Chapter 2
Literature overview

2.1 Parkinson's Disease

Parkinson's disease (PD) is an age related, progressive disease that occurs sporadically. It is thought that environmental and endogenous toxins may play a role in the etiology of PD, (Dauer & Przedborski, 2003). For example, exposure to insecticides and herbicides, such as rotenone and paraquat, may result in an increased occurrence of PD (Tanner *et al.*, 2011; Bove *et al.*, 2005). A combination of different environmental, genetic and endogenous factors could lead to PD. About 5% of PD cases are genetic (Dauer & Przedborski, 2003).

The most perceptible manifestation of PD is the movement disorders associated with this disease: tremor during rest, rigidity, akinesia, postural instability and freezing, (Jankovic, 2008). On a cellular level, PD is characterized by the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) of the brain and the subsequent depletion of dopamine (DA) (Uhl *et al.*, 1994). The depleted DA levels ultimately result in the observed movement disorders, since DA is responsible for the regulation of normal movement in the body. Another characteristic trait of PD is the presence of Lewy Bodies. Lewy Bodies are protein-like inclusions in the cytoplasm of cells in the affected brain regions and may contribute to neuronal death in PD (Dauer & Przedborski, 2003).

2.1.1 Mechanism

There are various mechanisms that may contribute to the manifestation of PD. A minor cause of PD may be linked to genetic factors while other mechanisms involve the misfolding and aggregation of proteins. Neurotoxicity, caused by exogenous and endogenous factors along with oxidative stress, may also contribute to the development of PD (Dauer & Przedborski, 2003). These mechanisms are discussed below.

2.1.1.1 Misfolding and aggregation of proteins

Misfolded and aggregated proteins can cause direct damage to cells by physically deforming cells and interfering with intracellular transfer in neurons. Certain proteins, like α-synuclein, are susceptible to oxidation by reactive oxygen species (ROS), resulting in a higher possibility of protein aggregation (Giasson & Duda, 2000). Herbicides and pesticides may also enhance the misfolding and aggregation of α-synuclein (Uversky *et al.*, 2001). It was found that α-synuclein is a major constituent of Lewy Bodies along with parkin, ubiquitin and neurofilaments (Spilantini *et al.*, 1997). These protein inclusions may be indicative that the clearance mechanism to remove soluble misfolded proteins from the cells are dysfunctional (Cummings *et al.*, 1999; Warrick *et al.*, 1999). In the elderly, the cellular mechanism responsible for the clearance of misfolded proteins is often impaired, contributing to the accumulation of misfolded proteins and ultimately, cellular damage (Figure 7) (Sherman & Goldberg, 2001).

Genetic mutations on α-synuclein, parkin and ubiquitin are associated with higher occurrences of hereditary PD (Polymeropoulos *et al.*, 1997). Mutations in the gene for parkin, that annihilates its function, were found in hereditary cases of early onset PD. In these cases, neurodegeneration of the dopaminergic neurons were observed while Lewy Bodies was not necessarily present (Mizuno *et al.*, 2008). Parkin is associated with the ubiquitin-proteasome system where it is responsible for the identification of misfolded proteins and the subsequent targeting of these proteins to the proteasomes for degradation (Sherman & Goldberg, 2001). Mutations of parkin may result in a decrease in protein clearance activity and the accumulation of parkin substrates. These accumulated proteins can contribute to dopaminergic neurodegeneration (Dauer & Przedborski, 2003). Another protein of which mutations may result in hereditary PD, is ubiquitin C-terminal hydrolase-L1 (UCH-L1) (Leroy *et al.,* 1998). UCH-L1 plays a role in the recycling of ubiquitin bound misfolded proteins after degradation by the proteasomes (Wilkinson, 2000). Mutations on UCH-L1 may thus lead to the accumulation and aggregation of misfolded proteins (Dauer & Przedborski, 2003).

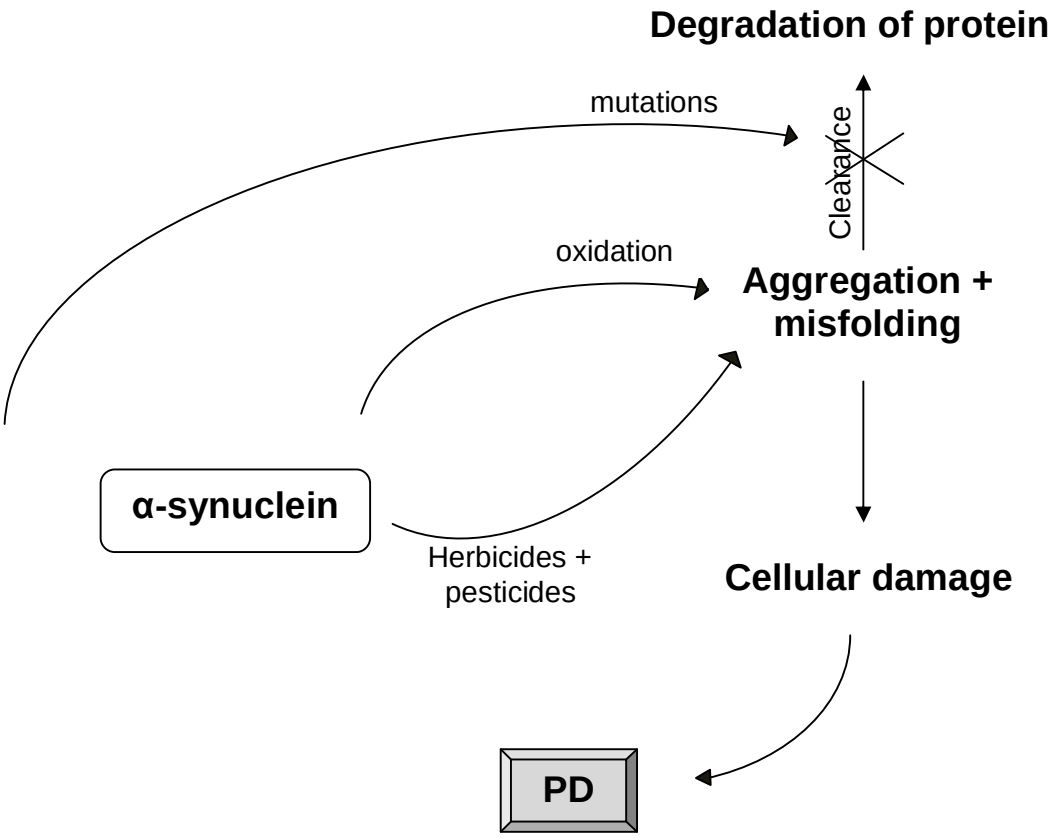


Figure 7 A schematic representation of the contribution of misfolded and aggregated proteins, specifically α-synuclein, to the etiology of PD.

2.1.1.2 Endogenous toxins

Endogenous toxins are generated within the body due to the impairment or over stimulation of natural processes. MAO-B is implicated in one of the major processes responsible for the generation of neurotoxins and harmful compounds within the body (Youdim & Bakhle, 2006).

Increased activity of MAO-B has been observed in the brains of PD patients (Zhou *et al*., 2001). This results in a higher rate of oxidative deamination of DA and could possibly lead to the depletion of DA as well as neurodegeneration. During the metabolism of DA the corresponding aldehyde is generated (Figure 8) (Youdim &

Bakhle, 2006). These aldehydes can be neurotoxic if allowed to accumulate. Under physiological conditions, aldehydes are further oxidized by aldehyde dehydrogenase to the corresponding carboxylic acid which is then excreted. Since PD patients are reported to have reduced levels of aldehyde dehydrogenase, these toxic aldehydes can accumulate and contribute to neurodegeneration (Grünblatt *et al*., 2004).

Hydrogen peroxide (H_2O_2) is also generated during the metabolism of DA. The inactivation of H_2O_2 is catalyzed by glutathione peroxidase in the brain (Youdim & Bakhle, 2006). Since PD patients may also possess decreased levels of glutathione peroxidase (Sian *et al.*, 1994), H_2O_2 can accumulate and contribute to oxidative stress. In addition, elevated levels of iron in the substantia nigra of PD patients has been observed (Dexter *et al.*, 1989). Free iron can react with H_2O_2 (Fenton reaction) to yield highly reactive hydroxyl radicals which cause oxidative stress and ultimately neurodegeneration (Figure 8) (Blum *et al.*, 2001).

Significant amounts of DA, H_2O_2 and free iron can be found in the dopaminergic neurons of the substantia nigra, allowing the Fenton reaction to take place. Even a small amount of hydroxyl radicals produced during the Fenton reaction may participate in a non-enzymatic reaction in physiological conditions to yield 6-hydroxydopamine (6-OHDA), a neurotoxin that causes PD like symptoms (Slivka & Cohen, 1985; Blum *et al.*, 2001). The consequent autoxidation of 6-OHDA produces toxic quinones, superoxide radicals, H_2O_2 and hydroxyl radicals (Figure 8) (Soto-Otero *et al.*, 2000).

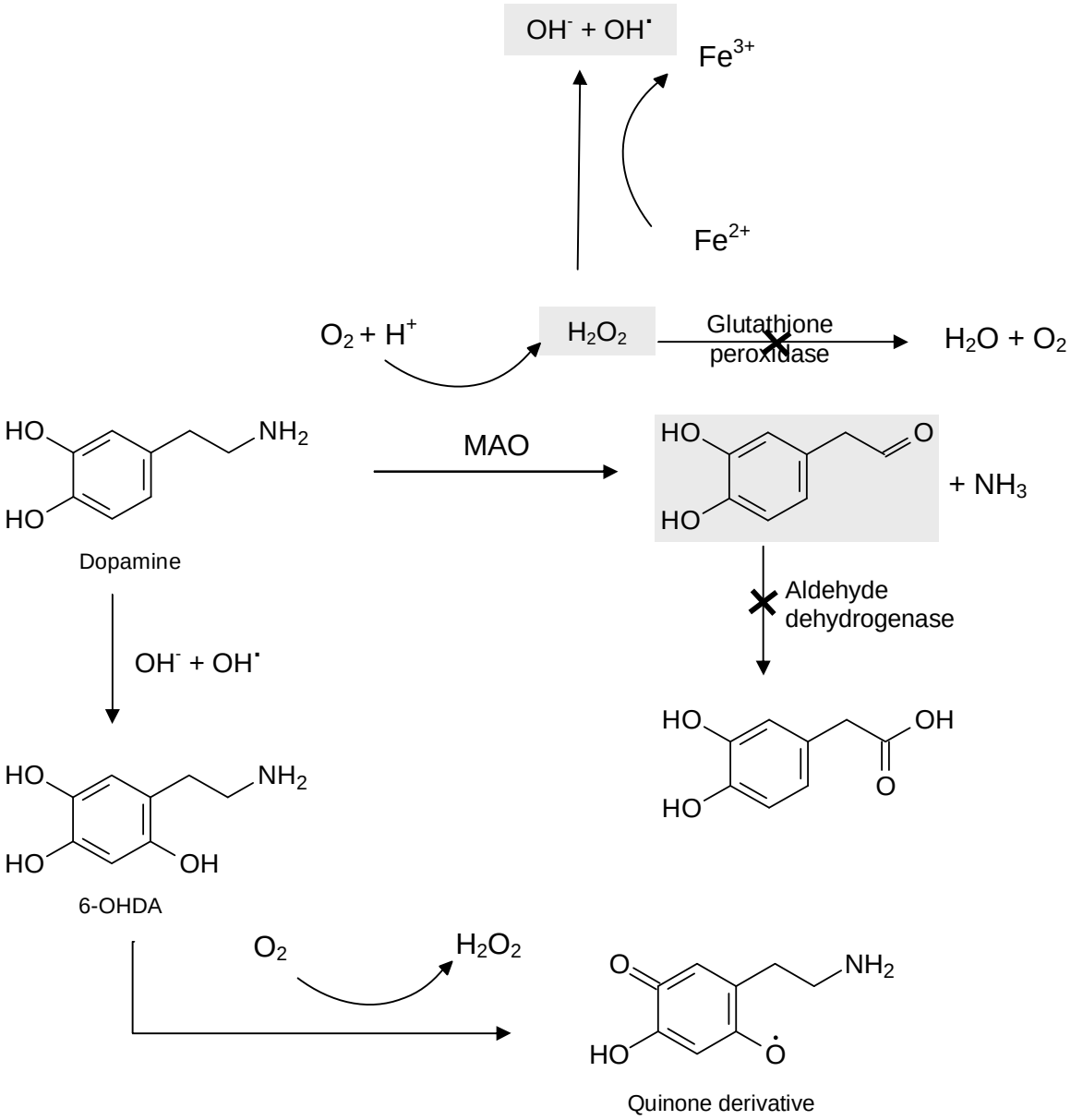


Figure 8 Schematic representation of the role of DA in the formation of endogenous toxins. The oxidative deamination of DA by MAO is illustrated with the production and accumulation of aldehydes, hydrogen peroxide and hydroxyl radicals shown by the highlighted areas. The autoxidation products of DA namely, 6-OHDA and the related DA quinone are also shown.

2.1.1.3 Exogenous toxins

Various exogenous neurotoxins are used to create animal models of PD. Some of the most common neurotoxins are 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-OHDA, rotenone and paraquat (Bové *et al.*, 2005).

MPTP

During the illicit synthesis of 1-methyl-4-phenyl-4-propionoxypiperidine (MPPP), MPTP was inadvertently produced as a by-product and caused parkinsonian-like symptoms, including tremor, rigidity, slowness of movement, postural instability and freezing. Improvement could be observed with anti-parkinsonian treatments. It was found that MPTP causes damage to the nigro-striatal pathway, resulting in DA depletion and the observed movement disorders. Successful animal models have been created using MPTP, but the formation of Lewy bodies are not observed in these models (Bové *et al.*, 2005; Dauer & Przedborski, 2003).

MPTP readily traverses the blood-brain barrier (BBB) after which it is taken up in the glial cells. Within the glial cells MAO-B oxidizes MPTP to 1-methyl-4-phenyl-2,3-dihydropyridinium ($MPDP^+$) which then spontaneously disproportionates to yield the active toxin, 1-methyl-4-phenylpyridinium (MPP^+). MPP^+ is actively transported out of the glial cells and taken up in the dopaminergic neurons by means of the dopamine transporter (DAT). From here MPP^+-mediated mitochondrial inhibition occurs, leading to a decrease in cellular ATP production and ultimately cell death (Figure 9) (Blum *et al.*, 2001; Smeyne & Jackson-Lewis, 2005; Dauer & Przedborski, 2003). In addition to the damage caused by MPP^+, further neuronal damage can be caused by the formation of H_2O_2-derived oxygen species during the oxidation of MPTP (Zang & Misra, 1993; Przedborski *et al.*, 2004). The discovery that MAO-B converts MPTP to the neurotoxin MPP^+, supports the notion that endogenous processes may play a role in activating exogenous toxins (Elbaz & Tranchant, 2007).

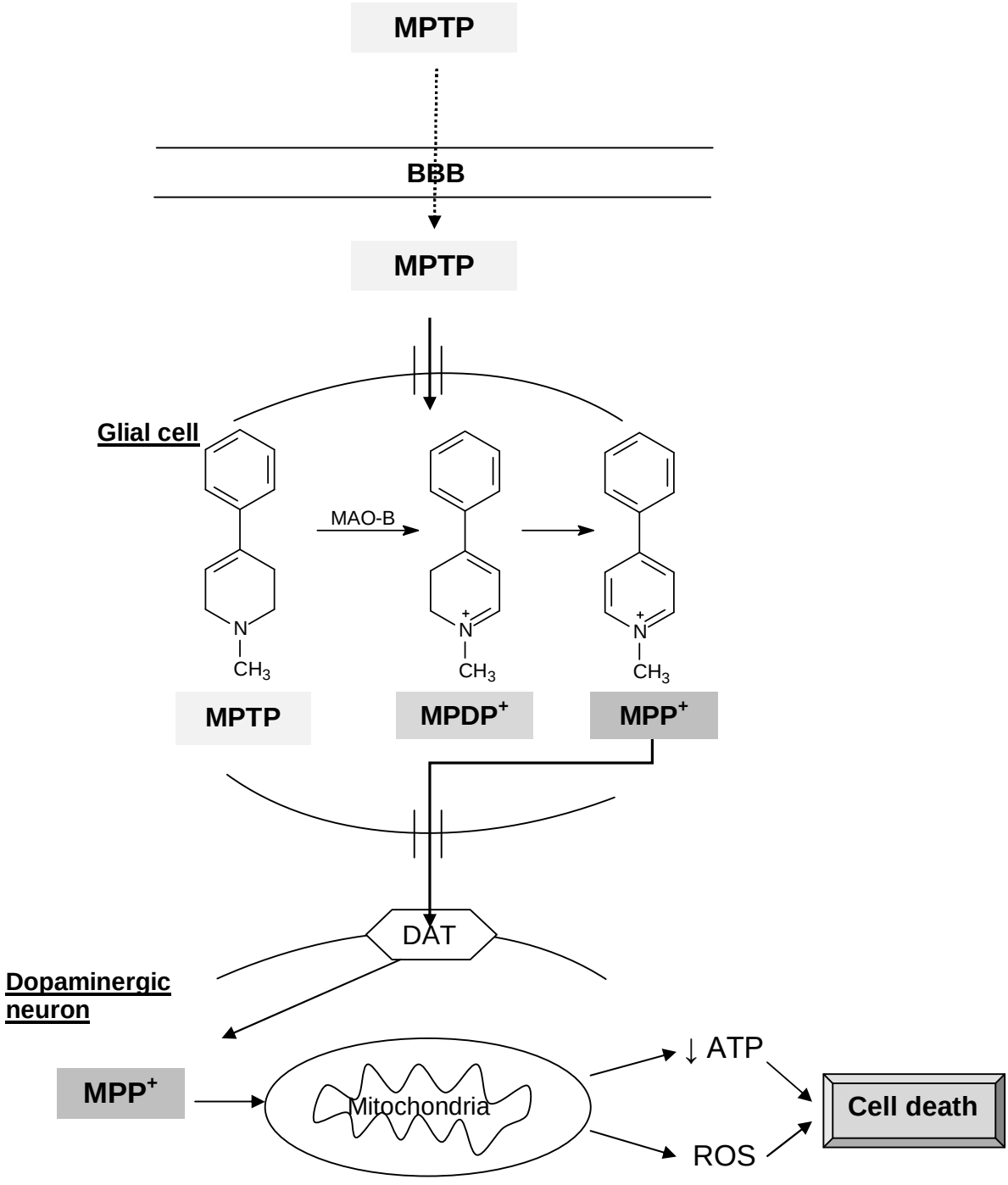


Figure 9 Schematic representation of the mechanism by which MPTP exert its neurotoxic effect on the dopaminergic neurons.

6-OHDA

Animal models of PD using 6-OHDA have also been created in the past. Systemic administration of 6-OHDA does not result in high enough levels in the brain, since 6-OHDA crosses the BBB poorly. Direct injection into the substantia nigra is thus necessary to obtain specific damage to the nigro-striatal dopaminergic neurons (Ungerstedt, 1968). There is a close structural resemblance of 6-OHDA with DA as well as with norepinephrine (Figure 10). This implies a high affinity of 6-OHDA for the DA and norepinephrine transporters and consequently accumulation of 6-OHDA in these neurons (Bové *et al.*, 2005). Rat models of 6-OHDA display characteristic rotational behaviour after treatment with dopaminergic drugs. This behaviour is related to the degree of the lesion (Przedborski *et al.*, 1995). An improvement in rotational behaviour could be observed after administration of L-dopa (Bové & Perier, 2012). Lewy bodies also could not be demonstrated convincingly with this model (Bové *et al.*, 2005).

Lesions of the dopaminergic neurons were observed and may be attributed to the actions of hydroxyl radicals, superoxide radicals and hydrogen peroxide. Since 6-OHDA is very similar to DA, 6-OHDA also acts as a substrate for MAO. During MAO activity and the autoxidation of 6-OHDA, hydrogen peroxide is formed, which reacts with free iron to produce highly reactive hydroxyl radicals (Blum *et al.*, 2001; Soto-Otero *et al.*, 2000) . Similar to MPTP, 6-OHDA impairs mitochondrial function and decreases ATP production by inhibiting mitochondrial complex I (Blum *et al.*, 2001).

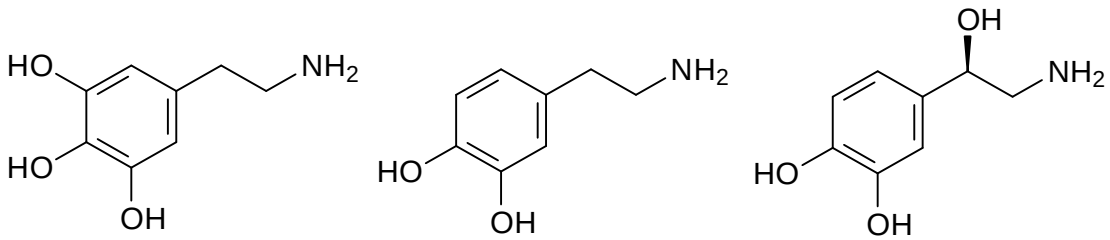
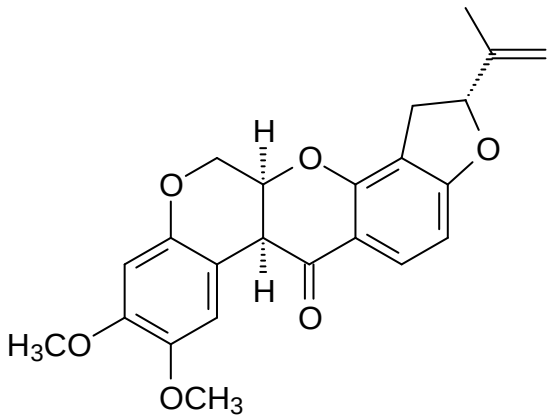


Figure 10 The chemical structures of 6-OHDA, DA and norepinephrine.

Rotenone

Rotenone (Figure 11) is an insecticide and piscicide that produces parkinsonian motor symptoms, possibly due to the inhibition of mitochondrial complex I (Schuler & Casida, 2001). Although it has been shown that exposure to rotenone increases the risk of developing PD (Tanner *et al.*, 2011), it is thought unlikely that rotenone by itself causes PD. Rotenone is rapidly broken down by the sun and does not leach from the ground to pollute ground water. The rate of absorption from the stomach is also very slow and incomplete (Bové *et al.*, 2005). However, rotenone is very lipophilic and easily traverses the BBB after systemic administration where it freely crosses cellular membranes and can accumulate in the mitochondria (Bové & Perier, 2012).

In the rotenone rat model, neurodegeneration of the nigrostriatal dopaminergic neurons could be observed along with motor abnormalities. An improvement of the motor abnormalities was obtained after treatment with L-dopa. Proteinaceous inclusions, resembling Lewy bodies, were found in these models (Bové *et al.*, 2005; Bové & Perier, 2012). Of the available neurotoxic animal models of PD, the rotenone model is not favourable since the effect on the nigro-striatal pathway is inconsistent and unpredictable (Bové *et al.*, 2005).



Rotenone

Figure 11 Chemical structure of rotenone

Paraquat

Paraquat is a herbicide and share structural similarity with MPP^+ (Figure 12), the neurotoxic metabolite of MPTP (Bové & Perier, 2012). Lethal poisoning by paraquat has been reported due to ingestion or dermal exposure (Smith, 1988). The neurotoxic effect of paraquat is exerted through the induction of oxidative stress through ROS (Drechsel & Patel, 2008). Increased expression of α-synuclein was observed in animals treated with this compound (Bové *et al.*, 2005). A greater deleterious effect on the dopaminergic system was obtained when paraquat was used in combination with the fungicide, maneb (Wang *et al.*, 2011).

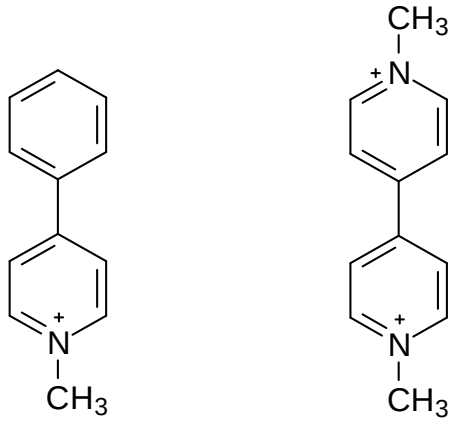


Figure 12 The chemical structures of MPP^+ and paraquat.

2.1.2 Treatment

There are various treatment options available for the management of PD. Since it is not possible to reverse the neurodegenerative process, treatments are mainly focused on the symptomatic management of PD and the improvement of the patient's quality of life. Symptomatic treatment of PD can be achieved by directly stimulating dopaminergic neurons, increasing the supply of DA in the brain or by the inhibition of the reuptake and metabolism of DA (Lees, 2005).

L-dopa

One of the most important treatment options in PD is the administration of the DA precursor, L-dopa. L-dopa undergoes decarboxylation by aromatic amino acid decarboxylase (AADC) in the brain to form DA (Hornykiewicz, 2002) (Figure 13). Administration of L-dopa can thus directly increase the supply of DA in the brain and consequently improves motor function. Unfortunately, long-term use of L-dopa is associated with fluctuating motor function, dyskineasias and neurobehavioural problems (Guttman *et al*., 2003; Olanow & Jankovic, 2005).

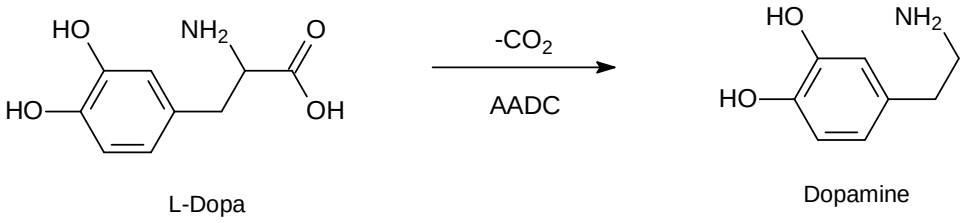


Figure 13 The decarboxylation of L-dopa to DA by aromatic amino acid decarboxylase (AADC).

Dopamine agonists

Dopamine agonists directly increase DA in the brain by the stimulation of dopaminergic receptors. Pramipexole (Figure 14) is one of the most popular DA agonists and selectively stimulates the dopamine D_3 receptors. Activation of the dopamine D_3 receptors results in an inhibitory effect on motor function and can bring about symptomatic relief for PD patients (Bennett & Piercey, 1999). Unlike L-dopa, the DA agonists do not display side effects associated with long-term use. The DA agonists can be used as mono-therapy or as an adjunct to L-dopa. When used in combination with L-dopa, especially in the initial treatment of PD, the required dose of L-dopa can be lowered (Holloway, 2004).

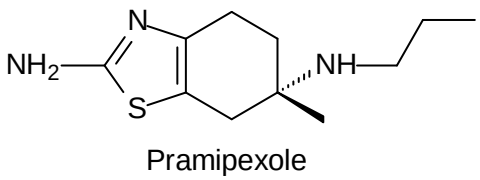
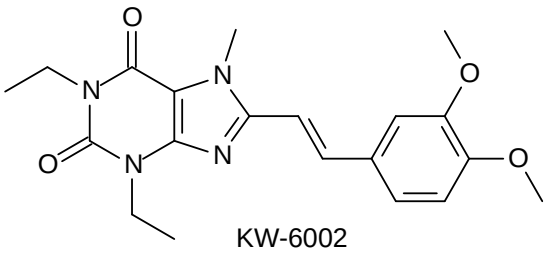


Figure 14 The chemical structure of the dopamine agonist, pramipexole.

Adenosine A_{2A} receptor antagonists

These compounds have the ability to decrease the effects of reduced post synaptic DA in PD. Adenosine A_{2A} antagonists can thus reduce motor discrepancies and bring about symptomatic relief for PD patients. In addition to the symptomatic effects, adenosine A_{2A} antagonists also possess neuroprotective properties. The mechanism of neuroprotection is still uncertain, but one of the hypotheses is that A_{2A} antagonism reduces the release of glutamate in the brain and prevents the neurotoxic effects that are associated with elevated glutamate levels in the brain (Schwarzschild *et al.*, 2006; Prediger, 2010). One of the well-defined antagonists of adenosine A_{2A} receptors is KW-6002 (Figure 15), a xanthine derivative. KW-6002 has been used in combination with a reduced dose of L-Dopa in animal models of PD. The combination of KW-6002 with L-dopa resulted in symptomatic relief equivalent to that of a full dose of L-dopa with the further advantage of reduced dyskinesia (Hickey & Stacy, 2012; Morelli *et al.*, 2012). The mechanism of action of adenosine A_{2A} antagonists involves mainly the indirect GABAergic pathway. Adenosine A_{2A} antagonism opposes the activity of the dopamine D_2 receptors and consequently inhibits the GABAergic striatopallidal neurons (indirect pathway) (Figure 16) (Schwarzschild *et al.*, 2006; Morelli *et al.*, 2012).



KW-6002

Figure 15 Chemical structure of the adenosine A_{2A} receptor antagonist, KW-6002.

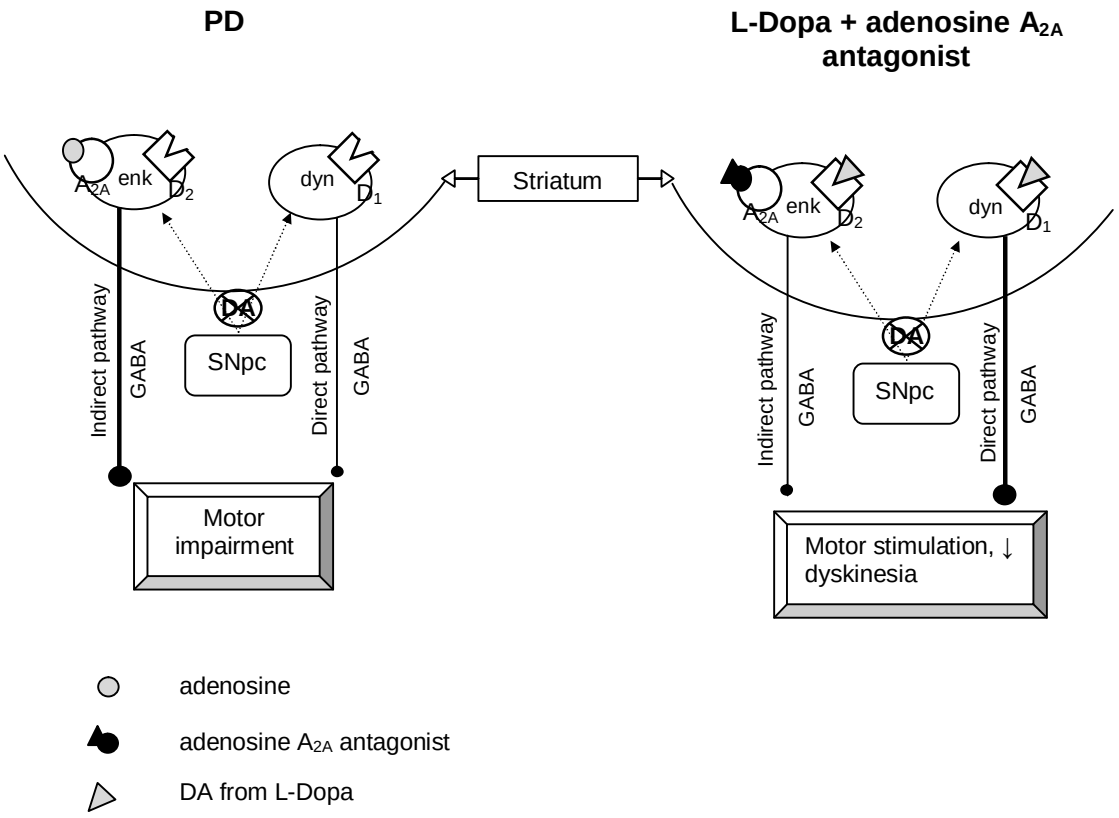


Figure 16 Schematic representation of the mechanism of action of adenosine A_{2A} antagonists. The dopaminergic input from the substantia nigra pars compacta (SNpc) to the striatum is impaired in PD. The direct pathway co-expresses dopamine D_1 receptors with the neuropeptide dynorphin (dyn) while the indirect pathway expresses adenosine A_{2A} receptors, dopamine D_2 receptors and the neuropeptide enkephalin (enk). The scheme on the left shows the effect of DA depletion on the direct and indirect pathways in PD that leads to motor impairment. The scheme on the right shows the effect of treatment with L-Dopa and an adenosine A_{2A} antagonist. The indirect pathway is inhibited to effect motor stimulation with decreased dyskinesia (Morelli *et al.*, 2012).

Amantadine

Amantadine prolongs the action of DA by preventing it from being taken up pre-synaptically. It can be administered as adjunct therapy with L-dopa or anticholinergic drugs or it can be used on its own. Although amantadine shows a better long-term safety profile than L-dopa, some patients may develop tolerance for the effects of amantadine (Schwab *et al.*, 1972; Adler *et al.,* 1997).

Anti-cholinergic drugs

The use of anti-cholinergic drugs in PD is restricted, especially with elderly patients, due to the numerous side effects associated with this class. Some of the most common anti-cholinergic drugs used are trihexyphenidyl, benztropine and procyclidine. These drugs reduce tremor associated with PD and are reserved as last resort for patients with debilitating tremor resistant to dopaminergic treatments (Rezak, 2007; Rao *et al.*, 2006).

Catechol-O-methyltransferase (COMT) inhibitors

Entacapone and tolcapone are the two most commonly known COMT inhibitors. COMT is responsible for the metabolism of L-dopa in the gastrointestinal tract. Inhibition of COMT can thus increase the concentration of L-dopa that reaches the substantia nigra and ultimately the levels of DA in the brain. COMT inhibitors are often co-administered with L-dopa since they are able to reduce the 'wearing off' effect of L-dopa. The use of tolcapone should be closely monitored, since fatal hepatotoxicity was observed for this drug (Rezak, 2007; Rao *et al.*, 2006).

MAO inhibitors

These compounds may possibly conserve DA and as a result provide symptomatic relief for PD patients. MAO inhibitors may also reduce neurodegeneration by restricting the production of neurotoxic metabolic products. The use of MAO inhibitors in early PD may delay the need for L-dopa (Youdim *et al.*, 2006). Previously non-selective, irreversible inhibitors such as tranylcypromine (figure 17) were used in the treatment of PD, but these drugs may lead to hypertensive crisis when combined with tyramine containing foods. Since DA is the preferred substrate of MAO-B, selective MAO-B inhibitors such as selegiline and rasagiline (figure 17) were developed which proved more successful (Yamada & Yasuhara, 2004). Unfortunately selegiline has unfavourable psychotoxic and cardiovascular side effects. Irreversible inhibitors have the added disadvantage of a slow recovery rate of enzyme activity after termination of drug treatment (Tipton *et al.*, 2004). A new reversible, selective MAO-B inhibitor, safinamide (figure 17), is currently in clinical trials and promises to have a better safety profile than the irreversible MAO-B inhibitors (Fernandez & Chen, 2007).

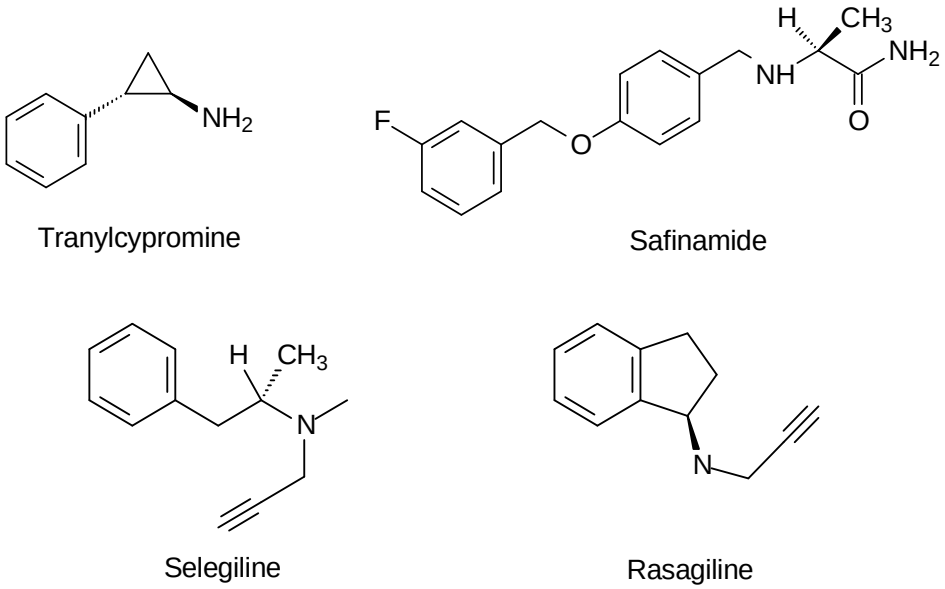


Figure 17 Chemical structures of the non-selective MAO inhibitor, tranylcypromine, and the selective, reversible MAO-B inhibitor, safinamide. The structures of the selective, irreversible MAO-B inhibitors, selegiline and rasagiline are also given.

2.2 Monoamine oxidase

MAO is the main enzyme responsible for the oxidative deamination of various neurotransmitters and dietary amines. There are two isoforms of MAO, MAO-A and MAO-B. Both these isozymes are tightly bound to the outer membrane of mitochondria. The two MAO isoforms share a 70% sequence identity and both contain a flavin adenine dinucleotide (FAD) co-factor (Nagatsu, 2004; Youdim & Bakhle, 2006).

MAO-A and –B have different substrate and inhibitor specificities. MAO-A preferably catalyzes the oxidation of serotonin and is inhibited by low concentrations of clorgyline. MAO-B on the other hand, catalyzes the oxidation of benzylamine and 2-phenylethylamine and is inhibited by low concentrations of selegiline. Both MAO-A and –B use DA as substrate (Youdim *et al.*, 2006).

The two isoforms are also differently expressed in tissues throughout the body. Human placenta is a rich source of MAO-A while high concentrations of MAO-B can be found in the blood platelets. In the brain, MAO-A is mainly localized to the catecholaminergic neurons while MAO-B is expressed in the serotoninergic neurons, the histaminergic neurons and the glial cells (Nagatsu, 2004). Although both isoforms are present in the brain, MAO-B is mainly responsible for DA metabolism in this region (Lees, 2005; Yamada & Yasuhara, 2004). An age related increase of MAO-B levels in the brain was observed, especially for patients between the ages of 50 and 60 (Saura *et al.*, 1997). MAO-A, on the other hand, already reaches mature levels at birth (Nicotra *et al.*, 2004). Since PD is an age related disease, increased MAO-B levels in elderly PD patients may further increase DA metabolism which would result in the depletion of DA in the brain and an elevation in the production of metabolic by-products.

Inhibition of MAO may therefore be an effective strategy of increasing DA levels in the PD brain as well as reducing neurodegeneration by limiting the production of potentially neurotoxic metabolic by-products (Youdim *et al.*, 2006; Youdim & Bakhle, 2006).

2.2.1 Catalytic mechanism of MAO

MAO-A and –B are thought to employ the same catalytic mechanism, since the active sites of these two isoforms are similar (De Colibus *et al.*, 2005). Oxidation of amine substrates occur at the 5 or 4a positions of the isoalloxazine ring of the FAD co-factor. When a substrate binds to the active site of MAO, α-CH cleavage of the amine substrate occurs, to produce the relevant imine. During this process the FAD co-factor is reduced. In the case of a primary amine substrate, the imine is hydrolyzed to an aldehyde and ammonia. Imines from secondary and tertiary amine substrates are hydrolyzed to form another amine. The FAD is re-oxidized with the concurrent production of hydrogen peroxide (Figure 18) (Binda *et al.*, 2002a; Edmondson *et al.*, 2004).

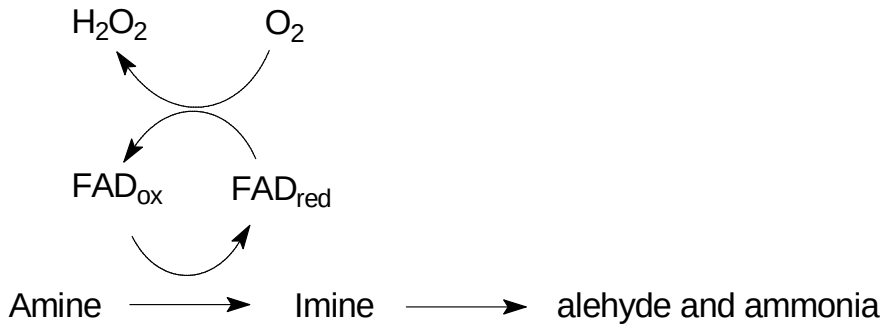


Figure 18 The FAD cycle during the oxidation of amines. FAD is firstly reduced and then re-oxidized with the concurrent production of hydrogen peroxide

The exact mechanism of MAO catalysis is still unknown, but a few possible mechanisms have been proposed. Silverman (1995) suggested a single electron transfer mechanism where the first step of catalysis involves the single electron oxidation of the amine to produce the aminium cation radical. This step results in an intermediary product with an acidic α-proton which allows for proton abstraction by a basic amino acid residue in the active site (Figure 19a). Unfortunately there exists no direct evidence to support the formation of a flavin radical intermediate. Also no basic amino acid residues are found in the MAO-A and –B active sites.

A more likely mechanism is the polar nucleophilic mechanism. In this mechanism the amine substrate binds covalently to the 4a position of the flavin in a nucleophilic manner. This activates and enables position N5 to act as a strong active site base (Figure 19b) (Binda *et al.*, 2002a; Edmondson *et al.*, 2004). More recently it was suggested that a hydride transfer mechanism cannot be excluded since there is no direct evidence against this mechanism. It is also known that other amine oxidazes, like the D-amino acid oxidazes, employ a single hydride transfer mechanism (Figure 19c) (Fitzpatrick, 2010).

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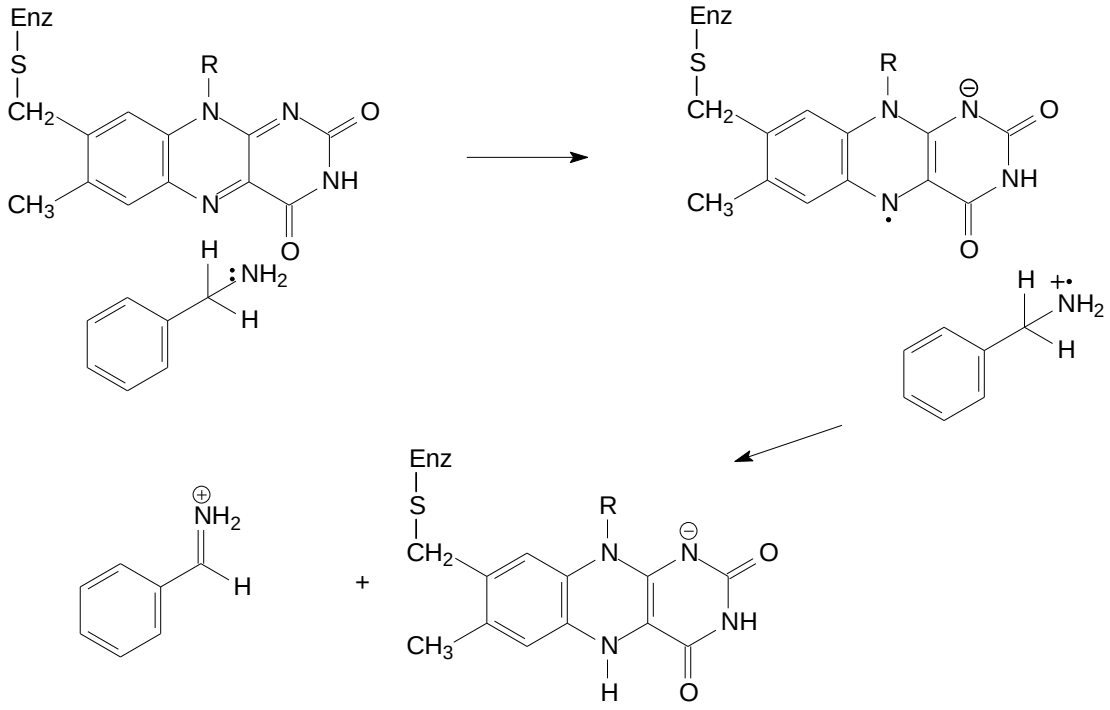

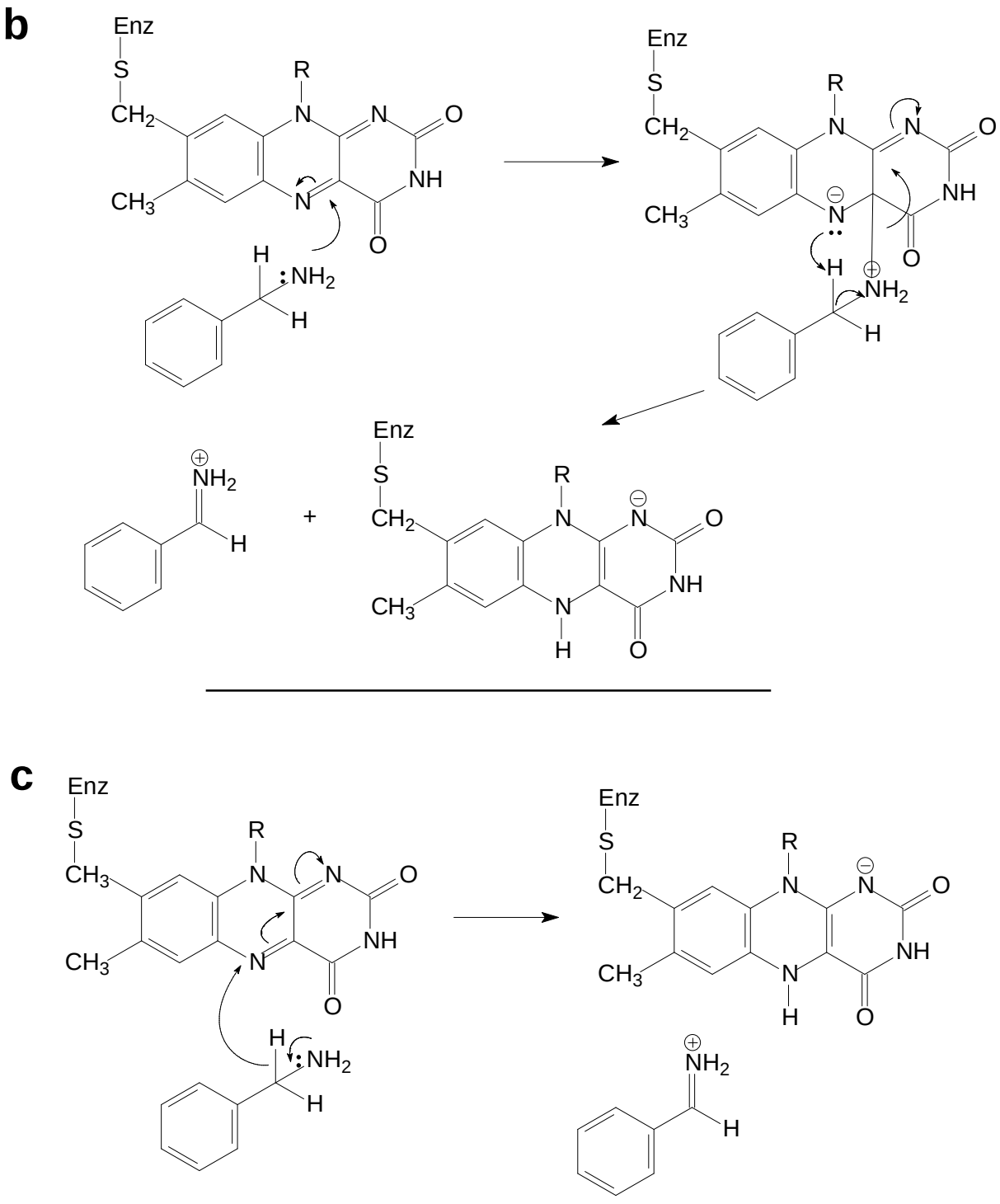

Figure 19 a) The single electron transfer mechanism. b) The polar nucleophilic mechanism. c) The hydride transfer mechanism.

2.2.2 MAO inhibition

MAO inhibition may provide symptomatic relief to PD patients as well as a measure of neuroprotection by limiting the production of toxic metabolites. MAO inhibitors generally consist of reversible and irreversible inhibitors, among which there are selective and non-selective inhibitors. Irreversible inhibition involves the covalent attachment of an inhibitor to the FAD co-factor of the enzyme. This is associated with unfavourable side effects, since it may require weeks to regain normal enzyme activity after termination of treatment. With reversible inhibition, enzyme recovery is almost immediate after drug withdrawal (Riederer *et al.*, 2004; Tipton *et al.*, 2004). Since MAO-B is mainly responsible for DA metabolism in the brain, focus has been on the development of selective MAO-B inhibitors. However, it has been shown that, in the brain, MAO-A can still sufficiently metabolize DA when MAO-B is inhibited (Kalaria *et al.*, 1988; Fowler *et al.*, 1980). Thus, there may be merit in the development of reversible, non-selective inhibitors of MAO for the treatment of PD.

2.2.2.1 MAO-A inhibitors

MAO-A inhibitors are widely used as antidepressant agents, since they elevate serotonin levels by inhibiting the metabolism of serotonin in the brain. Initially non-selective MAO inhibitors such as tranylcypromine were used as anti-depressants, but severe side effects, due to the 'cheese reaction', hampered its further use. The cheese reaction is caused by the accumulation of tyramine and other sympathomimetic amines taken in by food. Usually these amines are metabolized by MAO-A in the gut wall. Non-selective, irreversible inhibition of MAO consequently causes the accumulation of tyramine which then enters the circulation and increases the release of noradrenaline (NA) (Figure 20) (Youdim & Bakhle, 2006). This can result in the occurrence of a hypertensive crisis and could be fatal (Finberg *et al.*, 1981).

More recently, reversible inhibitors of MAO, such as moclobemide, were developed for the treatment of depressive illnesses. These inhibitors have better safety profiles since they do not precipitate the 'cheese reaction'. Inhibition of MAO-A may find application in the management of PD since research has showed that PD patients often suffer from depression (Slaughter *et al.*, 2001; Leentjies *et al.*, 2011)

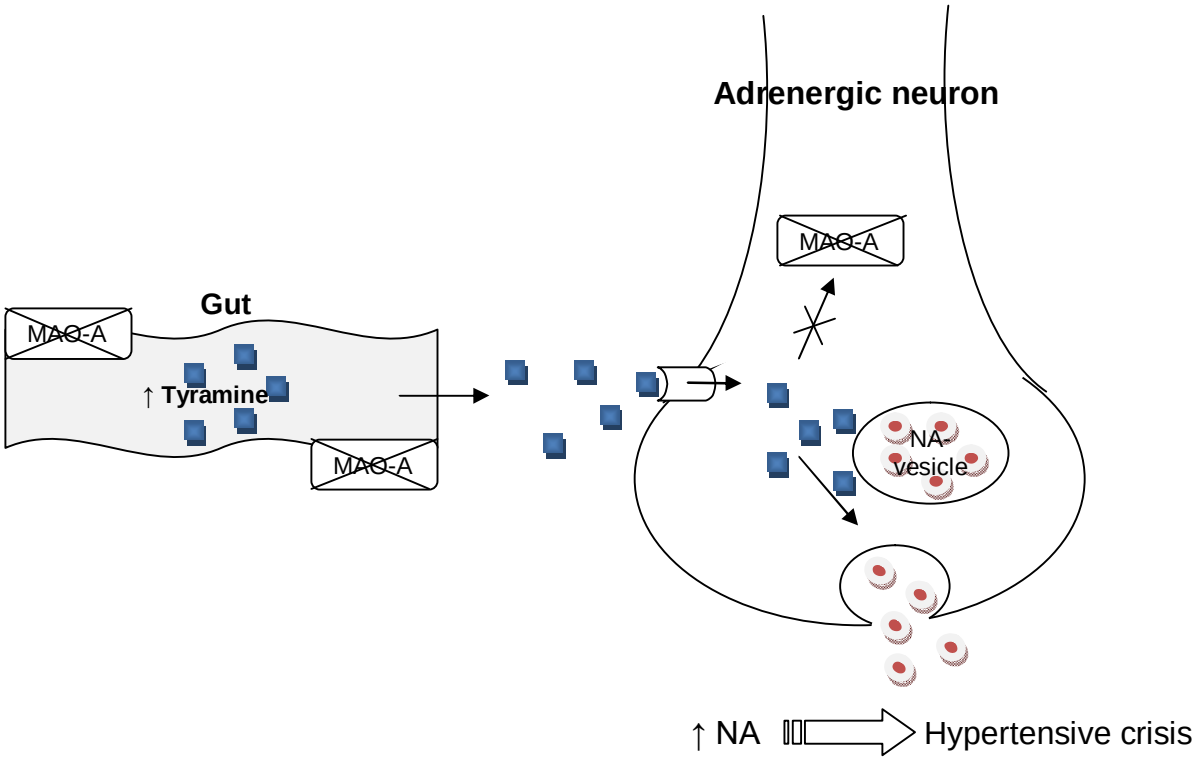

Figure 20 Illustration of the mechanism of the cheese reaction. MAO-A inhibition results in increased levels of tyramine in the circulation which then enters the adrenergic neurons. In the adrenergic neurons, tyramine can increase the release of noradrenaline (NA) and result in hypertensive crisis.

2.2.2.2 MAO-B inhibitors

MAO-B inhibitors are mainly used as mono-therapy or adjunct therapy to L-dopa in the treatment of PD. There are currently two MAO-B inhibitors in use, namely selegiline and rasagiline. Both of these inhibitors are selective, irreversible inhibitors of MAO-B (Riederer *et al.*, 2004). They are mechanism-based inhibitors of MAO-B and form covalent adducts with the N5 or C(4a) positions of the FAD co-factor. Rasagiline, for instance, bind covalently to N5 of the FAD co-factor (Figure 21) (Edmondson *et al.*, 2004).

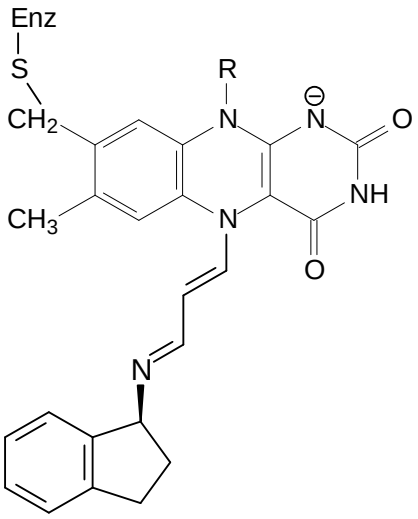

Figure 21 Chemical structure of rasagiline covalently bound to FAD

Selegiline undergoes extensive first pass metabolism and has a low bioavailability. Amphetamine and methamphetamine metabolites are produced during the metabolism of selegiline (Figure 22) and may result in neurotoxicity, cardiovascular as well as psychiatric side effects. The possible neuroprotective effects of selegiline are reduced due to the side effects associated with the metabolites of this drug (Riederer *et al.*, 2004; Youdim & Bakhle, 2006; Chen *et al.*, 2007). Rasagiline has a better safety profile compared to selegiline since no amphetamine-like compounds are produced when rasagiline is metabolized (Zhu *et al.*, 2008). One of the major metabolites of rasagiline, aminoindan, may indeed have neuroprotective properties (Figure 22) (Bar-Am *et al.*, 2007). The mechanism of neuroprotection by MAO inhibitors is unknown, but the reduction of oxidative stress and the prevention of apoptosis may play a role (Riederer *et al.*, 2004; Youdim & Bakhle, 2006). Other irreversible inhibitors of MAO-B are the cyclopropylamines (tranylcypromine) and hydrazines, which are non-selective MAO inhibitors and therefore have limited use in PD.

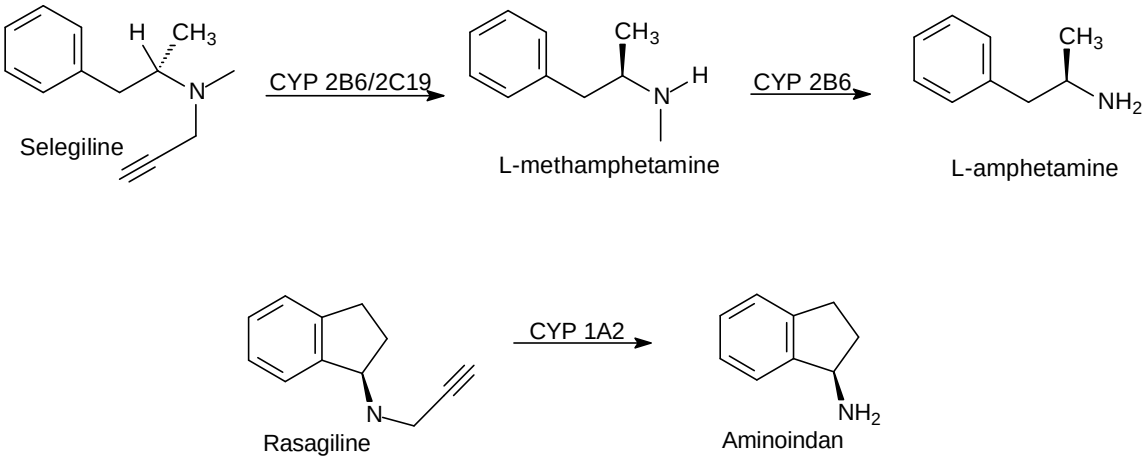

Figure 22 The metabolic pathway of selegiline to yield methamphetamine and amphetamine as metabolites. The metabolism of rasagiline to aminoindan by cytochrome P450 (CYP) is also shown.

Safinamide is a selective, reversible inhibitor of MAO-B which also inhibits DA reuptake. Since safinamide is a reversible inhibitor, it does not interact covalently with the FAD co-factor and enzyme recovery after withdrawal is almost immediate. Safinamide binds in an extended conformation within the active site of MAO-B with the primary amide moiety oriented towards the FAD co-factor (Fernandez & Chen, 2007; Binda *et al.*, 2007).

2.2.3 MAO active sites and rational inhibitor design

MAO-A and –B have different substrate and inhibitor specificities, since the structures of their active site cavities are different. By gaining a better understanding of each of these active sites (MAO-A and –B), a rational approach can be employed in the design of specific inhibitors for these enzymes.

2.2.3.1 MAO-B

The crystal structure of human MAO-B was initially solved to a resolution of 3 Å which made it possible to predict and explain the behaviour of specific inhibitors within the active site of MAO-B. The crystal structure can be used to design new inhibitors of MAO-B (Binda *et al.*, 2002b).

It was found that MAO-B consists of 520 amino acids and naturally occurs as a dimer, bound to the outer mitochondrial membrane. The enzyme attaches itself to the

mitochondria by a transmembrane α-helix at the C-terminal of MAO. The C-terminal consists of 32 amino acids and is hydrophobic in nature. It protrudes perpendicularly into the phospholipid bilayer of the mitochondrial membrane where it anchors the enzyme (Binda *et al.*, 2002b; Edmondson *et al.*, 2004). There are two theories concerning the conformation of the C-terminal within the mitochondrial membrane. One theory suggests that the C-terminal traverses the membrane with 5 amino acids protruding on the other side. The other hypothesis proposes that the C-terminal folds within the membrane so that the end of the helix protrudes on the same side as the enzyme (Figure 23) (Binda *et al.*, 2004). The anchoring of the C-terminal into the mitochondrial membrane might be crucial for MAO catalytic activity. The substrate cavity faces the membrane surface and has a hydrophobic region near the C-terminal which may assist in membrane binding and the stabilization of the enzyme. Residues 99 – 112 forms a loop which acts as a gate to the active site. This loop is believed to be partly embedded in the membrane which may control its function as a gate to the active site. The mitochondrial membrane is anionic and possibly concentrates the positively charged amine substrates near the active site entrance (Binda *et al.*, 2002b; Edmondson *et al.*, 2004; Binda *et al.*, 2004).

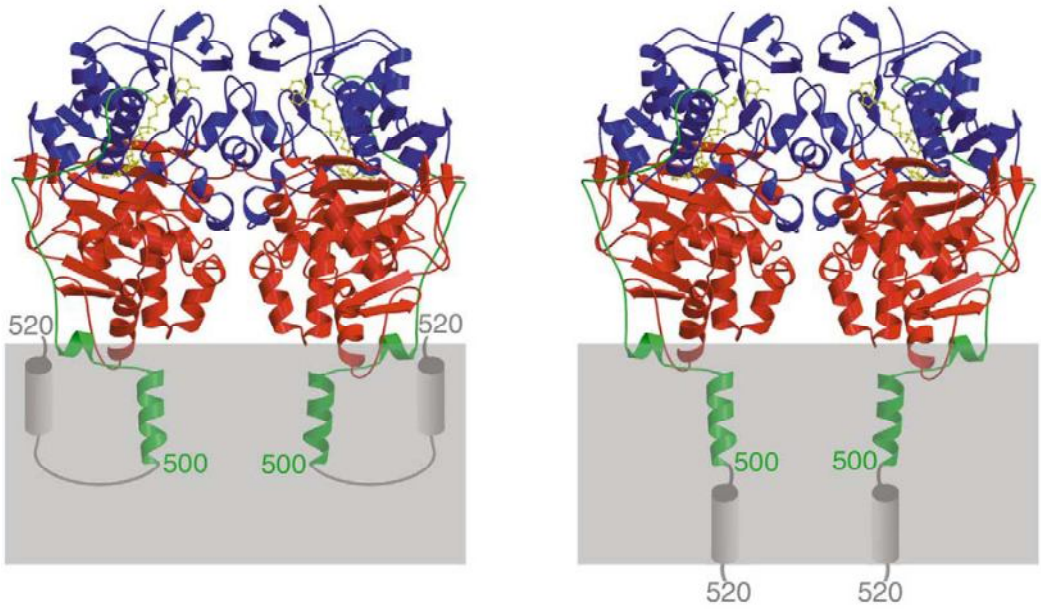

Figure 23 The two proposed conformations of C-terminal anchorage of MAO-B within the mitochondrial membrane. The first panel shows the C-terminal folding on itself to protrude on the same side as the enzyme. The second panel shows the suggestion that the C-terminal traverses the membrane to protrude on the other side (Binda *et al.*, 2004).

The active site of MAO-B consists of two cavities (Figure 24a). Both of these cavities, the substrate and entrance cavities, are hydrophobic in nature. The substrate cavity appears to be flat in comparison to the smaller entrance cavity. The two cavities are separated by residues Lue 171, Ile 199 and Tyr 326. Rotation of the side chain of Ile 199 can either fuse the two cavities or separate them, allowing this residue to act as a gate between the cavities (Figure 24b). When smaller substrates or inhibitors bind to the substrate cavity of MAO-B, Ile 199 is in a 'closed' conformation. In the case of larger inhibitors that traverse both cavities, Ile 199 is in an 'open' conformation (Binda *et al.*, 2002b; Binda *et al.*, 2003).

Within the substrate cavity, the FAD co-factor is situated to the back of the cavity flanked by the residues Tyr 398 and Tyr 435 to form an aromatic cage (Figure 24b). The aromatic cage is more hydrophilic in nature and plays a role in the orientation and recognition of amine groups (Binda *et al.*, 2002b; Li *et al.*, 2006).

One of the unique features of MAO is the fact that the isoalloxazine moiety of the FAD, in its oxidized form, is bent and twisted, not planar. This twisted form may possibly assist the FAD co-factor in substrate binding by allowing the N5 and C(4a) positions to exhibit more sp^3 character. The N5 position can thus act as a better nucleophile while the C(4a) position act as a better nucleophile target (Li *et al.*, 2006). Another feature is the *cis* conformation of the peptide bond of the FAD-linking cysteinyl residue (Cys 397-Tyr 398). The *cis* conformation is energetically much more unfavourable than the *trans* conformation but it allows the phenolic ring of Tyr 398 to be perpendicular to the flavin ring and thus form the aromatic cage which plays a critical role in substrate recognition and orientation (Binda *et al.*, 2003; Edmondson *et al.*, 2004).

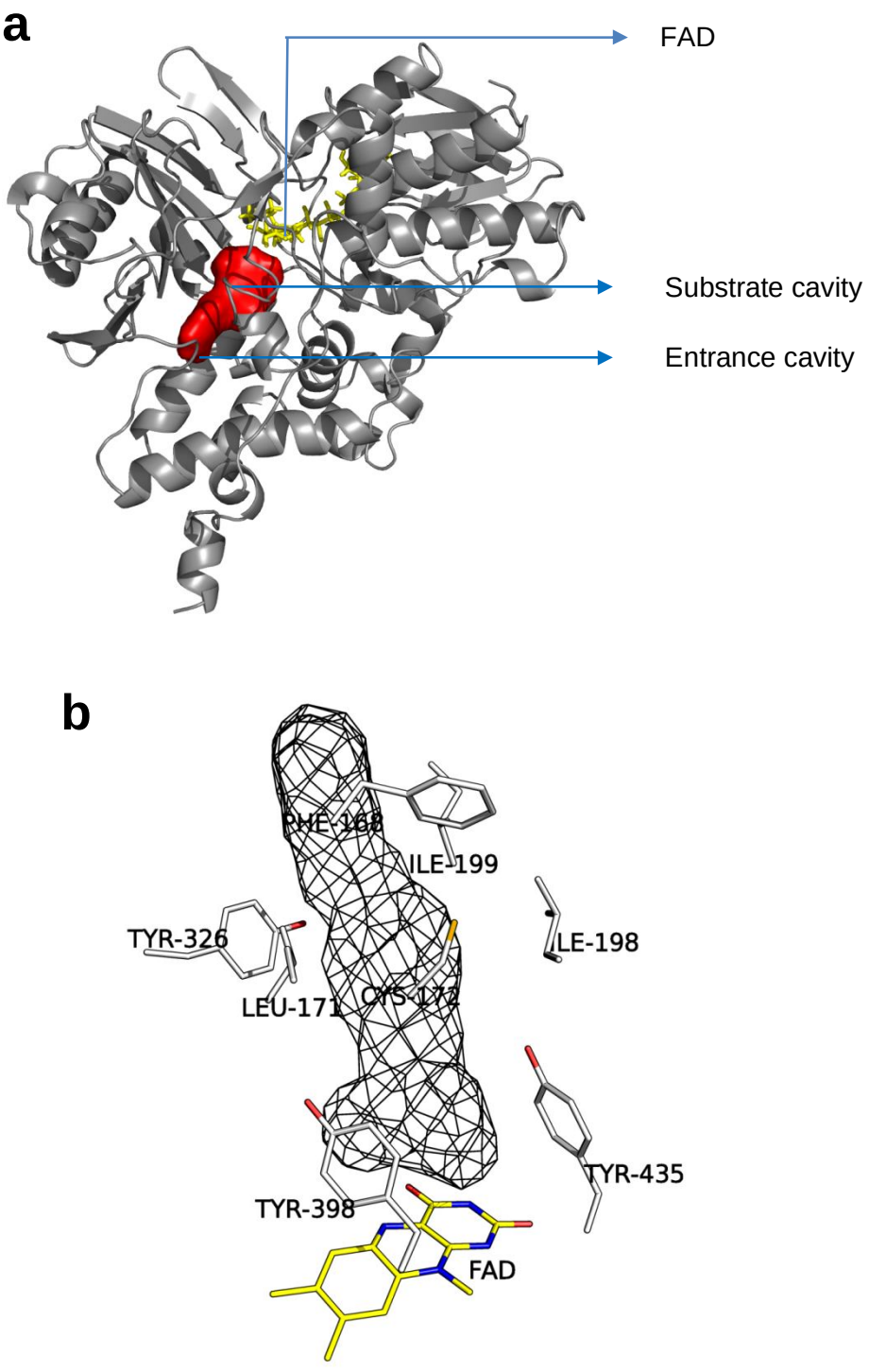

Figure 24 a) An illustration of MAO-B. The transmembrane helix is pointed downward with the entrance and substrate cavities indicated in red. The FAD co-factor is situated to the back of the substrate cavity and is indicated in yellow. b) The active site cavity of MAO-B. The two cavities are fused with Ile 199 in an 'open' conformation. Tyr 435 and Tyr 398 form the aromatic cage (Binda *et al.*, 2007).

2.2.3.2 MAO-A

The crystal structure of human MAO-A, co-crystallized with harmine, has been solved to a resolution of 2.17 Å (Son *et al.*, 2008). It was found that MAO-A and MAO-B are very similar in their structures, in fact the catalytic regions of these two isoforms, containing the FAD co-factor and the aromatic cage are almost identical. This implies the same catalytic mechanism for the two isoforms. Another feature similar to that of MAO-B is the transmembrane helix that anchors the enzyme to the outer mitochondrial membrane (Figure 25a) (De Colibus *et al.*, 2005). It appears that transmembrane anchorage also play a critical role in the catalytic activity of MAO-A, the same as with MAO-B (Son *et al.*, 2008). There are 16 major residues in the active site cavities of MAO-A and –B, of which 6 MAO-A residues differ from that found in the active site of MAO-B (Son *et al.*, 2008).

A unique feature of MAO-A is that this enzyme, unlike MAO-B, naturally occurs as a monomer (Figure 25a). The active site cavity of MAO-A is shaped by residues 210 – 216 which forms a loop that distinguishes the perimeter of the cavity and stretches from the entrance to the FAD co-factor at the back. Compared to MAO-B, the active site cavity of MAO-A is shorter, wider and much smaller (Figure 25b). This cavity is also hydrophobic in nature with a hydrophilic pocket in front of the FAD in the aromatic cage. The difference in active site residues between MAO-A and MAO-B may contribute to the different substrate and inhibitor specificities of the two isoforms. For example, the residue Phe 208 in MAO-A is in the homologues position to residue Ile 199 in MAO-B. In MAO-B Ile 199 is able to rotate out of the way of larger inhibitors, such as safinamide, in order to fuse the two cavities of the active site. Phe 208 on the other hand is a much larger residue and is not able to rotate to accommodate larger inhibitors. MAO-A also has residue Ile 335 in the place of Tyr 326 in MAO-B. Where Ile 335 would allow the binding of certain inhibitors, such as clorgyline, within MAO-A, the residue Tyr 326 may restrict such binding within MAO-B (De Colibus *et al.*, 2005; Son *et al.*, 2008).

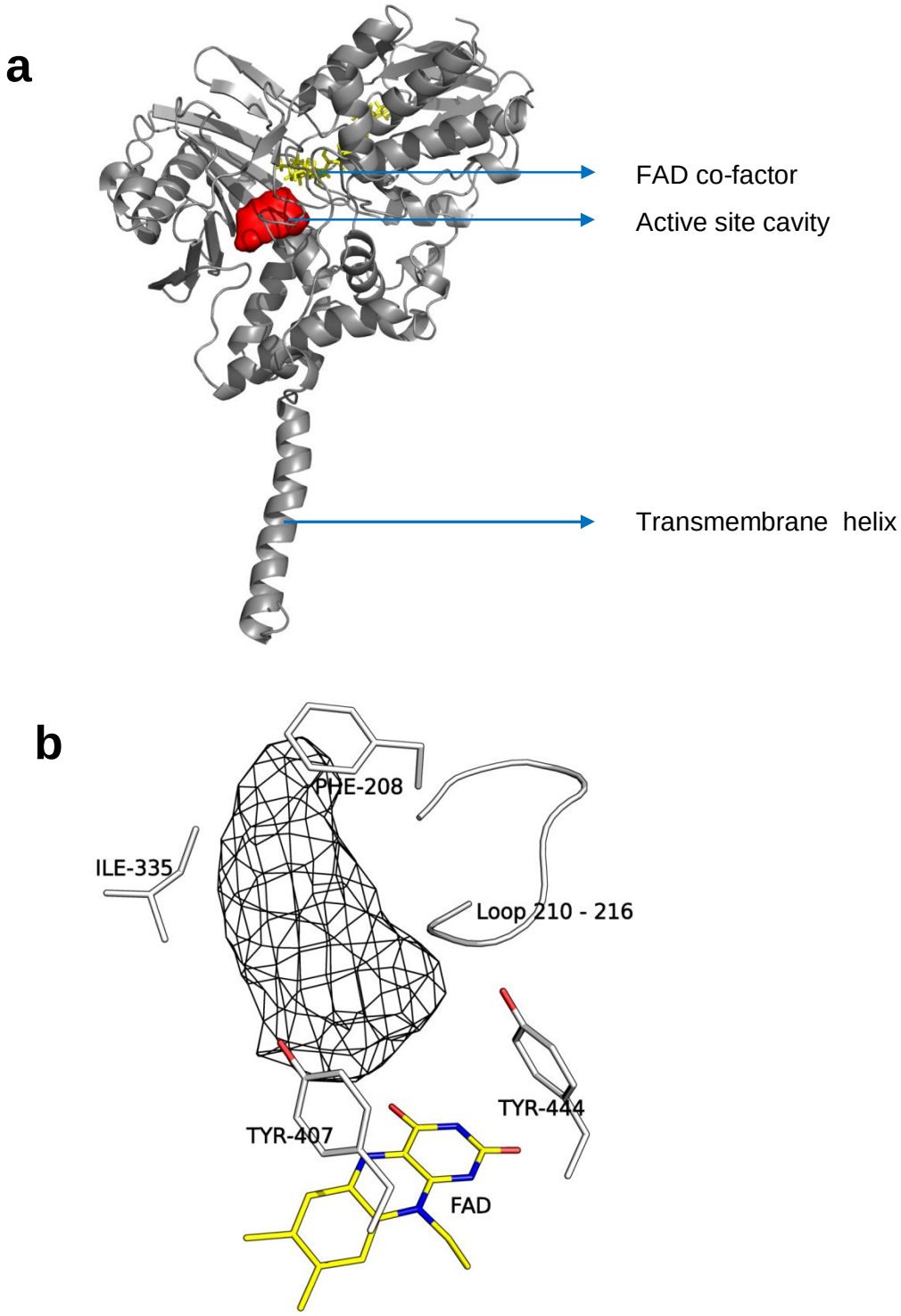

Figure 25 a) The overall structure of the human MAO-A monomer. The FAD co-factor is situated to the back of the single active site cavity. b) An illustration of the active site cavity of MAO-A. FAD is indicated in yellow at the bottom with Tyr 407 and Tyr 444 forming the aromatic cage. Loop 210 – 216 define the outer perimeter of the active site (Son *et al.*,2008).

2.2.3.3 Rational inhibitor design

Considering the differences of the active site cavities of MAO-A and –B, different structural properties are required for compounds to act as inhibitors of the respective enzymes. Thus, the shape, size and composition of the active site cavity determines the structure of the inhibitor.

The larger, bipartite active site cavity of MAO-B is able to accommodate larger molecules, since Ile 199 can rotate to fuse the two cavities. This mode of binding, with the inhibitor traversing both entrance and substrate cavities, may be an important determinant of selective MAO-B inhibition (Hubálek *et al.*, 2005). Since the active site cavity of MAO-A is much smaller and Phe 208 restricts the binding of larger inhibitors, smaller molecules are more suited for the MAO-A active site. For example, safinamide, that traverses both the entrance and the substrate cavity, is a selective MAO-B inhibitor. Safinamide would not be able to bind within the smaller active site cavity of MAO-A, since unfavourable interactions with Phe 208 may occur (Figure 26) (Binda *et al.*, 2007; Son *et al.*, 2008). Harmine, on the other hand, is a selective MAO-A inhibitor and binds easily within the smaller active site cavity of MAO-A with Ile 335 oriented to accommodate this molecule (Son *et al.*, 2008). Within the active site of MAO-B, however, harmine might undergo unfavourable interactions with Tyr 326 which is in the homologues position to Ile 335 (Figure 26).

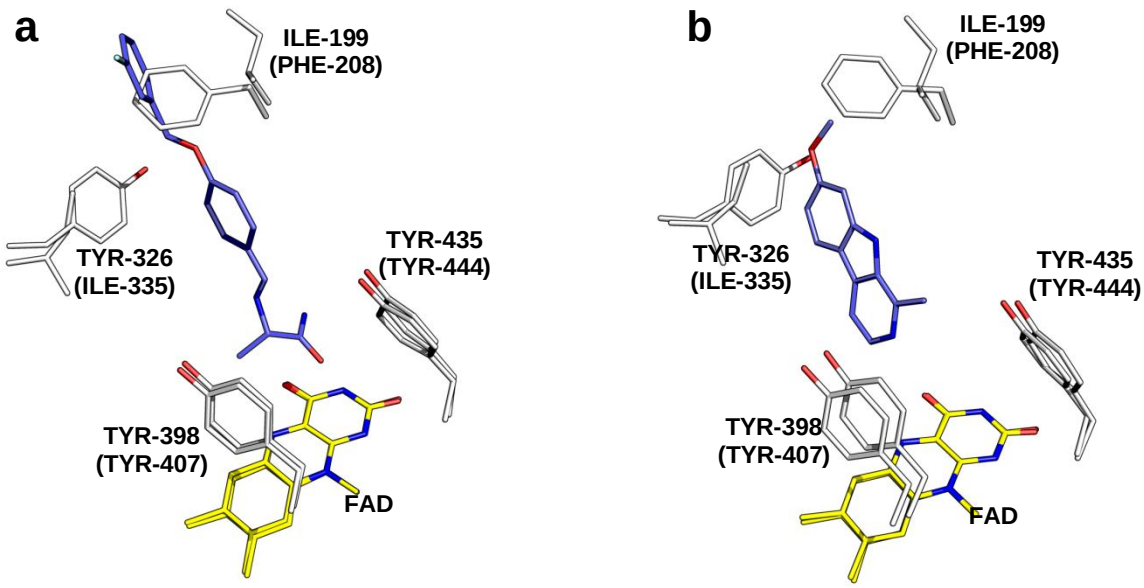

Figure 26 Overlays of the MAO-A active site on that of MAO-B. The identifiers of the residues for MAO-A are indicated in parenthesis. a) The orientation of safinamide within MAO-B (Binda *et al.*, 2007). Safinamide traverses both the entrance and active site cavities of MAO-B with the side chain of Ile 199 in an open conformation. The same orientation of safinamide would not be possible within MAO-A since the inhibitor would overlap with Phe 208 as indicated. b) The orientation of harmine within MAO-A (Son *et al.*, 2008). The same orientation of harmine within MAO-B may result in unfavourable interactions with the phenolic ring of Tyr 326.

It has been shown that larger inhibitors, which possess a high to moderate degree of flexibility, may bind within the smaller active site of MAO-A. Although not considered to be the optimal binding mode, larger inhibitors may bind in a folded conformation in the MAO-A active site, thus avoiding unfavourable interactions with the MAO-A active site residues (Van der Walt *et al.*, 2009; Strydom *et al.*, 2010). Since the cavities of both isoforms are lipophilic in nature, the binding of lipophilic compounds will be favoured (De Colibus *et al.*, 2005; Binda *et al.*, 2002b).

As mentioned earlier, there may be merit in developing non-selective inhibitors of MAO. Non-selective inhibition has the possible advantage of anti-depressive action via MAO-A inhibition in addition to the antiparkinsonian effects of MAO-B inhibition (Slaughter *et al.*, 2001; Leentjies *et al.*, 2011). Since DA is a substrate for both MAO-A and –B, non-selective inhibition of MAO may further increase DA levels (Kalaria *et*

al., 1988; Fowler *et al.*, 1980). In order to develop non-selective inhibitors, the differences in the active sites of the two isoforms have to be considered. The required inhibitor would need the necessary flexibility to orient itself optimally within both enzymes. For example, 8-benzyloxycaffeine is a potent inhibitor of MAO-B with activity towards MAO-A. 8-Benzyloxycaffeine consists of a caffeine moiety which binds within the substrate cavity in front of the FAD co-factor and a benzyloxy side chain which extends into the entrance cavity of MAO-B. In order to allow this extended binding conformation, Ile 199 undergoes a rotation of its side chain to fuse the two cavities. Within MAO-A the benzyloxy side chain undergoes a considerable conformational change to allow binding of 8-benzyloxycaffeine within the smaller active site cavity of MAO-A and avoid overlapping with active site residues. In fact, the benzyloxy side chain bends at the ether oxygen bond by about 34 degrees (Figure 27). Therefore the benzyloxy moiety can be considered as a suitable substituent to enhance the non-selective character of an inhibitor by increasing the conformational freedom of a molecule (Van der Walt *et al.*, 2009; Strydom *et al.*, 2010).

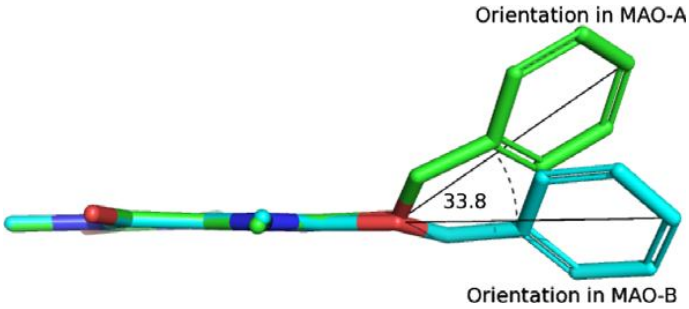

Figure 27 The orientation of 8-benzyloxycaffeine within MAO-B superimposed on the orientation within MAO-A. The benzyloxy side chain is bent at an angle of about 34º at the ether oxygen linkage. This flexible mode of binding enables 8-benzyloxycaffeine to act as an inhibitor of both MAO-A and –B (Strydom *et al.*, 2010).

2.3 Conclusion

This chapter discussed the pathology, etiology and treatment of PD. MAO inhibitors are of particular interest and the mechanism for symptomatic and neuroprotective effects of these compounds were discussed. The protein structure and active site cavities of MAO-A and –B were investigated to facilitate in rational inhibitor design for these target enzymes.

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