl & Felker, 2008). Endogenously, tyramine is formed as a by-product from tyrosine, in the catecholamine biosynthesis (see Figure 3.2, Chapter 3). Tyramine is also ingested via food which include cheese, red-wine or related food produced via fermentation (Standaert & Young, 2001). Tyramine causes the release of noradrenaline and can thus elevate blood pressure. Normally, tyramine is metabolized by MAO in the small intestine (MAO-A = 80%; MAO-B = 20%) (Youdim *et al.*, 2006) and tyramine cannot accumulate to dangerous levels. Thus, MAO, in particular MAO-A, in the intestine prevents tyramine from entering the circulation (Youdim *et al.*, 2006). However, if MAO-A is inhibited, excessive amounts of tyramine enter the systemic circulation and as a result noradrenaline is released in excessive amounts which may lead to a hypertensive response (Stahl & Felker, 2008).

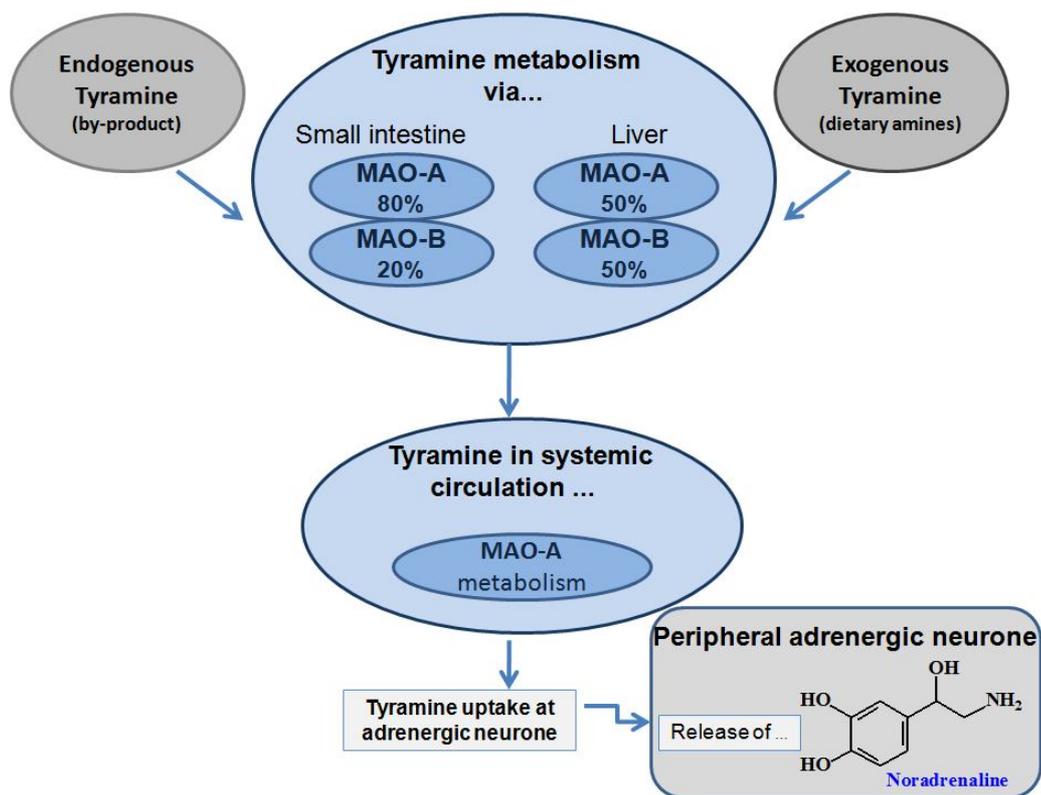


Figure 4.6: The “cheese reaction”. The reaction induced by either an irreversible non-selective inhibitor of MAO-A and MAO-B (MAO-A/MAO-B) or an irreversible inhibitor of MAO-A in combination with ingested food containing high quantities of tyramine.

With MAO-B inhibitors, the “cheese reaction” is not generally observed, except where MAO-B is taken at high doses and subsequently loses its selectivity (Youdim & Bakhle, 2006; Youdim *et al.*, 2006). Reversible inhibitors of MAO-A may also avoid the “cheese reaction” as the inhibitor may be displaced from peripheral MAO-A by dietary tyramine resulting in normal metabolism of tyramine (Youdim & Bakhle, 2006; Youdim *et al.*, 2006).

The neurotransmitters, noradrenaline and serotonin, are implicated in depression and anxiety (Yamada & Yasura, 2004). Since MAO-A deaminates these neurotransmitters, MAO-A inhibitors are used as pharmacological antidepressants and anti-anxiety drugs (Alcaro *et al.*, 2010). Even though, MAO-A also metabolizes brain dopamine, inhibition of MAO-A alone does not sufficiently enhance the brain dopamine levels, since MAO-B can still metabolize dopamine (Stahl & Felker, 2008). In PD, the use of MAO-A inhibitors may be employed to treat the non-motor symptoms of Parkinson’s disease such as depression and anxiety (Youdim & Bakhle, 2006; Prediger *et al.*, 2012). Moclobemide, a reversible MAO-A inhibitor, has been used for this purpose in clinical studies of PD (Youdim & Bakhle, 2006).

4.3.3 MAO-B inhibitors

MAO-B inhibition is not effective as antidepressant treatment. This may be attributed to the fact that MAO-B does not directly influence the metabolism of noradrenaline and serotonin (Stahl & Felker, 2008). MAO-B primarily deaminates dopamine and the dietary amine, β -phenylethylamine (Youdim *et al.*, 2006). MAO-B inhibitors are thus used in the therapy of PD. Selective inhibitors of MAO-B conserve the depleted dopamine supply by blocking the metabolism of dopamine in the basal ganglia (Di Monte *et al.*, 1996; Finberg *et al.*, 2000). Since MAO-B inhibitors may enhance the levels of dopamine obtained from L-DOPA, MAO-B inhibitors are either used as monotherapy or in combination with L-DOPA (Alcaro *et al.*, 2010). Additionally, MAO-B inhibitors may increase dopamine levels by inhibiting the termination of β -phenylethylamine (Youdim *et al.*, 2006). β -Phenylethylamine is an amine that does not only stimulate dopamine release (Youdim *et al.*, 2006; Fernandez & Chen, 2007), but also inhibits active neuronal dopamine uptake (Fernandez & Chen, 2007). MAO-B inhibitors may also protect against the neurodegenerative processes associated with PD. Oxidative deamination of dopamine via MAO-B results in the formation of H_2O_2 and the accumulation of toxic aldehyde metabolites of dopamine (Nagatsu & Sawada, 2006; Youdim & Bakhle, 2006). H_2O_2 produces highly reactive oxygen species via the Fenton reaction, a reaction that is catalyzed by iron and neuromelanin (Nagatsu & Sawada, 2006). The formation of H_2O_2 therefore aggravates neurodegeneration (Armentero *et al.*, 2011). Inhibitors of MAO-B, such as selegiline and rasagiline may be neuroprotective by blocking the formation of H_2O_2 and aldehyde species (Youdim & Bakhle, 2006; LeWitt & Taylor, 2008; Armentero *et al.*, 2011). Selegiline and rasagiline have been documented to also block MPTP induced neurotoxicity (LeWitt & Taylor, 2008; Armentero *et al.*, 2011). Therefore, MAO-B inhibitors are not only important for the inhibition of dopamine metabolism (a symptomatic effect), but these inhibitors may also exert a neuroprotective effect by preventing the production of neurotoxic dopamine metabolites (Nagatsu & Sawada, 2006).

Currently, the irreversible MAO-B inhibitors selegiline and rasagiline (Figure 4.7) are licensed in Europe and North America for treatment of PD (Shapira, 2011). According to literature, selegiline and rasagiline are used as monotherapy or adjunct therapy in treatment of early PD. It is reported that with the use of these drugs the incidence of motor fluctuations is reduced without substantial side-effects (Simonson *et al.*, 2007; Shapira 2011). In the later stages of PD, the off-time is reduced compared to the baseline by using these MAO-B inhibitors as adjunct therapy to L-DOPA (Simonson *et al.*, 2007; Shapira 2011).

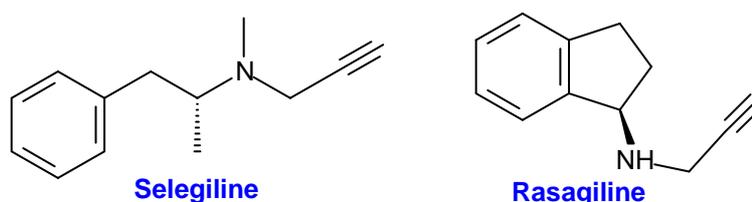


Figure 4.7: Chemical structures of irreversible MAO-B inhibitors, selegiline and rasagiline.

Selegiline is less selective compared to rasagiline (Onofrij *et al.*, 2008). The difference between selegiline and rasagiline is mainly in their drug properties. Selegiline metabolizes to yield amphetamine metabolites and rasagiline to yield aminoindan (Tazik *et al.*, 2009). It has been documented that aminoindan display neuroprotective effects, whereas the metabolite of selegiline, L-methamphetamine, exhibits neurotoxic effects that may counter the neuroprotective effect of selegiline (Tazik *et al.*, 2009).

The two reversible inhibitors of MAO-B that have been studied in clinical trials are lazabemide and safinamide (Figure 4.8). Unlike selegiline, lazabemide is not a propargylamine compound and does not metabolize to amphetamine. Clinical studies with lazabemide have shown that this drug is responsible for postponing the necessity for initiating dopaminergic treatment (LeWitt & Taylor, 2008). Unfortunately, despite the positive findings, the development of lazabemide has been discontinued by the study sponsor (LeWitt & Taylor, 2008; Shapira, 2011). Safinamide is currently studied in a phase III clinical trial and is a water-soluble, orally active aminoamide derivative with various actions (Shapira, 2011). Safinamide was shown to be a potent reversible MAO-B inhibitor with a documented IC_{50} value of $0.08 \mu M$ (Binda *et al.*, 2007).

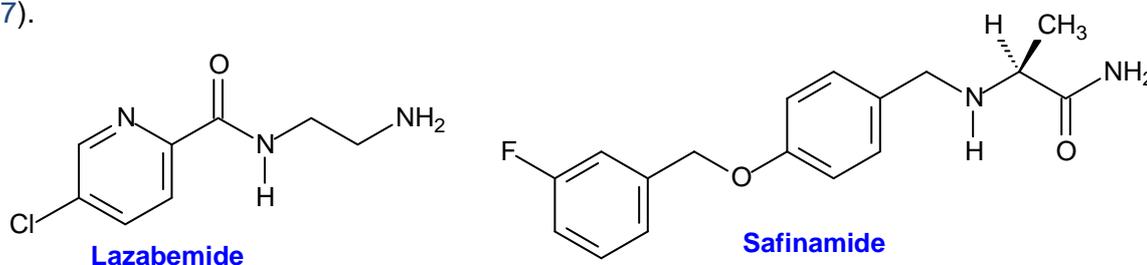
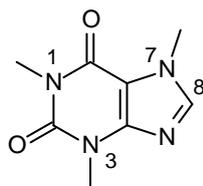


Figure 4.8: Chemical structures of reversible MAO-B inhibitors, lazabemide and safinamide.

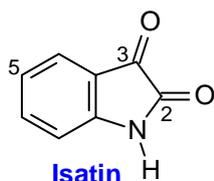
During the past decades, the role of MAO inhibitors has been investigated with regards to their therapeutic role as antidepressants and antiparkinsonian drugs. Many efforts have been made to develop novel compounds exhibiting MAO-A and/or MAO-B inhibition activities. Some of the recent studies focused on the small molecules caffeine (Figure 4.9) and isatin (Figure 4.10), as scaffolds for the design of MAO inhibitors. These molecules are of particular interest in this thesis. Both of these molecules, caffeine ($K_i = 3.6 \text{ mM}$) and isatin ($K_i = 3 \mu M$), are weak inhibitors of MAO-B (Van der Walt *et al.*, 2009). The inhibition potency of these small molecules may, however, be enhanced by several magnitudes of order with the appropriate substitution.

For example, isatin analogues containing C5- and C6-benzyloxy substituents were found to be potent MAO-B inhibitors with IC_{50} values of 0.103 and 0.138 μM , respectively (Manley-King *et al.*, 2011). Caffeine analogues bearing substitution on the C8 position of the caffeine ring were also shown to act as potent MAO-B inhibitors (Strydom *et al.*, 2010; Booysen *et al.*, 2011; Van der Walt *et al.*, 2009). For example, both 8-sulfanylcaffeine analogues (Booyesen *et al.*, 2011) and 8-benzyloxycaffeine analogues (Strydom *et al.*, 2010) are potent MAO-B inhibitors with IC_{50} values in the nanomolar range. Interestingly, a decrease in the inhibition activity towards MAO-A for the 8-sulfanylcaffeine analogues was noted (Booyesen *et al.*, 2011) compared to the 8-benzyloxycaffeine analogues (Strydom *et al.*, 2010).



Caffeine

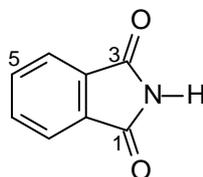
Figure 4.9: Chemical structure of caffeine.



Isatin

Figure 4.10: Chemical structure of isatin.

Furthermore, it has been demonstrated that phthalimide analogues also exhibit MAO inhibitory activities. Phthalimide (Figure 4.11) is structurally related to isatin and is a very weak MAO-A ($IC_{50} = 165 \mu\text{M}$) and MAO-B ($IC_{50} = 134 \mu\text{M}$) inhibitor. In a recent publication, phthalimide analogues containing a series of benzyloxy side chains were found to be very potent reversible inhibitors of recombinant human MAO-B, with IC_{50} values ranging from 0.007 to 2.49 μM (Manley-King *et al.*, 2011).



Phthalimide

Figure 4.11: Chemical structure of phthalimide.

Substitution with a benzyloxy side chain on isatin, caffeine and phthalimide was thus proven to be a favourable strategy for increased MAO-B inhibition (Strydom *et al.*, 2010; Booysen *et al.*, 2011; Manley-King *et al.*, 2011). Furthermore, halogen substitution on the benzyloxy ring has

additionally been documented to improve inhibition potencies (Strydom *et al.*, 2010; Booysen *et al.*, 2011; Manley-King *et al.*, 2011).

Among the various types of structures that have been reported to inhibit the MAO enzymes are nitrile containing compounds. It is thought that nitrile functional groups, because of their polar nature, are likely to interact with the polar region of the substrate cavity of the enzyme. Recently, analogues of phthalonitriles and benzonitriles have been documented to act as potent and selective MAO-B inhibitors. Significantly, these nitriles are also substituted with the benzyloxy side chain (Manley-King *et al.*, 2012).

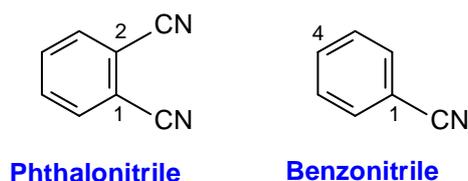


Figure 4.12: Chemical structures of phthalonitrile and benzonitrile.

Therefore, several potent MAO-B inhibitors possess the benzyloxy side chain. Furthermore, modelling studies have suggested that the binding modes of the benzylsulfanyl and benzyloxy side chains within the entrance cavity of MAO-B are highly comparable. For example, the IC_{50} values of 8-benzylsulfanylcaffeine analogues and 8-benzyloxycaffeine analogues are very similar. The above mentioned considerations prompted us to develop novel reversible and selective MAO-B inhibitors. The rational, design, synthesis, and evaluation of novel sulfanylphthalimide, sulfanylphthalonitrile and sulfanylbenzonitrile analogues as MAO-B inhibitors are discussed in Chapters 6 and 7 (article 1 and 2).

4.4 Conclusion

MAO plays an essential role in the oxidative deamination of important neurotransmitters. Literature shows that MAO-A and MAO-B have specific substrate and inhibitor preferences (Binda *et al.*, 2007; Son *et al.*, 2008). The two identified isoforms, MAO-A and MAO-B, are attractive drug targets in the therapy of neurodegenerative diseases (MAO-B) and depression (MAO-A).

The deamination of serotonin, adrenaline and noradrenaline is favoured by MAO-A and neurological disorders such as clinical depression and anxiety are treated with selective MAO-A inhibitors (Yamada & Yasuhara, 2004). MAO-B preferentially deaminates the neurotransmitter dopamine and inhibitors of MAO-B are useful for the treatment of PD (Yamada & Yasuhara, 2004; Nagatsu & Sawada, 2006). MAO-B inhibitors may be divided into two categories namely, reversible and irreversible inhibitors. Irreversible inhibitors of MAO-B are less desirable as therapeutic agents and the aim is thus to develop reversible inhibitors of MAO-B with potential neuroprotective properties.

As mentioned previously, current symptomatic treatments are focused on restoring dopaminergic functions, especially with the dopamine precursor L-DOPA. Unfortunately, prolonged treatment with this drug may induce motor related complications such as dyskinesia and motor fluctuations (Fernandez & Chen, 2007). In addition, evidence show that treatment with L-DOPA does not ameliorate non-motor symptoms (including depression and anxiety). Symptomatic treatment of PD may be provided by MAO-B selective inhibitors, as these inhibitors are frequently co-administered with L-DOPA in order to enhance the levels of dopamine derived from L-DOPA. Moreover, inhibitors of MAO-B are also employed as monotherapy in PD drug therapy and it is thought that MAO-B inhibitors may provide a neuroprotective effect.

This thesis, in part, focuses on the design and synthesis of novel MAO-B inhibitors that may be employed in the treatment of PD. Of particular interest are recent findings that appropriately substituted phthalimides, phthalonitriles and benzonitriles possess high potency MAO-B inhibitory activities. One of the aims of this thesis was to develop selective and potent MAO-B inhibitors by examining the MAO inhibitory properties of series of sulfanylphthalimdes, sulfanylphthalonitrile and sulfanylbenzonitrile analogues.

4.5 References

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